Journal of Chemical Ecology, Vol. 29, No. 10, October 2003 (© 2003)

ENANTIOMERS OF (*Z*,*Z*)-6,9-HENEICOSADIEN-11-OL: SEX PHEROMONE COMPONENTS OF *Orgyia detrita*

REGINE GRIES,[1,4] GRIGORI KHASKIN,[1] EUGENE KHASKIN,[1] JOHN L. FOLTZ,[2] PAUL W. SCHAEFER,[3] and GERHARD GRIES[1,*]

[1]*Department of Biological Sciences*
Simon Fraser University
Burnaby, British Columbia, Canada, V5A 1S6

[2]*Department of Entomology & Nematology*
University of Florida
Gainesville, Florida 32611-0620, USA

[3]*United States Department of Agriculture*
Agricultural Research Service
Beneficial Insects Introduction Research Laboratory
Newark, Delaware 19713, USA

(Received November 1, 2002; accepted June 17, 2003)

Abstract—(6*Z*,9*Z*,11*S*)-6,9-Heneicosadien-11-ol (*Z*6*Z*9-11*S*-ol-C21) and (6*Z*,9*Z*,11*R*)-6,9-heneicosadien-11-ol (*Z*6*Z*9-11*R*-ol-C21) were identified as major sex pheromone components of female tussock moths, *Orgyia detrita* Guérin-Méneville (Lepidoptera: Lymantriidae), on the basis of (1) analyses of pheromone gland extracts of female *O. detrita* by coupled gas chromatographic-electroantennographic detection (GC-EAD) and GC mass spectrometry, and (2) field trapping experiments with synthetic standards. *Z*6*Z*9-11*S*-ol-C21 and *Z*6*Z*9-11*R*-ol-C21 in combination, but not singly, attracted significant numbers of male moths. Racemic *Z*6*Z*9-11-ol-C21 was more attractive than the 1:3.5 (*R*:*S*) blend ratio found in pheromone gland extracts from female moths. Lower and higher homologues of *Z*6*Z*9-11-ol-C21 were also detected in GC-EAD recordings of pheromone extracts, and the racemic compounds enhanced attractiveness of *Z*6*Z*9-11-ol-C21 in field experiments. Because of trace amounts of these homologues in extracts, their enantiomeric composition could not be determined. This is the first report of secondary alcohols as pheromone components in the ditrysian (advanced) Lepidoptera.

Key Words—Ditrysian Lepidoptera, Lymantriidae, *Orgyia detrita*, sex pheromone, (6*Z*,9*Z*,11*S*)-6,9-heneicosadien-11-ol, (6*Z*,9*Z*,11*R*)-6,9-heneicosadien-11-ol, (6*Z*,9*Z*)-6,9-eicosadien-11-ol, (6*Z*,9*Z*)-6,9-docosadien-11-ol.

* To whom correspondence should be addressed. E-mail: gries@sfu.ca

[4] Dedicated to my Aunt Martha Dittmar in recognition of her contribution to my upbringing.

INTRODUCTION

The tussock moth, *Orgyia detrita* Guérin-Méneville (Lepidoptera: Lymantriidae), occurs in the southeastern United States, from New York south along the coast to Florida and west into Texas (Ferguson, 1978). This coastal distribution matches that of live oak, *Quercus virginiana* Mill., (Little, 1971), which together with bald cypress, *Taxodium distichum* (L.) Rich., serve as main food plants for *O. detrita* larvae (Ferguson, 1978). During an outbreak in coastal North Carolina, larvae also fed on sweetbay, *Magnolia virginiana* L., and cultivated azalea, *Rhododendron* spp., and a laboratory generation was successfully reared on red maple, *Acer rubrum* L. (Drooz et al., 1986).

Male *O. detrita* have never been captured in traps baited with synthetic pheromone components of congeners, suggesting that *O. detrita* employ a different pheromone blend. Moderate population densities of *O. detrita* around Gainesville (Florida) in the last several years provided the opportunity to collect egg masses for laboratory rearing of this tussock moth, and for studying its sex pheromone. We report identification and field testing of sex pheromone components of *O. detrita*.

METHODS AND MATERIALS

Experimental Insects and Pheromone Extraction. O. detrita eggs were collected in Gainesville, Florida, in January 1999, and sent to the USDA Beneficial Insect Introduction Research quarantine facilities, Newark, Delaware. There, hatching larvae were reared on gypsy moth diet (Bell et al., 1981) at 25°C, 60–80% RH, and a 14L:10D photoperiod. Pupae were extricated from their cocoons, and isolated by sex (Winter, 2000). Abdominal tips with pheromone glands of calling, 1–2-day-old females were removed and extracted for 15–30 min in high-performance liquid chromatography (HPLC) grade hexane. Ampoules with the supernatant of pheromone extracts and male pupae were sent by courier to Simon Fraser University.

Analyses of Pheromone Extracts. Aliquots of one female equivalent (FE) of pheromone gland extract, or synthetic standards, were analyzed by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Arn et al., 1975) with a Hewlett-Packard (HP) 5890A gas chromatograph equipped with a fused silica column (30 m × 0.25 or 0.32 mm i.d.) coated with either DB-5, DB-23, DB-210, or Cyclodex-B (J&W Scientific, Folsom, California, USA). The most antennal stimulatory and abundant candidate pheromone component was isolated by HPLC with a Waters LC 626 high-performance liquid chromatograph equipped with a Waters 486 UV-Visible detector set to 210 nm, and a reverse phase Nova-Pak C-18 column (300 × 3.9 mm i.d.) eluted with acetonitrile (1 ml/min). The (*R*)- and (*S*)-enantiomers of insect-produced (6*Z*,9*Z*)-6,9-heneicosadien-11-ol

(*Z*6*Z*9-11-ol-C21) were separated by GC on a custom-made chiral fused silica column coated with a 1:1 mixture of heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin and OV-1701 (König et al., 1992; Pietruszka et al., 1992).

For field experiments, the (*R*)- and (*S*)-enantiomers of synthetic racemic *Z*6*Z*9-11-ol-C21 were separated by HPLC (50–100 μg per injection) on a Chiralpak AD column (250 $\times$ 4.6 mm i.d.; Chiral Technologies Inc., Exton, Pennsylvania, USA) eluting with 0.9 ml/min of hexane: 2-propanol (99.9:0.1). Enantiomers eluted several minutes apart, resulting in >99% enantiomeric excess (ee) and >98% chemical purity of field tested chemicals. Mass spectra of synthetic or insect-produced compounds were obtained in full-scan electron impact ionization mode from a Varian Saturn II Ion Trap GC-MS (70 eV) fitted with a DB-5 column.

Synthesis of Racemic and Enantiomerically Pure 1-Tridecyn-3-ols and Corresponding Acetates. (Figure 1). Racemic 1-tridecyn-3-ol was obtained by stirring 10 g of lithium acetylide–ethylenediamine complex (**2**, 90% pure, 97.8 mmol) (Aldrich Chem. Co.) with freshly prepared undecanal (**1**, 8.36 g, 48.9 mmol) in DMSO-THF (1:1) at 65°C overnight under argon. Silica gel flash chromatography of the worked-up reaction mixture afforded alcohol **3** (3.22 g, 33.6% yield). Alcohol **3** (2.94 g, 15.0 mmol) was stirred with 2.12 ml (23.0 mmol) of vinyl acetate in 10 ml of hexane with 0.5 g of immobilized lipase Novozym 435 (Sigma Chemical Co.) (Xiao and Kitazume, 1997) at 40°C for 4 hr. Novozym-containing resin and solvents were removed by filtration and evaporation *in vacuo*, respectively. Separation of the reaction mixture by flash chromatography (25 g of SiO_2; 10% ether in hexane as eluent) gave alcohol **4** (1.26 g) and acetate **5** (1.97 g). Alcohol **4** (10 mg) was acetylated to **6** overnight at room temperature (rt) with acetic anhydride and pyridine. GC analyses of **6** and **5** on the Cyclodex-B column (150°C isothermal) determined that their ee's were 99.9 and 98.4%, respectively.

The absolute configurations of **4** and **5** were confirmed through an alternative synthesis, as follows: Sharpless asymmetric epoxidation of (*E*)-2-tridecenol (**7**) with L-(+)-diisopropyl tartrate (0.15 equivalent), titanium (IV) isopropoxide (0.15 equivalent), and *t*-butyl hydroperoxide (2.2 equivalent) in dichloromethane at -25°C for 3 days afforded the epoxy-alcohol **8**. Replacement of the hydroxy group of **8** with chlorine by refluxing **8** for 48 hr with an equivalent amount of triphenylphosphine and catalytic amounts of sodium bicarbonate in carbon tetrachloride, and treatment of the resulting epoxy-chloride **9** with a three-fold excess of lithium diisopropylamide (LDA) (Takano et al., 1989; Rodriguez and Spur, 2001, and references cited therein) gave the alcohol **10**. Acetylation of **10** with acetic anhydride and pyridine overnight produced (*S*)-1-tridecyn-3-yl acetate (**5**, overall yield 16%).

*Synthesis of (6Z, 9Z, 11R)-6,9-Heneicosadien-11-ol [(**R**)-**16**]* (Figure 1). (*R*)-1-Tridecyn-3-ol (**4**, 1.25 g, 6.42 mmol) was protected by overnight reaction with

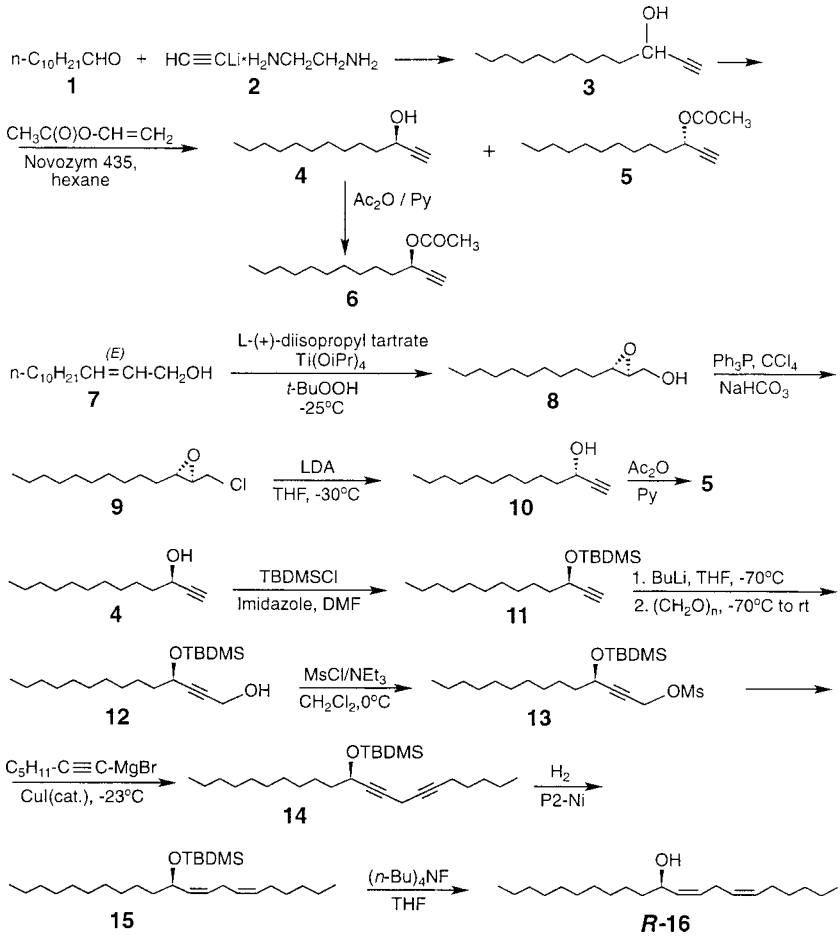

FIG. 1. Syntheses of (*R*)- and (*S*)-1-tridecyn-3-yl acetates and (6*Z*,9*Z*,11*R*)-6,9-heneicosadien-11-ol.

t-butyldimethylsilyl chloride (1.05 g, 7.00 mmol) and imidazole (7.00 mmol) in DMF at rt to afford after work-up ether **11** (1.79 g, 90% yield). Ether **11** (1.79 g, 5.77 mmol) in THF was extended by one carbon by treating it consecutively with butyllithium at −70°C and paraformaldehyde, warming the reaction mixture to rt, and stirring for 24 hr to form after work-up the mono-protected diol **12** (1.65 g, 84% yield). Mesylation of **12** (1.65 g, 4.85 mmol) with an equivalent amount of methanesulfonyl chloride and 1.5 excess of triethylamine in dichloromethane (0°C) for 30 min gave (*R*)-4-(*tert*-butyldimethylsilyloxy)-2-tetradecyn-1-yl

methanesulfonate (**13**). Immediate reaction of **13** in THF at −23°C with a two-fold excess of freshly prepared heptynylmagnesium bromide (from 1-heptyne and EtMgBr; 2 hr reflux in THF) with CuI catalyst (180 mg) gave 1.67 g (4.00 mmol, 82% yield) of the *tert*-butyldimethylsilyl ether of (11*R*)-6,9-heneicosadiyn-11-ol (**14**). Hydrogenation of **14** (0.89 g, 2.00 mmol) with P2-nickel catalyst in ethanol (Brown and Ahuja, 1973), and removal of the silyl protecting group by treatment with excess tetrabutylammonium fluoride in THF (rt, 12 hr) gave (6*Z*,9*Z*,11*R*)-6,9-heneicosadien-11-ol [**(*R*)-16**] [0.495 g, 1.61 mmol, 81% yield; >95% *ee* (conservative estimate considering that enantiomers were not completely baseline resolved)]. The overall yield of **(*R*)-16** (based on **4**) was 50%. The optical rotation of **(*R*)-16** was $[\alpha]_D^{22}+1.9^\circ$ (c 6.8, $CHCl_3$), and NMR data and retention characteristics were consistent with those reported for racemic (*Z*,*Z*)-6,9-heneicosadien-11-ol (Gries et al., 1997). The retention time of **(*R*)-16** on the custom-made chiral column (König et al., 1992; Pietruszka et al., 1992) was identical to that of the first eluting enantiomer of racemic (*Z*,*Z*)-6,9-heneicosadien-11-ol.

Field Experiments. Field experiments were conducted in a wooded area of Gainesville, Florida, latitude 29.688°N, longitude 82.388°W. Host trees in the area included water oak, *Quercus nigra* L., laurel oak, *Q. laurifolia* Michx., live oak, *Q. virginiana* Mill., and Shumard oak, *Q. shumardii* Buckl. Delta-type traps made from 2-l milk cartons (Gray et al., 1984) were coated with Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan, USA) and suspended from trees at a height of 2 m and spacings of 20–25 m in complete randomized blocks. Traps were baited with folded filter papers (Whatman # 1; Whatman International Ltd, Maidstone, England) or gray rubber septa (The West Company, Lionville, Pennsylvania, USA) impregnated with candidate pheromone components just prior to the field experiment.

Treatments for all experiments are listed in Table 1. Experiment 1 tested attractiveness of *Z*6*Z*9-11-ol-C21 versus that of unbaited controls. Experiment 2 tested the effect of dispenser types (rubber septum or filter paper) on the attractiveness of *Z*6*Z*9-11-ol-C21. Experiments 3 and 4 tested whether additional candidate pheromone components, including (*Z*,*Z*)-6,9-eicosadien-11-ol (*Z*6*Z*9-11-ol-C20), (*Z*,*Z*)-6,9-docosadien-11-ol (*Z*6*Z*9-11-ol-C22), and (*Z*)-6-heneicosen-11-one (*Z*6-11-one-C21), enhanced attractiveness of *Z*6*Z*9-11-ol-C21. Experiment 5 tested *Z*6*Z*9-11*S*-ol-C21 and *Z*6*Z*9-11*R*-ol-C21 (isolated from the racemate by chiral HPLC) singly and in combination. Experiment 6 tested the effect of ratio between *Z*6*Z*9-11*R*-ol-C21 and *Z*6*Z*9-11*S*-ol-C21 on blend attractiveness, and Experiment 7 compared the most attractive blend ratio (1:1) from Experiment 6 versus the blend ratio (1:3.5) found in pheromone gland extracts from female *O. detrita*.

Trap catch data were analyzed by nonparametric analyses of variance (Friedman's test) followed by comparison of means by Bonferroni (Dunn) *t* test (Zar, 1984; SAS/STAT, 1988). In all analyses, $\alpha = 0.05$.

TABLE 1. NUMBER OF ($\bar{x} + SE$) OF MALE *Orgyia detrita* CAPTURED IN EXPERIMENTS 1–8 IN TRAPS BAITED WITH CANDIDATE PHEROMONE COMPONENTS ALL EXPERIMENTS CONDUCTED NEAR GAINESVILLE, FLORIDA, USA

Exp. no.	Treatment[a]	Number ($\bar{x} + SE$) of males captured[b]	*N*	Dispenser type	Time period
1	Unbaited control	0 a	10	Filter paper	18–20 April 2000
	*Z*6*Z*9-11-ol-C21 (50)	3.3 + 0.45 b	10	Filter paper	
2	Unbaited control	0.2 + 0.2 c	5	Rubber septum	10–11 May 2000
	Unbaited control	0.2 + 0.5 c	5	Filter paper	
	*Z*6*Z*9-11-ol-C21 (25)	0.8 + 0.4 b	5	Rubber septum	
	*Z*6*Z*9-11-ol-C21 (25)	9.8 + 1.8 a	5	Filter paper	
3	Unbaited control	0.1 + 0.1 d	8	Filter paper	2–3 May 2000
	*Z*6*Z*9-11-ol-C21 (50)	7.6 + 1.9 c	8	Filter paper	
	*Z*6*Z*9-11-ol-C21 (50) + *Z*6*Z*9-11-ol-C20 (5)	12.4 + 3.3 c	8	Filter paper	
	*Z*6*Z*9-11-ol-C21 (50) + *Z*6*Z*9-11-ol-C22 (5)	15.6 + 2.2 b	8	Filter paper	
	*Z*6*Z*9-11-ol-C21 (50) + *Z*6*Z*9-11-ol-C20 (5) + *Z*6*Z*9-11-ol-C22 (5)	20.3 + 2.1 a	8	Filter paper	
4	Unbaited control	0 b	8	Filter paper	3–4 May 2000
	*Z*6*Z*9-11-ol-C21 (50)	19.3 + 6.8 a	8	Filter paper	
	*Z*6*Z*9-11-ol-C21 (50) + *Z*6-11-one-C21 (5)	19.6 + 2.8 a	8	Filter paper	
5	Unbaited control	2.8 + 0.9 b	5	Filter paper	29 April–1 May 2000
	*Z*6*Z*9-11*S*-ol-C21 (10)	3.6 + 1.3 b	5	Filter paper	
	*Z*6*Z*9-11*R*-ol-C21 (10)	3.2 + 0.8 b	5	Filter paper	
	Racemic *Z*6*Z*9-11-ol-C21 (20)	11.4 + 2.0 a	5	Filter paper	
6	Unbaited control	0 b	5	Filter paper	
	*Z*6*Z*9-11*S*-ol-C21 (25)	0.2 + 0.2 b	5	Filter paper	13–14 May 2000
	*Z*6*Z*9-11*R*-ol-C21 (5) + *Z*6*Z*9-11*S*-ol-C21 (25)	0 b	5	Filter paper	
	*Z*6*Z*9-11*R*-ol-C21 (25) + *Z*6*Z*9-11*S*-ol-C21 (25)	3.0 + 0.7 a	5	Filter paper	
7	*Z*6*Z*9-11*R*-ol-C21 (8.5) + *Z*6*Z*9-11*S*-ol-C21 (30)	4.2 + 0.6 b	10	Filter paper	27–28 April 2002
	*Z*6*Z*9-11*R*-ol-C21 (30) + *Z*6*Z*9-11*S*-ol-C21 (30)	10.3 + 1.5 a	10	Filter paper	

[a] Numbers in brackets refer to micrograms; all control treatments received the equivalent amount of solvent.
[b] In each experiment, numbers followed by different letters are significantly different; $P < 0.05$.

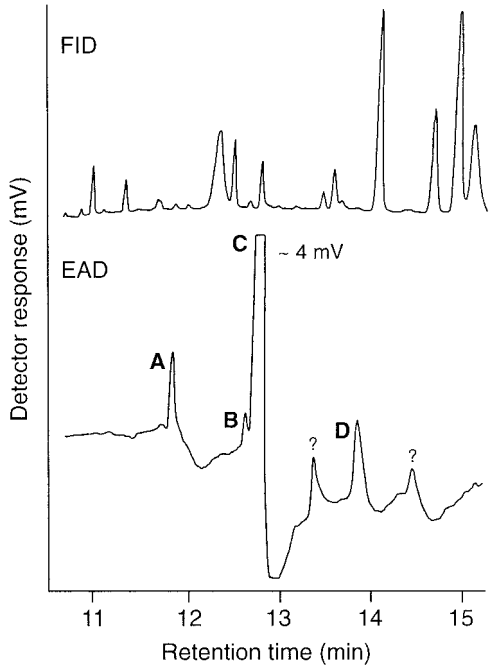

FIG. 2. Flame-ionization detector (FID) and electroantennographic detector (EAD: male *Orgyia detrita* antenna) responses to one female equivalent (FE) of pheromone extract from female *O. detrita*. Chromatography: DB-23 column; splitless injection; injector and FID detector: 240°C; temperature program; 100°C (1 min), then 10°C/min to 200°C (5 min). **A** = (*Z*,*Z*)-6,9-eicosadien-11-ol; **B** = (*Z*)-6-heneicosen-11-one; **C** = (*Z*,*Z*)-6,9-heneicosadien-11-ol; **D** = (*Z*,*Z*)-6,9-docosadien-11-ol; and **?** = unknown.

RESULTS

GC-EAD analyses of pheromone gland extracts from female *O. detrita* revealed four compounds (**A–D** in Figure 2) that consistently elicited responses from male antennae. Retention indices (RI) (van den Dool and Kratz, 1963) of the most antennal stimulatory and abundant component **C** [RI (versus alkane standards): 2240 (DB-5); RI: 2432 (DB-210); RI: 2727 (DB-23)] were consistent with those of previously synthesized *Z*6*Z*9-11-ol-C21 (Gries et al., 1997). The diagnostic fragment ion *m*/*z*171 in identical mass spectra [*m*/*z* (relative intensity): 41 (36), 55 (59), 69 (54), 83 (59), 97 (100), 111 (35), 152 (2), 171 (11), 312 (1)] of authentic heneicosan-11-ol and of HPLC-isolated and hydrogenated **C** confirmed that the hydroxy-group was on C11. With no mass spectra obtainable for components **A**, **B**, and **D**, their structural assignments were based on retention

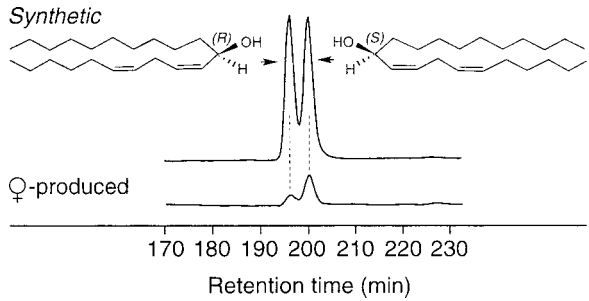

FIG. 3. *Top*: Chromatogram of synthetic (*Z*,*Z*)-6,9-heneicosadien-11-ol with (almost) baseline resolution of the enantiomers of (*Z*,*Z*)-6,9-heneicosadien-11-ol. *Bottom*: Chromatogram of insect-produced (*Z*,*Z*)-6,9-heneicosadien-11-ol (123 female equivalents). Chromatography: custom-made chiral column; splitless injection; injection port and FID: 240°C; temperature program: 150°C isothermal. Note: (*R*)- and (*S*)-enantiomers of (*Z*,*Z*)-6,9-heneicosadien-11-ol occurred in a 1:3.5 ratio in pheromone gland extracts from female *O. detrita*.

indices. For **A** and **D**, the retention indices on all three columns were 100 units lower and higher, respectively, than those of *Z*6*Z*9-11-ol-C21, suggesting that **A** and **D** were lower and higher homologues of *Z*6*Z*9-11-ol-C21. Retention indices of compound **B** were consistent with those of (*Z*)-6-heneicosen-11-one (*Z*6-11-one-C21), a pheromone component of *Orgyia pseudotsugata* (Smith et al., 1975). Comparative analyses of pheromone extract and authentic standards by GC-EAD for components **A–D**, and by GC-MS for component **C**, confirmed these structural assignments.

Chromatography of HPLC-isolated component **C** on a chiral stationary phase column revealed that female *O. detrita* produced *Z*6*Z*9-11*R*-ol-C21 and *Z*6*Z*9-11*S*-ol-C21 in a 1:3.5 ratio (Figure 3). Both the lower and higher homologues of **C** (compounds **A** and **D**) occurred in quantities too low to allow their isolation by HPLC and determination of their enantiomeric composition.

In field experiment 1 (Table 1), traps baited with racemic Z6*Z*9-11-ol-C21 captured significant numbers of male *O. detrita*. For unknown reasons, filter paper was a more suitable dispenser than rubber septa for release of *Z*6*Z*9-11-ol-C21 (when tested at a 25 μg dose) (Table 1; experiment 2). Lower and higher homologues of racemic *Z*6*Z*9-11-ol-C21 enhanced attractiveness of *Z*6*Z*9-11-ol-C21 (Table 1; experiment 3), whereas *Z*6-11-one-C21 had no effect (Table 1; experiment 4). Racemic *Z*6*Z*9-11-ol-C21, but neither enantiomer alone, attracted significant numbers of males (Table 1; experiment 5). Surprisingly, racemic *Z*6*Z*9-11-ol-C21 was also more attractive than other blend ratios (Table 1; experiments 6, 7), including the 1:3.5 (*R*:*S*) ratio found in pheromone gland extracts from female *O. detrita* (Figure 3).

DISCUSSION

(6*Z*,9*Z*,11*S*)-6,9-Heneicosadienol (*Z*6*Z*9-11*S*-ol-C21) and (6*Z*,9*Z*,11*R*)-6,9-heneicosadien-11-ol (*Z*6*Z*9-11*R*-ol-C21) are major sex pheromone components of female *O. detrita*. This conclusion is based on the following evidence: (1) *Z*6*Z*9-11*S*-ol-C21 plus *Z*6*Z*9-11*R*-ol-C21 were the most abundant and antennal stimulatory compounds in GC-EAD recordings from pheromone gland extracts (Figure 2); (2) identifications were based on comparative GC, GC-MS, and GC-EAD analyses of insect-produced and authentic standards; and (3) blends of the synthetic compounds attracted significant numbers of males in field experiments (Table 1).

Lower and higher homologues of racemic *Z*6*Z*9-11-ol-C21 seem to be part of the pheromone blend. They were detected in GC-EAD analyses of pheromone extract (Figure 2), and enhanced attractiveness of *Z*6*Z*9-11-ol-C21 in field experiments (Table 1). Whether their enantiomeric composition is equivalent to that of *Z*6*Z*9-11-ol-C21, and whether they were tested at the most appropriate blend ratio could not be determined because of their trace occurrence in pheromone gland extracts. Two as yet unknown compounds also elicited responses from male antennae (Figure 2). Although they may be part of the pheromone blend, their presence does not appear to be essential for significant attraction of male moths (Table 1).

Synergism between pheromonal enantiomers has been reported only rarely in the Lepidoptera. A racemate of *cis*-3,4-epoxy-(*Z*,*Z*)-6,9-heptadecadiene attracted three times more males of the geometrid moth *Epelis trunctaria* than did either enantiomer alone (Millar et al., 1990). Similarly, a 1:1 and more strongly 1:4 ratio of (6*R*,7*S*)- and (6S,7R)-*cis*-6,7-epoxy-(*Z*,*Z*)-3,9-nonadecadiene attracted males of the noctuid moth *Bleptina caradrinalis* (Millar et al., 1991). Finally, attraction of male pink gypsy moths, *Lymantria mathura*, in Japan not only required the presence but also the same ratio (1:4) of (9*S*,10*R*)- and (9*R*,10*S*)-*cis*-9,10-epoxy-(*Z*,*Z*)-3,6-nonadecadiene as produced by conspecific females (Gries et al., 1999b). Conversely, male *O. detrita* appeared to be more strongly attracted to the unnatural 1:1 ratio of *Z*6*Z*9-11*R*-ol-C21 and *Z*6*Z*9-11*S*-ol-C21 than to the 1:3.5 blend ratio found in pheromone gland extracts of conspecific females (Figure 3). Such an unexpected result was also obtained in pheromone studies of the Japanese giant looper, *Ascotis selenaria cretacea* (Ando et al., 1997), in which females produced nearly the racemate of (*Z*,*Z*)-6,9-*cis*-3,4-epoxy-nonadecadiene, whereas males responded most strongly to the pure (3*R*,4*S*)-enantiomer in a field experiment.

Secondary alcohol pheromone components, as produced by female *O. detrita*, contrast with the ketone pheromone components produced by most congeners. These ketones include (*Z*)-6-heneicosen-11-one (Smith et al., 1975), (*Z*,*E*)-6,8-heneicosadien-11-one (Gries et al., 1997), (*Z*)-6-heneicosen-9-one (Gries et al., 1999a), and (*Z*,*Z*)-6,9-heneicosadien-11-one (Liu, 1999). Production of *trans*-11,12-epoxy-(*Z*,*Z*)-6,9-heneicosadiene by female *O. postica* (Chow et al.,

2001; Wakamura et al., 2001) as a pheromone component seems to suggest a greater diversity of pheromone components in *Orgyia* spp. than previously realized.

Secondary alcohols as pheromone components are reported here for the first time in a species of the ditrysian (advanced) Lepidoptera. Secondary alcohols have previously been identified as pheromone components in several species of the nonditrysian Lepidoptera, including *Stigmella malella* [(*S*)-(*E*)-6,8-nonadien-2-ol and (*S*)-(*Z*)-6,8-nonadien-2-ol (Tóth et al., 1995)], *Eriocrania cicatricella* [(2*R*)-heptan-2-ol (Zhu et al., 1995)], *E. sangii* [(2*S*,6*Z*)-nonen-2-ol (Kozlov et al., 1996)], and *E. semipurpurella* [(2*S*,6*Z*)-nonen-2-ol and (2*R*,6*Z*)-nonen-2-ol (Kozlov et al., 1996)]. The short-chain pheromones of these primitive moth species, however, resemble more closely those of caddisflies than those of ditrysian Lepidoptera (Löfstedt et al., 1994; Zhu et al., 1995) such as *O. detrita.*

Acknowledgments—We thank Susan Barth for preparing insect diet and rearing of experimental insects, and Wittko Francke for lending a custom-made chiral column. PWS thanks the Foltz family for their hospitality during his visit to Gainesville. The research was supported, in part, by grants from Global Forest (GF-18-2000-95 and GF-18-2000-96), and the Natural Sciences and Engineering Research Council of Canada to GG. Experimental insects were maintained in SFU's Global Forest Quarantine Facility, construction of which was completed through financial support from Global Forest (GF-18-2000-SFU-1).

REFERENCES

ANDO, T., OHTANI, K., YAMAMOTO, M., MIYAMOTO, T., QIN, X. R., and WITJAKSONO. 1997. Sex pheromone of Japanese giant looper, *Ascotis selenaria cretacea*: Identification and field tests. *J. Chem. Ecol.* 23:2413–2423.

ARN, H., STÄDLER, E., and RAUSCHER, S. 1975. The electroantennographic detector—A selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch.* 30c:722–725.

BELL, R. A., OWENS, C. D., SHAPIRO, M., and TARDIF, J. R. 1981. Mass rearing and virus production, pp. 599–655, *in* C. C. Doane and M. L. McManus (eds.). The Gypsy Moth: Research Toward Integrated Pest Management. U. S. Dept. Agric. Tech. Bull. 1584, 757 pp.

BROWN, A. L. and AHUJA, V. K. 1973. "P-2 nickel" catalyst with ethylenediamine, a novel system for highly stereospecific reduction of alkynes to *cis*-olefins. *J. Chem. Soc., Chem. Commun.* 553–554.

CHOW, Y. S., TSAI, R. S., GRIES, G., GRIES, R., and KHASKIN, G. 2001. Preliminary identification of the sex pheromone of *Orgyia postica* (Walker) (Lepidoptera: Lymantriidae). *Entomol. Sin.* 8:13–20.

DROOZ, A. T., SMITH, T. F., and DOGGETT, C. A. 1986. Outbreak of a rare lymantriid, *Orgyia detrita*, in coastal North Carolina. U. S. Dept. Agric., Forest Service, Southeastern For. Exp. Stn., Res. Note, SE-340, 3 pp.

FERGUSON, D. C. 1978. The Moths of America North of Mexico, Fasc. 22.2, Noctuoidea (in Part): Lymantriidae. E. W. Classey Ltd., Oxfordshire, England, 110 p.

GRAY, T. G., SLESSOR, K. N., SHEPHERD, R. F., GRANT, G. G., and MANVILLE, J. F. 1984. European pine shoot moth, *Rhyacionia buoliana* (Lepidoptera: Tortricidae): Identification of additional pheromone components resulting in an improved lure. *Can. Entomol.* 116:1525–1532.

GRIES, G., CLEARWATER, J., GRIES, R., KHASKIN, G., KING, S., and SCHAEFER, P. 1999a. Synergistic sex pheromone components of white-spotted tussock moth, *Orgyia thyellina*. *J. Chem. Ecol.* 25:1091–1104.

GRIES, G., GRIES, R., SCHAEFER, P. W., GOTOH, T., and HIGASHIURA, Y. 1999b. Sex pheromone components of pink gypsy moth, *Lymantria mathura*. *Naturwissenschaften* 86:235–238.

GRIES, G., SLESSOR, K. N., GRIES, R., KHASKIN, G., WIMALARATNE, P. D. C., GRAY, T. G., GRANT, G. G., TRACEY, A. S., and HULME, M. 1997. (*Z*)-6,(*E*)-8-Heneicosadien-11-one: Synergistic sex pheromone component of Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae). *J. Chem. Ecol.* 23:19–34.

KÖNIG, W. A., GEHRCKE, B., ICHELN, D., EVERS, P., DÖNNECKE, J., and WANG, W. 1992. New selectively substituted cyclodextrins as stationary phases for the analysis of chiral constituents of essential oils. *J. High Resolut. Chromatogr.* 15:367–372.

KOZLOV, M. V., ZHU, J., PHILIPP, P., FRANCKE, W., ZVEREVA, E. L., HANSSON, B. S., and LÖFSTEDT, C. 1996. Pheromone specificity in *Eriocrania semipurpurella* (Stephens) and *E. sangii* (Wood) (Lepidoptera: Eriocraniidae) based on chirality of semiochemicals. *J. Chem. Ecol.* 22:431–454.

LITTLE, E. L., JR. 1971. Atlas of United States Trees, Vol. 1. Conifers and Important Hardwoods. U.S. Dept. Agric., Forest Service, Misc. Publ. No. 1146, 9 pp., 94 maps.

LIU, W. 1999. Semiochemistry of *Orgyia* and *Diatraea* lepidopteran species and affinity labelling of 2,3-oxidosqualene cyclase. PhD Thesis, Department of Chemistry, Simon Fraser University.

LÖFSTEDT, C., HANSSON, B. S., PETERSSON, E., VALEUR, P., and RICHARDS, A. 1994. Pheromonal secretions from glands on the Vth abdominal sternite of hydropsychid and rhyacophilid caddisflies (Trichoptera). *J. Chem. Ecol.* 20:153–170.

MILLAR, J. G., GIBLIN, M., BARTON, D., MORRISON, A., and UNDERHILL, E. W. 1990. Synthesis and field testing of enantiomers of 6*Z*,9*Z*-*cis*-epoxydienes as sex attractants for geometrid moths. Interaction of enantiomers and regioisomers. *J. Chem. Ecol.* 16:2317–2339.

MILLAR, J. G., GIBLIN, M., BARTON, D., and UNDERHILL, E. W. 1991. Chiral lepidopteran sex attractants: Blends of optically active C_{20} and C_{21} diene epoxides as sex attractants for geometrid and noctuid moths (Lepidoptera). *Environ. Entomol.* 20:450–457.

PIETRUSZKA, J., HOCHMUT, D. H., GEHRCKE, B., ICHELN, D., RUNGE, T., and KÖNIG, W. A. 1992. Gas chromatographic enantioseparation of allenes. *Tetrahedron Asymm.* 3:661–670.

RODRIGUEZ, A. R. and SPUR, B. W. 2001. Total synthesis of aspirin triggered 15-lipoxin A4. *Tetrahedron Lett.* 42:6057–6060.

SAS/STAT. 1988. User's Guide, release 6.03 edition. SAS Institute, Cary, North Carolina.

SMITH, R. G., DATERMAN, G. E., and DAITES, G. D., JR. 1975. Douglas-fir tussock moth: Sex pheromone identification and synthesis. *Science* 188:63–64.

TAKANO, S., SAMIZU, K., SUGIHARA, T., and OGASAWARA, K. 1989. Asymmetric construction of optically active 3-hydroxyalkyne functionalities. *J. Chem. Soc., Chem. Commun*. 1344–1345.

TÓTH, M., SZÖCS, G., VAN NIEUKERKEN, E., PHILIPP, P., SCHMIDT, F., and FRANCKE, W. 1995. Novel type of sex pheromone structure identified from *Stigmella malella* (Stainton) (Lepidoptera: Nepticulidae). *J. Chem. Ecol.* 21:13–27.

VAN DEN DOOL, H. and KRATZ, P. D. 1963. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *J. Chromatogr.* 2:463–471.

WAKAMURA, S., ARAKAKI, N., YAMAMOTO, M., HIRADATE, S., YASUI, H., YASUDA, T., and ANDO, T. 2001. Posticlure: A novel *trans*-epoxide as a sex pheromone component of the tussock moth, *Orgyia postica* (Walker). *Tetrahedron Lett.* 42:687–689.

WINTER, W. D., JR. 2000. Basic techniques for observing and studying moths and butterflies. The Lepidopterists' Society, Memoir No. 5, Los Angeles, California, 444 pp.

XIAO, L. and KITAZUME, T. 1997. Optically active propargylic and allylic alcohols with a difluoromethyl group at the terminal carbon. *Tetrahedron Asymm.* 8:3597–3601.

ZAR, J. H. 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, New Jersey.

ZHU, J., KOZLOV, M. V., PHILIPP, P., FRANCKE, W., and LÖFSTEDT, C. 1995. Identification of a novel moth sex pheromone in *Eriocrania cicatricella* (Zett.) (Lepidoptera: Eriocraniidae) and its phylogenetic implications. *J. Chem. Ecol*. 21:29–43.