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CHARACTERIZATION OF A BEHAVIORALLY ACTIVE, GENDER-SPECIFIC VOLATILE COMPOUND FROM THE MALE ASPARAGUS FLY *Plioreocepta poeciloptera*

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Abstract—Adult male asparagus flies exhibit typical calling behaviors (suggestive of pheromone production) during which they emit a single volatile compound that was identified as isopropyl (*S*)-5-hydroxyhexanoate. In laboratory bioassays, synthetic samples elicited an arrestant response in females, but did not appear to attract females. On the other hand, the synthetic material attracted conspecific males in olfactometer bioassays.

Key Words V *Plioreocepta poeciloptera*, asparagus fly, male-produced volatile, isopropyl (*S*)-5-hydroxyhexanoate.

INTRODUCTION

The asparagus fly, *Plioreocepta (Platyparea) poeciloptera*, is a temperate univoltine monophagous tephritid, the maggots of which are serious pests of asparagus crops in Europe (Lesne, 1913). Adults appear in France in late March to early April (Reulet, 1991). Courtship and mating behaviors of this fly, as observed in both laboratory and field situations (unpublished observations), are similar to those of *Chaestostomella undosa*, wherein males patrol, or remain on the top of the host plant (Steck, 1984). In both of these species, encounters between males lead to agonistic behavior, and encounters between males and

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females occurred without the necessity of lek formation, the females approaching the males perched on their host plant. In the majority of tephritid species (Bateman, 1972; Sivinski and Burk, 1989), the male emits a sex pheromone that attracts females. We earlier described a calling behavior exhibited by adult male *P. poeciloptera*, and described attraction of virgin females to the "calling^ males as well as to dissected lateral abdominal pouches, the likely source of pheromone (Seguy, 1951; Thibout and Auger, 1999). We also described experiments with solid-phase microextraction (SPME) fibers, wherein a single volatile compound was collected from calling males and tentatively identified on the basis of its mass and infrared spectra. The structural assignment (Auger et al., 1998) of the presumed pheromone, as demonstrated by synthesis of the proposed structure (Casaña-Giner et al., 2001), turned out to be incorrect, however.

This paper reports our continuing studies of the asparagus fly, including positive identification of the major male-produced volatile. We have determined the optimum age and circadian rhythm of male calling, individually collected male- and female-produced volatiles, and again observed the presence of a major volatile produced exclusively by calling males. Additional gas chromatography-mass spectrometry studies (GC/MS) contributed to a revised structure for this compound, which has now been established as isopropyl (*S*)-5-hydroxyhexanoate. Laboratory behavioral assays and chiral gas chromatography support the assigned structure and stereochemistry of this sex-specific compound.

METHODS AND MATERIALS

Insects. The establishment of laboratory colonies of *P. poeciloptera* (Diptera: Tephritidae) has not been successful (Thibout and Auger, 1999), and test insects were obtained by collecting pupae from asparagus fields in early October. Pupae were collected at the base of spears in the Loire Valley, near Chinon, France, and were maintained in the laboratory at 8 ± 2°C, in a humid atmosphere, until the end of March, after which they were gradually introduced into Petri dishes at a rate of 10Y15 pupae per day, in synchronous photoperiod and thermoperiod: 16 hr at 26°C in photophase and eight hr at 17°C in scotophase. About 12 d later, adults (which were isolated by gender) emerged, and were individually maintained in Petri dishes on aqueous sucrose solutions.

Male Specific Volatiles Collection. Collection of volatiles was timed to coincide with the male calling period, determined by hourly observations on 80 isolated males during the 16 hr photophase from day 1 (emergence day) to day 8. The characteristic male calling position (lifted abdomen, protruded abdominal pouches, spread wings) has been previously described (Thibout and

Auger, 1999). SPME-GC was used to trap, transfer, and analyze the collected components of the volatiles. To collect volatiles, a male was placed in a 10-ml glass vial stoppered by a plastic cap with a hole allowing insertion of the SPME fiber. The fiber was positioned a few mm from the abdominal extremity of the immobile male for 1 min. Analysis of the adsorbed sample was carried out within 5 min of sampling by desorption for 2 min in the injection port of the GC. One-, 2-, 4-, and 8-d-old males were used regardless of whether they displayed calling behavior or not. Two- to 4-d-old females were used as controls. Up to seven repetitions were carried out for adults of each sex and age.

Isolation of Male-Specific Volatiles. Volatiles of 4-d-old males and females were trapped on Tenax cartridges (SKC Inc., Eighty-Four, PA). Each adult was introduced into a 20-ml glass vial for aeration. Incoming air (10 ml/min, a rate that did not disturb the flies) was precleaned by passing successively through two Tenax and two activated charcoal cartridges. Exiting air passed through the Tenax cartridge trap (50 mg Tenax). In the morning, a control and a male were aerated for 4 hr. In the afternoon, a female and another male were similarly aerated for 4 hr. This was repeated on 10 consecutive days to obtain four experimental Tenax cartridges, each containing the volatiles from 40 hr of trapping. The entire procedure was repeated a second time with another batch of four cartridges. The two batches of four cartridges were then eluted with pentane at 4°C. Only the two first drops eluting from the cartridge were collected and stored at −20°C until being analyzed by GC/MS.

We first compared the volatiles from females and from a control (vial containing no insects) to those from the two types of male samples (morning and afternoon collections).

Identification of the Male Volatile. ^{1}H NMR spectra were recorded in $CDCl_3$ or C_6D_6 on a Bruker QE-300 spectrometer. In the U.S. laboratory, GC was performed with a Shimadzu Model GC-17A instrument equipped with a 30 m × 0.25 mm SPB-35 capillary column (Supelco, Bellefonte PA) and a flame ionization detector, or on an Agilent 6890N instrument equipped with a 30 m × 0.25 mm HP-5 column (Agilent Avondale PA). Chiral gas chromatography was performed with a 30-m Chiraldex B-DM column (Advanced Separation Technologies, Whippany, NJ). In the French laboratory, solid-phase microextraction-GC (SPME-GC) experiments were conducted with a Varian 3300 instrument with a FID detector equipped with a splitYsplitless injector, an 80-μl liner for SPME fiber desorption, and a fused-silica HP-1 capillary column (Agilent, 20 m × 0.22 mm I.D., 0.33 μm film thickness). The conditions were as follows: injection temperature 200°C, carrier gas helium, column flow 1.0 ml/min, splitless mode. The column temperature program was 2°C/min from 40 to 200°C.

In the French laboratory, mass spectral experiments were conducted with a benchtop Perkin-Elmer Turbomass system with a splitYsplitless injector and a 25-m fused-silica HP-1 capillary column similar to that described above. The carrier

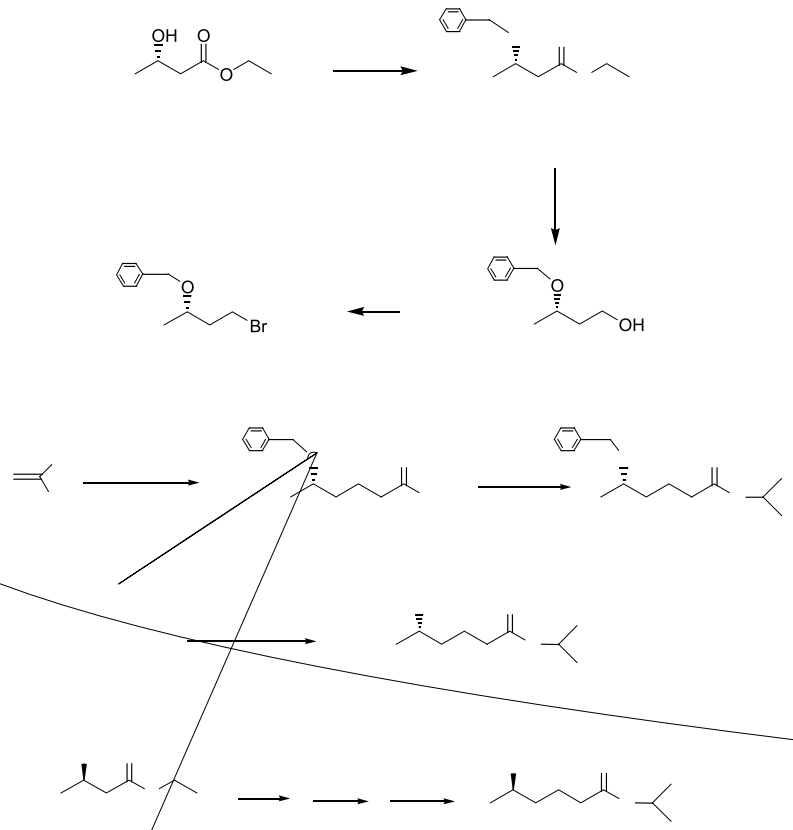
gas was helium at 2 ml/min, and the column temperature program was 5°C/min from 60 to 180°C then 15°C/min from 180 to 280°C. Total ion chromatograms (TICs) and mass spectra were recorded in the electron impact ionization mode at 70 eV. In the U.S. laboratory, mass spectra were obtained from a Shimadzu GCMS-QP 5050A equipped with a 30 m × 0.25 mm DB-5 column (J&W Scientific, Fulsom CA). Chemical ionization spectra were obtained using ammonia as reagent gas. Additional mass spectrometric experiments on the trapped male compound added little to those already published (Auger et al., 1998). Rotations were measured with a Perkin Elmer Model 241 polarimeter.

Syntheses. Described below are a series of reactions leading to the (*S*)-enantiomer of the major volatile, **4a** (Figure 1) An identical series, beginning with the benzyl ether of ethyl (*R*)-3-hydroxybutyrate (Malher et al., 1988), was conducted to prepare the (*R*)-enantiomer **4b**.

*(S)-3-(Benzyloxy)butyric acid ethyl ester **6a*** (Keck and Murry, 1991). To a solution of ethyl (*S*)-3-hydroxybutanoate **5a** (0.9 g, 6.8 mmol) and *O*-benzyltrichloroacetimidate (2.4 g, 7.5 mmol) in 2:1 cyclohexane/dichloromethane (36 ml) was added trifluoromethanesulfonic acid (80 μl). The mixture was stirred at room temperature for 24 hr, then filtered. The filtrate was added to saturated aqueous $NaHCO_3$, and the mixture was extracted with dichloromethane (3 × 8 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Flash chromatography (9:1 hexanes/EtOAc) afforded **6a** (1.37 g, 91%) as a light yellow liquid.

*(S)-3-(Benzyloxy)butan-1-ol **7a***. Dry ether (11 ml) was cooled with an ice bath, and $LiAlH_4$ (0.15 g, 3.9 mmol) was added. The mixture was warmed to room temperature and stirred for 30 min, then was again cooled with an ice bath, and a solution of **6a** (1.15 g, 5.18 mmol) in dry ether (3 ml) was added dropwise. After stirring for 1 hr, water (0.15 ml), 15% NaOH (0.15 ml), and water (0.45 ml) were added dropwise in that order, and the mixture was stirred at 20°C for 15 min, then was filtered through Celite. The filtrate was washed with 1:1 brine/sat. $NaHCO_3$, dried over $MgSO_4$, and concentrated *in vacuo* to yield **7a** (0.75 g, 84%) as a light yellow liquid. EI-MS, *m/z* (%): 161 (10), 108 (10), 107 (52), 92 (18), 91 (100), 79 (26), 77 (20), 65 (32), 56 (12), 51 (14), 43 (19), 42 (13).

*(S)-3-(Benzyloxy)-1-bromobutane **8a***. Bromine (260 μl, 5 mmol) in dichloromethane (2 ml) was added dropwise to a solution of triphenylphosphine (1.3 g, 5 mmol) in dichloromethane (5 ml) at about −10°C. The solution was warmed to room temperature and stirred 20 min. After cooling to −30°C, a solution of **7a** (0.75 g, 4.2 mmol) in dichloromethane (2 ml) was added dropwise, then the mixture was stirred at room temperature for 16 hr. After concentration *in vacuo* to near dryness, 10 ml pentane was added. The mixture was filtered and the solids were rinsed with several portions of pentane. The combined filtrates were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in*



vacuo. Flash chromatography (9:1 hexanes/EtOAc) afforded **8a** (0.68 g, 67%) as a light yellow liquid. EI-MS, *m/z* (%), 135 (31), 107 (15), 91 (100), 89 (13), 79 (28), 77 (25), 65 (30), 63 (13), 55 (23), 51 (20), 43 (18), 41 (15).

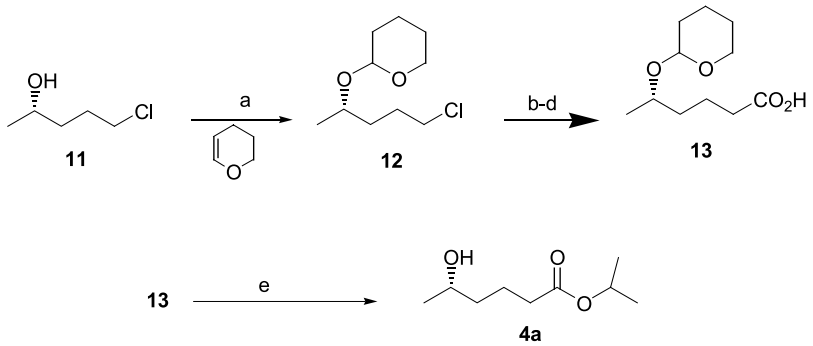
(S)-5-(Benzyloxy)hexanoic Acid ***9a***. Lithium diisopropylamide (LDA) was prepared from diisopropylamine (1.18 ml, 8.46 mmol) and 2.5 M *n*-butyllithium (3.38 ml, 8.46 mmol) in THF (5 ml) at −10°C. The newly prepared LDA was added by syringe to a solution of acetic acid (240 µl, 4.23 mmol) and NaI (2 mg) in THF (5 ml) at −20°C. Hexamethyl phosphoramide (HMPA) (1.47 ml, 8.46 mmol) was added, and after the mixture was stirred at 0°C for 30 min,

it was cooled to −20°C, and a solution of **8a** (0.685 g, 2.82 mmol) in THF (1 ml) was added. The reaction mixture was allowed to warm slowly to room temp and stirred 16 hr, after which time it was added to ice and extracted with ether (2 × 10 ml). The aqueous phase was acidified with 1 N HCl and extracted with 1:1 ether/hexane (3 × 10 ml). The ether/hexane extracts were washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The crude liquid was filtered through a plug of silica gel to afford **9a** (0.56 g, 89%) as a yellow liquid. EI-MS (of the methyl ester, formed by diazomethane treatment): 135 (19), 91 (100), 79 (19), 77 (16), 65 (28), 55 (16), 51 (13), 43 (12).

*(S)-5-(Benzyloxy)hexanoic acid isopropyl ester **10a**.* Oxalyl chloride (270 μl, 2.8 mmol) and DMF (2 μl) were added to a solution of **9a** (0.57 g, 2.55 mmol) in benzene. After stirring 1 hr, the reaction mixture was concentrated *in vacuo*, redissolved in benzene, and added to a solution of pyridine (270 μl, 3.2 mmol) and isopropanol (490 μl, 6.4 mmol) in benzene (2 ml). After stirring overnight, the mixture was diluted with ether (15 ml) and extracted with 1 N HCl (10 ml). The organic phase was washed with water, then with saturated aqueous $NaHCO_3$, and finally with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Flash chromatography (20:1 hexanes/EtOAc) afforded **10a** (0.345 g, 51%) as a light yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.36Y7.20 (m, 5H), 5.00 (sept, J = 6.43 Hz, 1H), 4.50 (m, 2H), 3.52 (sex, J = 6.05 Hz, 1H), 2.26 (t, J = 6.81 Hz, 2H), 1.78Y1.38 (m, 4H), 1.21 (m, 9H). EI-MS: 158 (39), 117 (11), 116 (49), 115 (20), 113 (20), 107 (29), 97 (16), 91 (100), 87 (24), 79 (15), 77 (13), 73 (26), 69 (21), 65 (32), 55 (21), 43 (39), 42 (20), 41 (35).

*(S)-5-Hydroxyhexanoic acid isopropyl ester **4a**.* To a solution of **10a** (0.345 g, 1.3 mmol) in ethyl acetate (10 ml) was added 10% Pd/C (34 mg). The solution was stirred under H_2 for 16 hr, then filtered through Celite and concentrated to provide **4a** (218 mg, 96%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$), 4.99 (sept, J = 6.43 Hz, 1H), 3.79 (sex, J = 6.06 Hz, 1H), 2.29 (t, J = 7 Hz, 2H), 1.68 (m, 2H), 1.57 (br s, 1H), 1.46 (m, 2H), 1.21 (t, J = 6.06 Hz, 3H). EI-MS: 115 (23), 114 (13), 99 (20), 97 (23), 89 (12), 88 (27), 73 (18), 71 (19), 70 (20), 69 (39), 68 (10), 60 (75), 55 (36), 45 (75), 44 (12), 43 (100), 42 (62), 41 (65). Under CI-MS conditions, the compound degraded to form 5-caprolactone. CI-MS m/z (%): 132 (54) ($M + NH_4^+$), 115 (100) ($M + H^+$). $[\alpha]_D^{25} = +7$ (c = 2.9, $CHCl_3$). The trimethylsilyl ether of this compound gave only a single peak on a chiral column, for an estimated enantiomeric excess (ee) of >99%.

*Alternate synthesis (Figure 2). (S)-5-Chloro-2-pentanol tetrahydropyranyl ether **12**.* A solution of (*S*)-5-chloro-2-pentanol (Daicel, Inc.; 9.47 g, 77 mmol) in dry dichloromethane (40 ml) and freshly distilled dihydropyran (8 ml, ca. 88 mmol) was treated with pyridinium *p*-toluenesulfonate (PPTS, 210 mg, 0.9 mmol). An exotherm was observed. The solution was allowed to stand undisturbed for 45 min, then most of the solvent was removed with a rotary evaporator and the residue was partitioned between cold aqueous sodium



a. dihydropyran, PPTS, b. Mg/THF, c. CO_2, d. H_3O^+, e. i-PrOH, PPTS, Sonicator

FIG. 2. Alternate synthesis of **4a** from (*S*)-5-chloro-2-pentanol.

carbonate (1 M) and 1:1 ether/hexane. The crude product (17 g) was distilled to give 12.3 g (77%) of **12**, b.p. 56°C, 0.03 Torr. ^{1}H NMR (300 MHz, $CDCl_3$, two diastereomers) 4.67 (m, 1H), 4.61 (m, 1H), 3.83 (m, 2H), 3.54 (m, 4H), 1.99Y1.41 (m, ~24H), 1.22 (t, J = 6.43 Hz, 3H), 1.12 (d, J = 6.06 Hz, 3H). This compound has been reported previously (Keinan et al., 1986).

*(S)-5-Hydroxyhexanoic acid tetrahydropyranyl ether **13***. Magnesium (1.56 g, 65 mmol) was covered with dry THF in an argon atmosphere. After activating the metal with a few microliters of 1,2-dibromoethane, THF was heated to reflux and a solution of **12** (6.01 g, 29 mmol) in THF (20 ml) was added dropwise over 0.5 hr. Refluxing was continued an additional 4.5 hr, then the Grignard solution was cooled to room temperature and transferred by cannula onto an excess of powdered dry ice in an argon-flushed flask. After standing about 45 min, ether and water were added, the mixture was acidified with aqueous $KHSO_4$, and the organic products were partitioned into ether. The ether solution was rinsed with water, then extracted with 1 M aqueous sodium carbonate (25 ml, then 2 × 5 ml). The combined extracts were rinsed with ether, then acidified with aqueous $KHSO_4$ and the acidic product was extracted into ether. After rinsing with water and brine, the solution was dried ($MgSO_4$), and concentrated to provide 5.25 g (84%) of **13** as a colorless liquid. ^{1}H NMR (300 MHz, benzene-d_6, mixture of diastereomers) 4.64 (m, 2H), 3.82 (m, 2H), 3.64 (m, 1H), 3.39 (m, 1H), 3.34 (s, 3H), 3.33 (s, 3H), 2.18 (m, 2H), 2.08 (m, 2H), 1.86Y1.25 (m, ~22H), 1.22 (d, J = 6.43 Hz, 3H), 0.97 (d, J = 6.05 Hz, 3H). EI-MS: 115 (83), 101 (13), 97 (54), 85 (100), 84 (10), 73 (27), 70 (13), 69 (69), 67 (27), 60 (21), 57 (39), 56 (48), 55 (74), 45 (41), 44 (85), 43 (80), 42 (42), 41 (82).

*Conversion of **13** to **4a***. A 100-ml flask containing a solution of **13** (5.25 g, 24.3 mmol) in 2-propanol (50 ml) plus pyridinium *p*-toluenesulfonate (0.61 g,

2.4 mmol) was fitted with a reflux condenser and introduced into a sonic bath that was operated overnight at room temperature (moderate warming of the water bath occurred during the procedure). The solvent was removed *in vacuo*, and the residue was flash chromatographed on 150 g silica gel, eluting with 10Y50% ethyl acetate in hexane. The earlier-eluting fractions provided 1.6 g (38%) of **4a** of 98% purity (GC), identical to **4a** described above. Slightly later-eluting material consisted of mixtures of a little more **4a** and lactone **1** plus some later-eluting (GC) material that appeared to be a dimeric product resulting from intermolecular transesterification of **4a** (or **4a** + **1**) (1.4 g). From chiral GC, the ee of **4a** was estimated to be >98%.

Adult Fly Behavioral Responses to Synthetic Compounds. Behaviors of 4-d-old males and females were observed in an olfactometer similar to that previously described (Thibout and Auger, 1999). Air was passed at 20 cm/sec through a 90 × 9 cm glass cylinder opening into control and test channels. The center of the test channel contained a 4-cm^2 filter paper square treated with 50 μg of a candidate compound dissolved in 5 μl of paraffin oil. One adult was released at the downwind opening of the tube, and its movements and displacements were observed for 2 min. The fly distributions at the end of the observation period were recorded for both sexes, using three classifications: (1) adults moving less than 5 cm; (2) adults moving between 5 and 25 cm; and (3) adults moving more than 25 cm. A second behavior was evaluated in females, that is, the number of flies remaining immobile with spread wings, a posture typically observed when a female is in the proximity of a calling male.

Thirty repetitions were performed, with each fly being used only once. Two similar experiments were carried out, one in 2002 and the other in 2003. In the latter, the *S*-enantiomer **4a** was also tested at a higher concentration (200 μg in paraffin oil). All behavioral data were analyzed, when necessary, with a χ^2 test at $!$ = 0.05.

RESULTS

Influence of Male Age on Calling. The study of the number of males that were calling according to the time of day, and of the ages of calling males, showed that 1-d-old males were sometimes able to call, but usually did not (Figure 3). The percentage of 2-d-old males calling was similar to that of older ones (65%). There was a shift in time of calling with age, with young males calling in the middle of the day (mean 8.22 hr in 2-d-old males), earlier than more mature males that called in the early afternoon (9.76 hr in 8-d-old males; $P < 0.001$ using a $\#^2$ test). All the male distributions were significantly different except between 6 and 8 d.

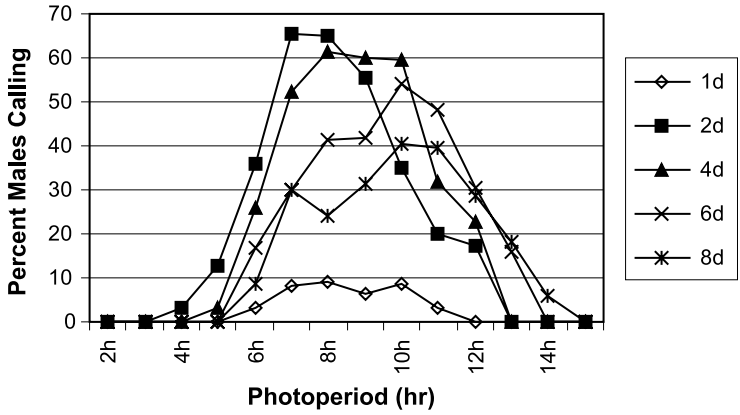


FIG. 3. Calling rhythm of *Plioreocepta poeciloptera* males at various ages. Distributions are statistically different except between 6 and 8 days ($\#^2$ test).

Identification of the Major Male Specific Volatile. Results obtained by SMPE/GC from individual flies showed that a major GC peak appeared at 13 min only in samples from calling males of 2-d-old or older (Figure 4). This peak was absent from volatiles from females, absent, or nearly so, from samples obtained from 1-d-old males, and absent from volatiles from noncalling males. The GC/MS results of the Tenax trapping of volatiles from males and females paralleled those of the SPME experiments, but reproducibly indicated additional components, particularly one with retention time 7.75 min, present in all extracts including the control (Figure 5). The mass spectra of these components indicated them to be sulfur-containing plant volatiles originating from the asparagus plants, the major being identified as dipropyl disulfide. As had been the case in the SPME/GC study, only one male-specific compound, corresponding to the 13-min eluting peak just discussed, was observed from males aerated in the afternoon, the time at which they were seen calling. In the control, in the female-derived samples, and in the samples from males aerated in the morning (when few or none were calling), no peak with this retention time was observed (Figure 5).

The electron impact ionization mass spectrum of the male-produced unknown was an excellent match for that of δ-caprolactone **1**. A chemical ionization spectrum obtained with methane as reagent gas produced an ion with *m*/*z* 115, consistent with a protonated molecular ion, $M + H^+$, of **1** (MW 114). However, the GC retention time of the unknown was longer than that of **1**, and furthermore, lactone **1** is not consistent with the published infrared spectrum (Auger et al., 1998) that exhibited OYH stretching as well as a carbonyl absorption at 1729 cm^{-1}, more consistent with an aliphatic hydroxy-ester than

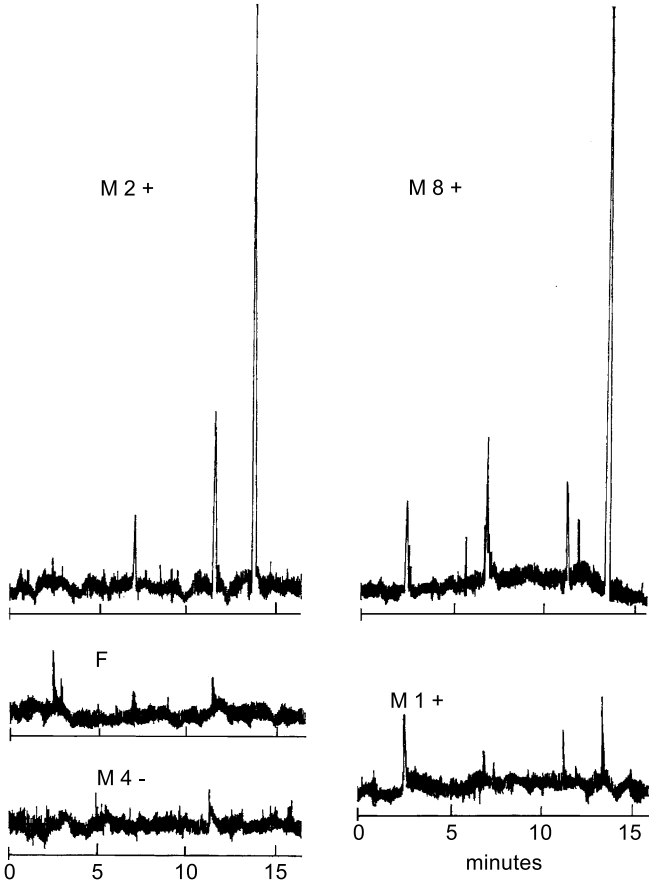


FIG. 4. GC analysis of *Plioreocepta poeciloptera* adult volatiles trapped by SPME at various ages. M1+: 1-d-old calling male; M2+: 2-d-old calling male; M4−: 4-d-old noncalling male; M8+: 8-d-old calling male; F: 4-d-old female.

with a lactone. We postulated that the volatile was possibly a somewhat labile compound that could decompose under MS ionization conditions to provide lactone **1**, but at the same time be stable enough to survive gas chromatography (to produce a retention time longer than that of **1** and a GCYFTIR inconsistent with **1**). We anticipated (incorrectly) that the less energetic CI reagent gas ammonia might improve our chances of determining molecular weight via CI-MS, but the NH_3-CI spectrum consisted cleanly and almost exclusively of ions with *m/z* 115 and 132 ($M + H^+$ and $M + NH_4^+$ for **1**, respectively).

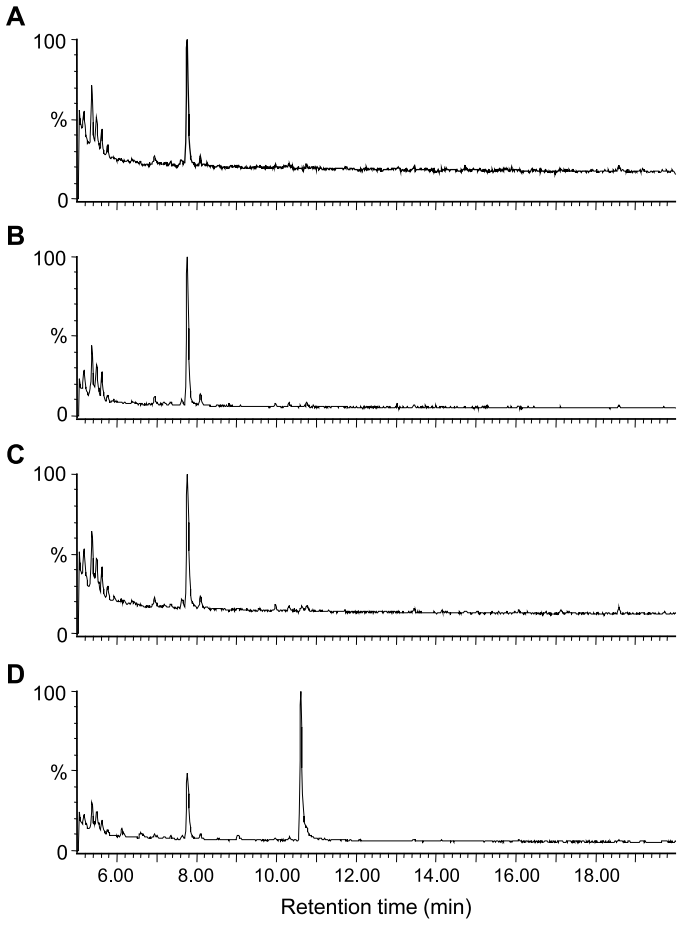


FIG. 5. GC/MS analysis of volatiles from 4-d-old *Plioreocepta poeciloptera* trapped on Tenax cartridges and eluted with pentane. **A** Control without insect; **B** Females, morning collection; **C** Males, morning collection; **D** Males, afternoon collection.

The most conservative explanation seemed to be an ester of 5-hydroxyhexanoic acid that could lose the alcohol component via intramolecular transesterification to form lactone **1**. Accordingly, we prepared ethyl ester **2** (Figure 4) by treatment of **1** with ethanol containing sodium ethoxide (Hernandez et al., 1996). The product **2**, a known compound (Lease and McElvain, 1933), eluted from the GC later than **1**, but still slightly earlier than the unknown; reassuringly, the mass spectra of **2** were also essentially identical to those of **1**. We next converted samples of **1** to the propyl and isopropyl esters **3** and **4**, respectively

(apparently unreported in the literature), and indeed, isopropyl ester **4** proved to be a perfect match for the insect-produced compound.

Racemic **1** was easily prepared by BaeyerŸVilliger oxidation of 2-methylcyclopentanone (Hernandez et al., 1996), and opening of the resulting lactone with sodium isopropoxide accordingly produced racemic **4** (Figure 6). By comparing racemate **4** with the insect-produced material by GC on a chiral column (analyses were conveniently conducted on the respective trimethylsilyl ethers, TMS), we established that the latter was a single enantiomer. To assign the absolute configuration of C-5 of the insect-produced compound, we synthesized the enantiomers from precursors in which the stereochemistry was already defined.

*Syntheses of the Isopropyl Ester of (S)-5-Hydroxyhexanoic Acid (**4a**).* Both enantiomers of ethyl 3-hydroxybutanoate are commercially available. The 3-(*S*)-enantiomer **5a** was protected as its benzyl ether **6a** (Keck and Murry, 1991), and **6a** was reduced with $LiAlH_4$ to provide the mono-protected diol **7a** (Figure 1). Treatment with Ph_3PBr_2 converted **7a** to bromide **8a**. Alkylation of the dianion of acetic acid (Pfeffer and Silbert, 1970) with **8a** gave (*S*)-5-hydroxyhexanoic acid benzyl ether **9a**, which was converted to the acid chloride and esterified with isopropanol to afford ester **10a**. Catalytic hydrogenation

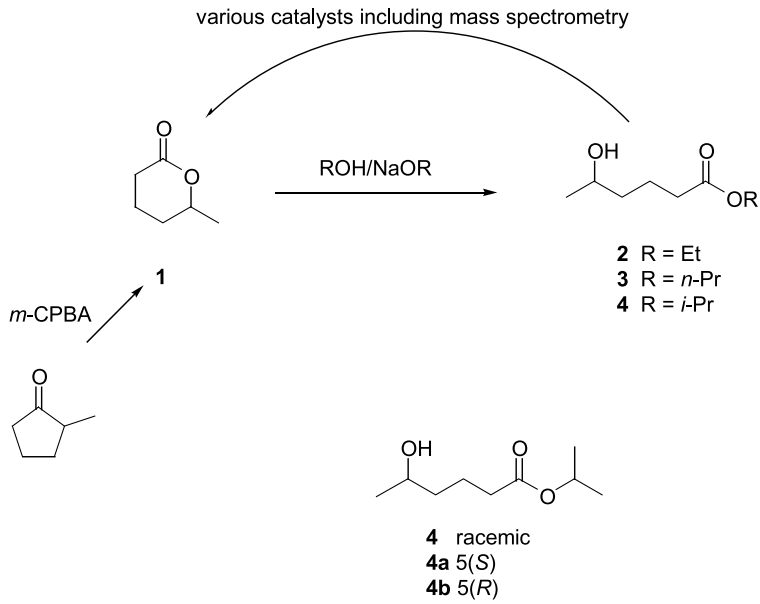


FIG. 6. 5-Hydroxyhexanoates from δ-caprolactone, and the reverse reaction.

removed the benzyl ether and produced the isopropyl ester of (*S*)-5-hydroxyhexanoic acid **4a**.

Repeating the above process beginning with the (*R*)-enantiomer of ethyl 3-hydroxybutanoate **5b**, similarly afforded **4b**, identical to **4a** except for retention time on a chiral GC column and specific rotation ($[\,!\,]_D^{25} = -8$ (c = 2.2, $CHCl_3$)).

Comparison by chiral GC proved that the 5-(*S*)-enantiomer **4a** matched the insect-produced compound, and that the insect produced compound gave only a single peak, with no trace of the (*R*)-enantiomer. Better GC results were obtained on the chiral column with TMS ethers of **4a** and **4b** than with the alcohols themselves. The TMS ether of the (*R*)-enantiomer **4b** eluted earlier than the corresponding TMS of the natural (*S*)-enantiomer **4a** from a Chiraldex B-DM column.

At about this time we became aware that (*S*)-5-chloro-2-pentanol **11** was becoming commercially available (Daicel Chemical Industries, Ltd.). Availability of this material suggested a convenient alternative synthesis as outlined in Figure 2. After protection of the secondary alcohol as its tetrahydropyranyl ether **12**, its Grignard reagent was carbonated to give THP acid **13**. Treatment of an isopropanol solution of **13** with Nafion-H™ or with pyridinium *p*-toluenesulfonate resulted in removal of the THP ether and esterification of the carboxylic acid, providing **4a** in a single step.

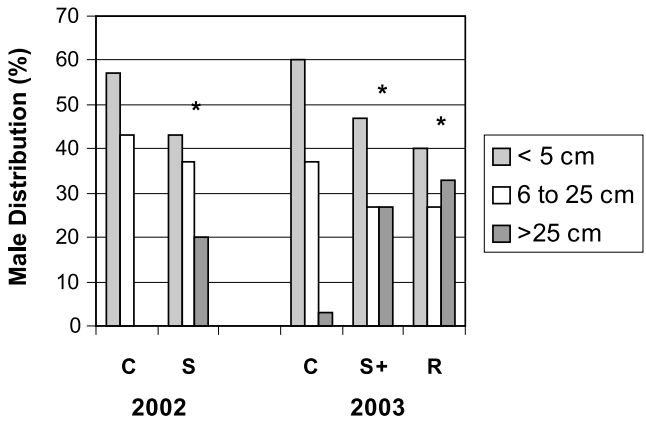
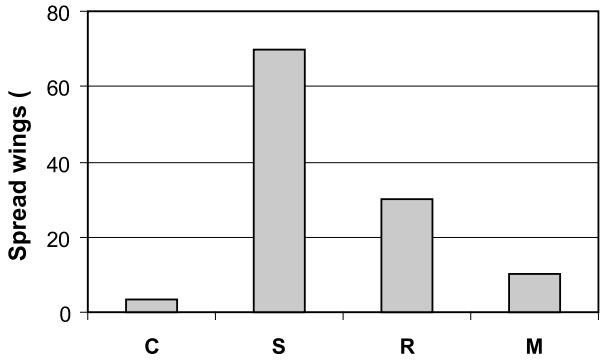


FIG. 7. Distributions in % of *Plioreocepta poeciloptera* males in the olfactometer in the presence of synthetic compounds recorded as distances moved toward the source (in cm). Two years' data are presented. *Distribution is statistically different from the control distribution ($\#^2$ test). C: Control without volatiles; R: *R*-enantiomer; S: *S*-enantiomer at 50 μg; S+: *S*-enantiomer at 200 μg.

Adult Fly Behavior in the Presence of Synthetic Compounds. The distributions of 4-d-old females and males in the olfactometer, in the presence of a single enantiomer **4a** or **4b**, or the racemate **4**, were compared in 2002 and 2003. The results were similar. Male distributions in response to both the *S*- and *R*-enantiomers were different from the control distributions. Males moved further in the presence of either of the two enantiomers, showing a higher chemo-anemotaxis (Figure 7). Female flies showed no significant upwind movement in response to either enantiomer or the racemate (data not shown).

However, the synthetic compounds were perceived by females and did produce behavioral responses. Females stimulated by the *S*-enantiomer often assumed a position of immobility with spread wings. This behavior was signifi-



cantly more frequent with the *S*-enantiomer than with the control or with the *R*-enantiomer in 2002 (Figure 8). In 2003, females responded more strongly to both doses of the (*S*)-enantiomer than to the control, the (*R*)-enantiomer, or the racemate. Responses to the latter two treatments were no different from responses to the control (Figure 8).

DISCUSSION

As observed previously, male *P. poeciloptera* called in the middle of the day and in the first half of the afternoon (Thibout and Auger, 1999). Calling behavior was rare in 1-d-old males but became frequent from the second d after emergence. The onset of calling and the peak calling time were delayed from age 1 to age 8 d. A time shift in calling corresponding to a time advance has been commonly seen in moth species (Swier et al., 1977; Gemeno and Haynes, 2000), where it was explained as an adaptation of older adults to increase their chances of mating (Swier et al., 1977). The adaptive advantage of delaying calling in mature *P. poeciloptera* males remains unclear.

The major male volatile appeared to be emitted during the afternoon calling period, and release of this compound coincided with eversion of glandular (Dingler, 1934) abdominal pouches (Thibout and Auger, 1999). In contrast to males, females were not attracted by the synthetic compound in the olfactometer. However, females did react behaviorally by spreading their wings, and only in response to the natural (*S*)-enantiomer. The induced wing spreading in females is believed to be a short range arrestant response. This olfactometer result is in contrast to our earlier field and laboratory observations that had indicated that females were attracted to a male pheromone (Thibout and Auger, 1999). The failure to respond in the olfactometer could suggest that factors other than the single compound may be involved in courtship and mating of this fly. We have looked for, but have been unable to find, other noteworthy male-specific volatiles. However, responses may be dose-dependent.

Unexpectedly, males were attracted in the olfactometer by both the *S*-enantiomer and the unnatural *R*-enantiomer. Cases of attraction to a nonnatural enantiomer have been observed in other insects such as cockroaches (Gemeno et al., 2003). However, we have never observed male *P. poeciloptera* attracted to other males or even to females in either the field nor the laboratory (Thibout and Auger, 1999), and the observed attraction of males to the synthetic compounds may result from unnaturally high concentrations, or possibly from some subtlety in the rhythm of emission. One could speculate that wild males could use the emission of invading males to detect their presence and chase them off, or, perhaps more likely, that invading males might exploit the

emission of native males to gain access to already-attracted females. The attraction of males to the male-produced compound, and the absence of attraction by females, can be compared to results previously observed in various tephritid species (Fletcher and Bellas, 1988; Landolt and Heath, 1990; Pike and Meats, 2003).

In summary, in spite of its release as a single, male-specific volatile compound coinciding with typical male calling behavior, and its elicitation of a typical arrestant response in females, isopropyl (*S*)-5-hydroxyhexanoate **4a** has not been established to be a true male sex pheromone of *P. poeciloptera*. Its behavioral effects, at least under laboratory conditions, do not correspond precisely to those observed previously with living males (i.e., lack of demonstrated motility toward a source). However, this volatile compound does attract males, which might be used in management of this insect. Because of the univoltinism of the species, additional studies must be undertaken in future years, particularly to study the activity of isopropyl (*S*)-5-hydroxyhexanoate in asparagus fields.

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