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SPECIES DISCRIMINATION IN FIVE SPECIES OF WINTER-FLYING GEOMETRIDS (LEPIDOPTERA) BASED ON CHIRALITY OF SEMIOCHEMICALS AND FLIGHT SEASON

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Abstract—Enantiomer separation of (6Z,9Z)-*cis*-3,4-epoxynonadecadiene and (3Z,9Z)-*cis*-6,7-epoxynonadecadiene could be achieved using chiral high-resolution gas chromatography and a cyclodextrin-bond column. (3Z,9Z)-(6*R*,7*S*)-Epoxynonadecadiene was identified from ovipositor extracts of *Colotois pennaria*, while in *Erannis defoliaria* the 6*S*,7*R*-enantiomer was found. In field trapping tests pure synthetic enantiomers caught only conspecific males of these species. (3Z,6Z,9Z)-Nonadecatriene was found in both species, while the presence of (3Z,6Z,9Z)-heneicosatriene was indicated in *C. pennaria* only. A 10:10:3 blend of (3Z,9Z)-(6*R*,7*S*)-epoxynonadecadiene, (3Z,6Z,9Z)-heneicosatriene, and (3Z,6Z,9Z)-nonadecatriene was found to be optimal for catching *C. pennaria*, while *E. defoliaria* males were optimally caught by a 1:1 mixture of (3Z,9Z)-(6*S*,7*R*)-epoxynonadecadiene and (3Z,6Z,9Z)-nonadecatriene. (6Z,9Z)-(3*S*,4*R*)-Epoxynonadecadiene was identified from ovipositor extracts of *Agriopis (Erannis) aurantiaria*. In field tests the pure enantiomer proved to be a highly specific sex attractant for both the late autumn/early winter flying *A. aurantiaria* and the late winter/early spring flying *A. leucophearia*. Males of *Agriopis marginaria*, which fly in late win-

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ter/early spring, were attracted to (3Z,9Z)-(6*S*,7*R*)-epoxynonadecadiene. The addition of (3Z,6Z,9Z)-nonadecatriene to the *S*,*R*-enantiomer increased captures. Optimal catches were recorded with a 10:3 epoxide–hydrocarbon blend. Enantiomer specificity in all species was confirmed in EAG measurements.

Key Words—Chirality, enantiomers, sex pheromones, (3Z,9Z)-(6*R*,7*S*)-epoxynonadecadiene, (3Z,9Z)-(6*S*,7*R*)-epoxynonadecadiene, (6Z,9Z)-(3*S*,4*R*)-epoxynonadecadiene, *Colotois pennaria*, *Erannis defoliaria*, *Agriopis aurantiaria*, *A. marginaria*, *A. leucophearia*, Lepidoptera, Geometridae, electroantennogram, field trapping.

INTRODUCTION

The group of winter-flying geometrids (Lepidoptera) includes a few genera with a limited number of species. Adults are adapted to low ambient temperature: some of the species fly in late autumn/early winter, others in late winter/early spring. Females cannot fly: they have no or only rudimentary wings. In mate finding, males search for chemically signaling females. In order to reveal the chemical basis of species specificity in the communication channels, we studied the pheromone systems of the most frequently occurring Middle-European species of the *Erannis*/*Agriopis* genus, as well as of *Colotois pennaria* L. Among these, *Erannis defoliaria* Cl., *Agriopis aurantiaria* Hbn., and *C. pennaria* fly in late autumn/early winter, while the flight season for *A. marginaria* F. and *A. leucophearia* Den. et Schiff. is late winter/early spring (Vojnits, 1980). Since these species are regarded as occasional pests of deciduous forests and orchards in large areas in Europe (Escherich, 1931), the establishment of specific sex attractants for practical monitoring by means of pheromone traps was another objective of our study.

Earlier, (3Z,9Z)-*cis*-6,7-epoxynonadecadiene (3Z,9Z-*cis*-6,7-epo-19Hy) and (3Z,6Z,9Z)-nonadecatriene (3Z,6Z,9Z-19Hy) were identified as pheromone components of *E. defoliaria* (Hansson et al., 1990). The presence of the epoxide in *C. pennaria* was also indicated (Hansson et al., 1990). A mixture of the synthetic racemic epoxide and the triene was found to attract males of *E. defoliaria*, *A. marginaria*, and *C. pennaria* (Hansson et al., 1990). At that time the absolute configuration of the natural compounds remained unknown. Here we report on the identification of the absolute configuration of chiral epoxydienes found as female pheromone components of *E. defoliaria*, *C. pennaria*, and *A. aurantiaria*, and on the biological activity of enantiomerically pure synthetic samples in electroantennogram measurements and field trapping tests. In addition, the EAG response of *A. marginaria*, and field attraction of *A. marginaria* and *A. leucophearia* males towards enantiomerically pure epoxydienes were also tested.

METHODS AND MATERIALS

Insects. Cultures of *C. pennaria* and *E. defoliaria* were initiated from adults collected in forests near Budapest, Hungary. Adult males and females were kept outdoors in glass jars with a sheet of filter paper for egg-laying. Pieces of filter paper with some overwintered eggs were fastened to twigs of pear (*C. pennaria*) or cherry (*E. defoliaria*) trees before bursting of the buds in the experimental orchard of the Plant Protection Institute at Julianna-major (near Budapest). Subsequently, the whole twig was covered by a linen bag. When the larvae reached the last instar, the twigs were cut and placed in plastic containers (diameter: 42 cm; height: 16 cm). For pupation, a small amount of sterilized soil was added. Pupae were taken out of the soil in September, sexed, and placed in separate Petri dishes on sheets of slightly moistened filter paper. Emerging adults were collected daily and placed in glass jars. All developmental stages were kept outdoors under natural ambient temperature and photoperiod.

A culture of *A. aurantiaria* was initiated from last (or penultimate) instar larvae collected from oak trees at Síkfőkút, Bükk Mountains, Hungary. This species was reared on oak trees at Síkfőkút, in a similar way as described above.

Extracts. Ovipositors of unmated calling females were extracted in redistilled hexane or pentane as described earlier (Hansson et al., 1990).

Synthesis. Pure enantiomers **6** and **7** were prepared as shown in Figure 1. The approach is based on the acetylene methodology used by Mori and Ebata (1986) and Mori and Takeuchi (1989) and resembles that of Becker et al. (1990) and Millar et al. (1990a). Nonylbromide **1** was elongated three times with protected propargyl alcohol to yield the triynol derivative **4.** Hydrogenation over P-2-Ni (Brown and Ahuja, 1973) followed by Sharpless epoxidation of the resulting trienols (30 hr at $-30°C$) afforded the enantiomers of the epoxy alcohol **5.** Treatment of the corresponding tosylates with lithium dimethylcuprate yielded the target epoxydienes **6** and **7,** which were chromatographed on silica gel (230–400 mesh, elution with *n*-hexane–ethyl acetate 100 : 1). The final products showed a chemical purity of ca. 98% containing ca. 2% of epoxyenes produced by partial overhydrogenation of the epoxydienes. Chiral gas chromatography (see below) showed the enantiomeric purity of the epoxides to be ca. 95% ee (ee = enantiomeric excess; here: 97.5 : 2.5) $\{[\alpha]_D^{21}$ (6) $= -3.7°$ ($c = 0.96$, CH_2Cl_2) and $[\alpha]_D^{21}$ (7) $= +3.7°$ ($c = 1.18$, CH_2Cl_2)$\}$. As compared to results reported in the literature (Millar et al., 1990a), our products showed higher optical purities, which may be due to the lower temperatures used in our procedure. The synthesis of both enantiomers of 3*Z*,9*Z*-*cis*-6,7-epo-19Hy has been described in detail by Mori and Brevet (1991). The products showed a chemical purity of higher than 99.5% and an optical purity of at least 99% (chiral gas chromatography).

Chemical Analysis. Structure elucidation of the target compounds was car-

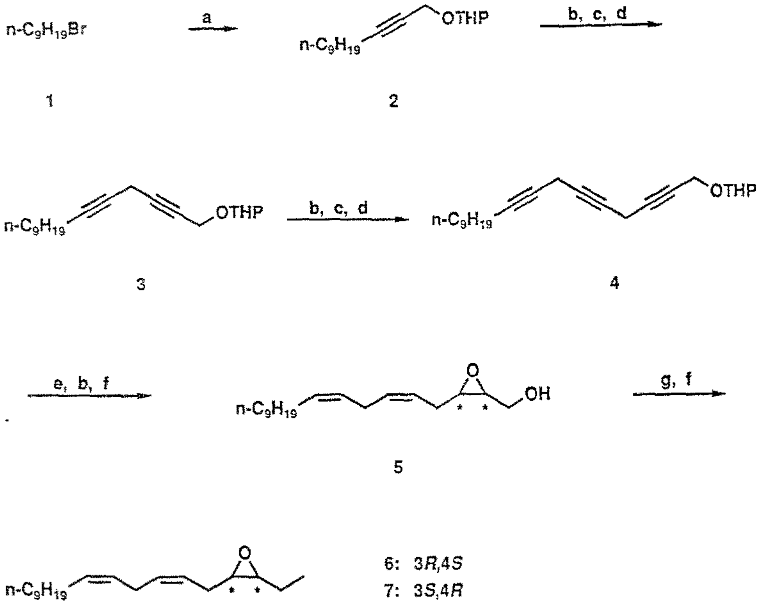

FIG. 1. Syntheses of pure enantiomers of (6Z,9Z)-*cis*-3,4-epoxydienes: (a) $THPOCH_2C{\equiv}CLi$/liq.NH_3/DMSO/−33°C → 20°C; (b) *p*-TsOH/MeOH/20°C; (c) PPh_3Br_2/CH_2Cl_2/−20°C; (d) $THPOCH_2C{\equiv}CMgBr$/CuCl/THF/40°C; (e) H_2 P2-Ni/EtOH/20°C; (f) $Ti(OiPr)_4$/diethyl tartrate/*t*BuOOH/CH_2Cl_2/−30°C/30hr; (g) TsCl/KOH/Et_2O/0°C; (h) Me_2CuLi/Et_2O/−60°C → −30°C.

ried out by combined gas chromatography–mass spectroscopy (GC-MS) under the same conditions as described earlier (Hansson et al., 1990). Mass spectra and gas chromatographic retention times (coinjection) of racemic samples served as references. Enantiomeric separation of chiral epoxides was achieved by gas chromatography using a 1 : 1 mixture of heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin and OV-1701 as the stationary phase and hydrogen (2 ml/min) as the carrier gas. Synthesis of the cyclodextrin as well as preparation of our tailor-made columns have been described in detail (König et al., 1992, Pietruszka et al., 1992). Separation conditions were carefully optimized. (3Z,9Z)-*cis*-6,7-Epoxynonadecadiene (3Z,9Z-*cis*-6,7-epo-19Hy) was analyzed on a 50-m, 0.25-mm-ID fused silica column held at 135°C for 1 hr, programmed to 155°C within 2 min, then held at 155°C. Under these conditions the enantiomers showed an α value, i.e., $R_t(6R,7S):R_t(6S,7R)$, of 1.02 (Figure 2). Assignment of absolute configuration of naturally occurring epoxydienes was performed by chiral gas chromatography using synthetic optically active reference compounds (Figure 2B and C). The (6Z,9Z)-*cis*-3,4-epoxynonadecadienes were analyzed

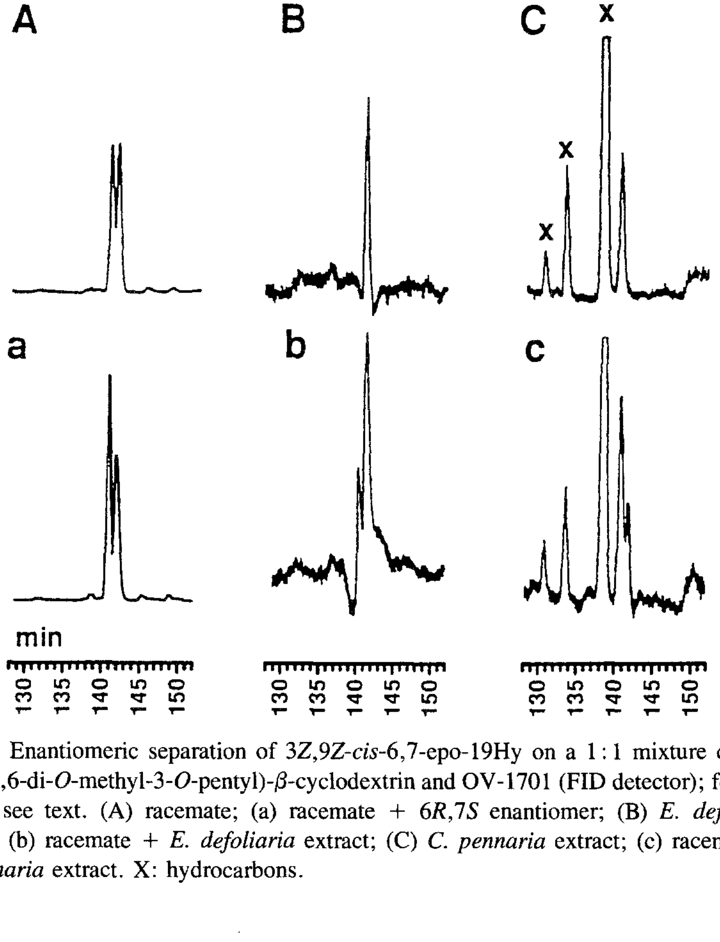

FIG. 2. Enantiomeric separation of 3*Z*,9*Z*-*cis*-6,7-epo-19Hy on a 1 : 1 mixture of heptakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin and OV-1701 (FID detector); for conditions see text. (A) racemate; (a) racemate + 6*R*,7*S* enantiomer; (B) *E. defoliaria* extract; (b) racemate + *E. defoliaria* extract; (C) *C. pennaria* extract; (c) racemate + *C. pennaria* extract. X: hydrocarbons.

on a 25-m, 0.25-mm-ID fused silica column programmed from 80°C to 145°C within 4 min, then held at 145°C.

Under these conditions, the enantiomers showed an α value, i.e., R_t(3 *S*,4*R*):R_t(3*R*,4*S*), of 1.02. An α value of 1.02 at a retention time of 140 min (as in the present case) represents a difference in retention times of almost 3 min, which enables one to clearly distinguish between the enantiomers.

Due to slight tailing of the peaks, the detectable amounts of enantiomeric impurities contained in the test compounds depend on whether they elute before or after the corresponding main stereoisomer. The first-eluting enantiomers were easily detectable in less than 0.1% contained in the synthetic samples and in ca. 0.5–1% in the gland extracts, while the minimum detectable amounts of later-eluting enantiomers was 0.5–1% in the synthetic samples and 2–3% in the gland extracts. As compared to synthetic compounds, determination of the enantiomeric composition of natural products is less exact due to less material being available (higher signal-to-noise ratio) and the presence of other contaminants.

Electroantennograms. Electroantennograms (EAG) were recorded from excised male antennae, with platinum electrodes set at the tip and the basal part of the antenna. Connection between the electrodes and the insect tissues was maintained by an electrically conducting gel (Valleylab, Boulder, Colorado). Responses were amplified by a high-impedance amplifier (Rumbo, 1981), and displayed on an OH 850 chart recorder (Radelkis, Budapest, Hungary). Test compounds in required amounts were applied on a 10 × 10-mm piece of filter paper positioned inside a Pasteur pipet. Stimuli were provided by injecting 1 ml of air through the Pasteur pipet into an airstream (0.3 m/sec) flushing over the antenna. The interval between stimuli was at least 1 min. Responses were normalized against responses to a common standard, which was administered before and after the series of stimuli. In addition to males reared according to the above-mentioned procedure, feral males collected by a live-catching light trap were used in these experiments.

Field Trapping. Triangular traps with sticky bottoms (sticky material: Tanglefoot, Tanglefoot Co., Grand Rapids, Michigan) were used, similar in shape and size as described by Arn et al. (1979), but made of transparent plastic sheets. Test compounds were applied in hexane solution to 1 × 1-cm pieces of rubber tubing (Taurus, Budapest, Hungary, MSZ 9691/6). In the field, traps were suspended from branches at a height of 1–1.5 m above ground level in a complete block design (Tóth et al., 1992). At inspections, captured moths were recorded, sticky bottoms exchanged, and traps moved one position further within the block. Trappings were conducted in mixed oak forests, near Budapest, Hungary. In statistical analysis, catches recorded at each trap inspection were regarded as replicates. Trap capture data were statistically analyzed after transformation to log $(x + 1)$, tested for homogenity (Bartlett's test), and then submitted to a one-way ANOVA. If the F value was significant, differences between mean catches were then tested for significance by Duncan's new multiple-range test (Steel and Torrie, 1960).

RESULTS

Colotois pennaria (Feathered Thorn)

Chemical Analysis. Using ovipositor extracts, GC-MS analysis on a DB-5 column (Hansson et al., 1990) showed the presence of (3Z,6Z,9Z)-nonadecatriene (3Z,6Z,9Z-19Hy), 3Z,9Z-*cis*-6,7-epo-19Hy, and (3Z,6Z,9Z)-heneicosatriene (3Z,6Z,9Z-21Hy) in a ratio of about 1:2:0.1. Traces of (3Z,9Z)-*cis*-6,7-epoxyheneicosadiene (3Z,9Z-*cis*-6,7-epo-21Hy) and (3Z,6Z)-*cis*-9,10-epoxyheneicosadiene (3Z,6Z-*cis*-9,10-epo-21Hy) were also detected. Gas chromatography on a modified cyclodextrin phase proved the natural epoxynonadecadiene to be the 6*R*,7*S*-enantiomer showing high optical purity (Figure 2C).

Electroantennograms. Higher responses were elicited from male antennae by (3Z,9Z)-(6*R*,7*S*)-epoxynonadecadiene (3Z,9Z-6*R*,7*S*-epo-19Hy) as compared to its enantiomer, in a dose range of 1–0.001 μg (Figure 3A). The dose–response relationship on a semilogarithmic scale showed a tight linearity with an increase at higher doses, characterized by a higher slope for the *R*,*S*-enantiomer.

Field Trapping. In a preliminary trial 3Z,9Z-6*R*,7*S*-epo-19Hy attracted males in large numbers, while no males were caught by the *S*,*R*-enantiomer (Figure 4).

In optimizing the ratio of 3Z,9Z-6*R*,7*S*-epo-19Hy, 3Z,6Z,9Z-21Hy, and 3Z,6Z,9Z-19Hy, the addition of any of the two hydrocarbons to the epoxide slightly enhanced captures over ranges of 30–1000%, and 30–100% for 3Z,6Z,9Z-21Hy and 3Z,6Z,9Z-19Hy, respectively (Figure 5). Combined addition of both hydrocarbons resulted in a superposed enhancement. The highest capture was recorded at the 10:10:3 ternary blend of 3Z,9Z-6*R*,7*S*-epo-19Hy, 3Z,6Z,Z-21Hy, and 3Z,6Z,Z-19Hy. The addition of 1.5, 5, 15, and 50 μg of 3Z,9Z-*cis*-6,7-epo-21Hy as a fourth component to the above-mentioned ternary mixture at a dose of 50:50:15 μg exerted no effect on captures (Nagy-Hárshegy, Budapest, Hungary, November 12–18, 1991, 10 traps/bait combination). In preliminary tests the two hydrocarbons on their own attracted no males into traps (Kétbükkfa-nyereg, Pilis Mountains, Hungary, November 11, 1990–January 27, 1991, six replicates).

Erannis defoliaria (Mottled Umber)

Chemical Analysis. Using ovipositor extracts, GC-MS analysis on a DB-5 column confirmed the presence of 3Z,6Z,9Z-19Hy and 3Z,9Z-*cis*-6,7-epo-19Hy in a ratio of about 1:3 (Hansson et al., 1990). Gas chromatography on a modified cyclodextrin proved the natural product to be the 6*S*,7*R*-enantiomer, showing high optical purity (Figure 2B).

Electroantennograms. Higher responses were elicited from male antennae by 3Z,9Z-6*S*,7*R*-epo-19Hy as compared to its enantiomer in a dose range of 1–0.01 μg (Figure 3B). The dose–response relationship on a semilogarithmic scale showed a tight linearity with an increase at higher doses, characterized by a higher slope for the *S*,*R*-enantiomer.

Field Trapping. In a test with pure synthetic enantiomers of 3Z,9Z-*cis*-6,7-epo-19Hy, only the *S*,*R*-enantiomer attracted *E. defoliaria* males to traps (Figure 4).

In a further test aimed at studying the effect of the addition of 3Z,6Z,9Z-19Hy to the activity of enantiomerically pure 3Z,9Z-6*S*,7*R*-epo-19Hy, signifi-

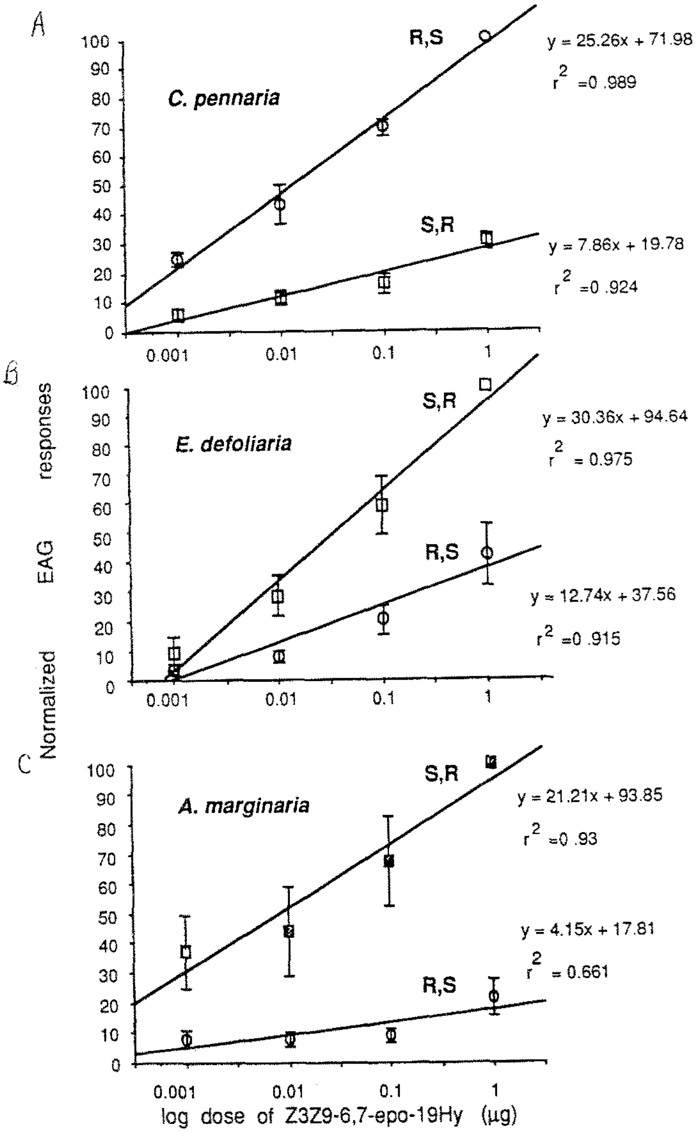

FIG. 3. Electroantennogram responses of *C. pennaria* (A), *E. defoliaria* (B), and *A. marginaria* (C) to *S*,*R* and *R*,*S* enantiomers of 3*Z*,9*Z*-*cis*-6,7-epo-19Hy. Responses were normalized against the response to 1 μg of 3*Z*,9*Z*-6*R*,7*S*-epo-19Hy (*C. pennaria*), or to 3*Z*,9*Z*-6*S*,7*R*-epo-19Hy (*E. defoliaria* and *A. marginaria*). Stimuli were tested consecutively on five male antennae. Bars represent SD.

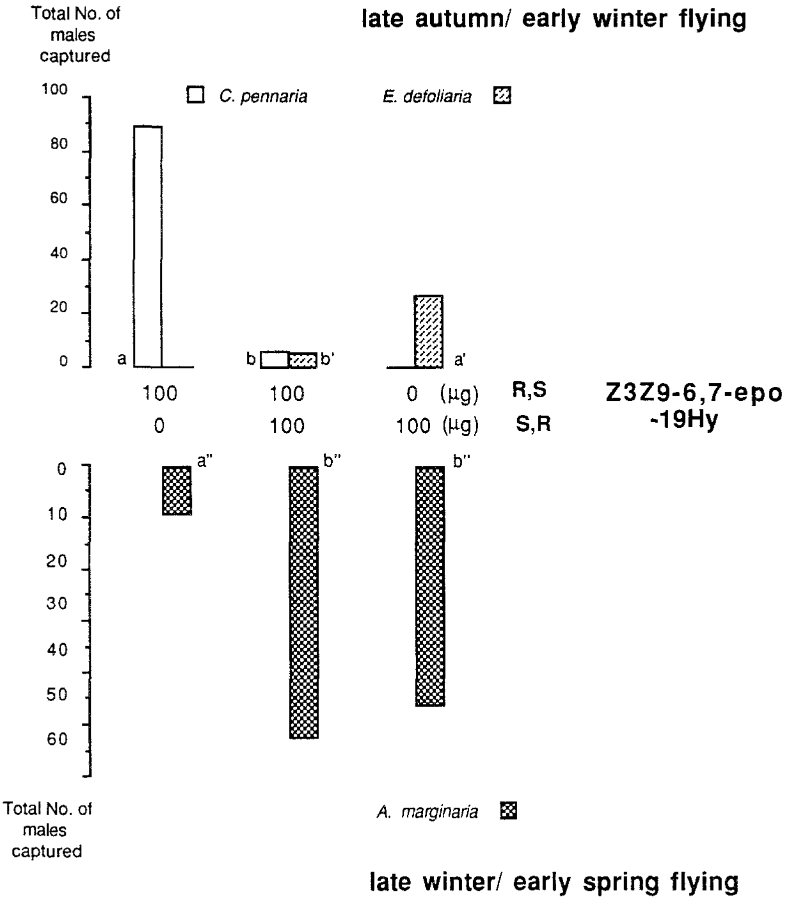

FIG. 4. Field captures of *C. pennaria*, *E. defoliaria*, and *A. marginaria* males in traps with *S,R* and *R,S* enantiomers of 3*Z*,9*Z*-*cis*-6,7-epo-19Hy. *C. pennaria* and *E. defoliaria*: Julianna-major, near Budapest, Hungary, November 2–29, 1991; 5 traps/bait combination. *A. marginaria*: Nagy-Hárshegy, Budapest, Hungary, February 27–March 26, 1992; 5 traps/bait combination. ANOVA—*C. pennaria*: $F = 36.93$, $P < 0.1\%$; *E. defoliaria*: $F = 9.95$, $P < 5\%$; *A. marginaria*: $F = 9.39$, $P < 0.1\%$. Captures followed by same letters within a species are not significantly different at the 5% probability level (Duncan's new multiple-range test). Catches significantly differ from zero catch within a species at the 5% probability level (Wilcoxon's signed-ranks two-tailed test).

cantly higher catch was recorded at the 1 : 1 mixture (Table 1) than the epoxide alone. In preliminary tests, 3*Z*,6*Z*,9*Z*-19Hy on its own attracted no males into traps.

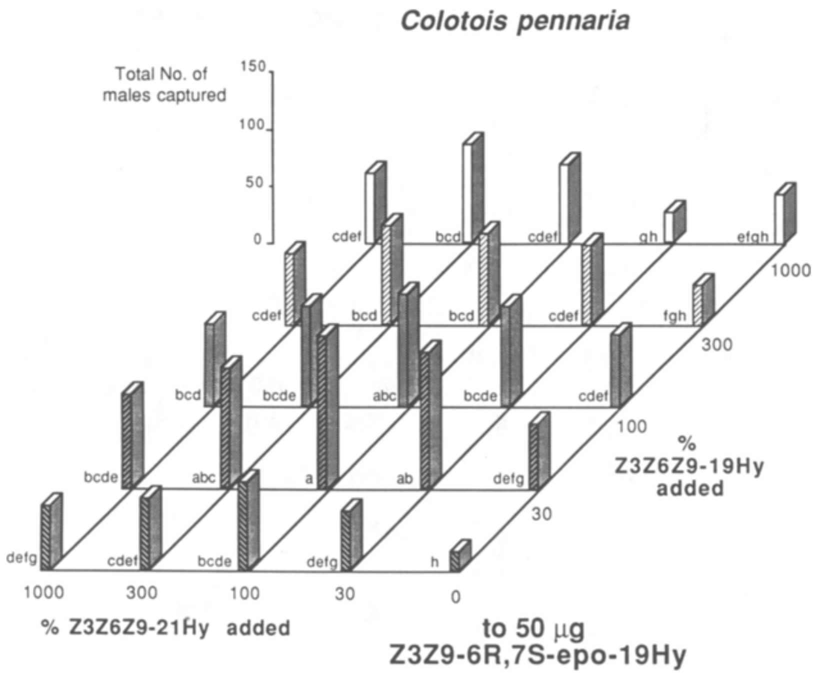

FIG. 5. Field captures of *C. pennaria* males by various ternary mixtures of 3*Z*,9*Z*-6*R*,7*S*-epo-19Hy, 3*Z*,6*Z*,9*Z*-21Hy, and 3*Z*,6*Z*,9*Z*-19Hy. Nagy-Hárshegy, Budapest, Hungary, October 24–November 11, 1991; 8 traps/bait combination. ANOVA—$F = 5.28$, $P < 0.1\%$. Captures followed by same letters are not significantly different at 5% probability level (Duncan's new multiple-range test).

Agriopis aurantiaria (Scarce Umber)

Chemical Analysis. Using ovipositor extracts, GC-MS analysis on a DB-5 column confirmed the presence of (6*Z*,9*Z*)-*cis*-3,4-epoxynonadecadiene (6*Z*,9*Z*-*cis*-3,4-epo-19Hy). Gas chromatography on a modified cyclodextrin revealed the compound to be the 3*S*,4*R*-enantiomer.

Electroantennograms. Higher responses were elicited from male antennae by 6*Z*,9*Z*-3*S*,4*R*-epo-19Hy as compared to its enantiomer at 1- and 0.1-μg doses (100 vs. 48.8, and 34.6 vs. 5.4, respectively, five antennae). Responses were generally low at lower doses and did not differ significantly from each other.

Field Trapping. Large catches were recorded with 6*Z*,9*Z*-3*S*,4*R*-epo-19Hy (Figure 6). While the *R*,*S*-enantiomer did not catch, the racemic mixture, produced by mixing equal amounts of the *S*,*R* and *R*,*S*-enantiomer, caught many fewer males as compared to catches with the *S*,*R*-enantiomer. When the same test was run in early spring, the *S*,*R*-enantiomer attracted males of the spring

TABLE 1. FIELD CAPTURES OF *E. defoliaria* AND *A. marginaria* AT VARIOUS BLENDS OF Z3,Z9-6S,7R-epo-19Hy AND Z3,Z6,Z9-19Hy[a]

Bait composition (μg)		Total No. of males captured	
Z3,Z9-6S,7R-epo-19Hy	Z3,Z6,Z9-19Hy	*E. defoliaria*	*A. marginaria*
50		28 bc	62 c
50	0.5	13 c	60 c
50	1.5	45 abc	77 c
50	5	51 ab	103 bc
50	15	84 ab	181 a
50	50	93 a	176 a
50	150	not tested	171 ab

[a] *E. defoliaria:* Nagy-Hárshegy, Budapest, Hungary, November 25–December 6, 1991; 10 traps/treatment. *A. marginaria:* Julianna-major, near Budapest, Hungary, March 2–16, 1992; 10 traps/bait composition. ANOVA—*E. defoliaria:* $F = 9.40$, $P < 0.1\%$; *A. marginaria:* $F = 4.83$, $P < 0.1\%$. Captures followed by same letters within a species are not significantly different at the 5% probability level (Duncan's new multiple-range test).

umber, *A. leucophearia* to traps (Figure 6). Four specimens were also caught by the racemic mixture.

Agriopis marginaria (Dotted Border)

Electroantennograms. Higher responses were elicited from male antennae by 3Z,9Z-6S,7R-epo-19Hy as compared to the R,S-enantiomer, in a dose range of 1–0.001 μg (Figure 3C). The dose–response relationship on a semilogarithmic scale showed a linearity with an increase at higher doses, characterized by a higher slope for the S,R-enantiomer.

Field Trapping. In a test with pure enantiomers of the 3Z,9Z-*cis*-6,7-epo-19Hy, the S,R-enantiomer attracted males in significantly higher numbers than the R,S-enantiomer (Figure 4). A racemic mixture attracted males in similar numbers as compared to the pure S,R-enantiomer.

Mixtures of 3Z,9Z-6S,7R-epo-19Hy with 3Z,6Z,9Z-19Hy in ratios of 10:3 to 1:3 caught significantly more males than the epoxide alone (Table 1). In preliminary tests, 3Z,6Z,9Z-19Hy on its own attracted no males into traps.

DISCUSSION

Gas chromatographic separation of enantiomers of chiral epoxydienes produced as pheromones by female geometrid species has been achieved for the first time. Our analysis enabled unambiguous assignment of the absolute con-

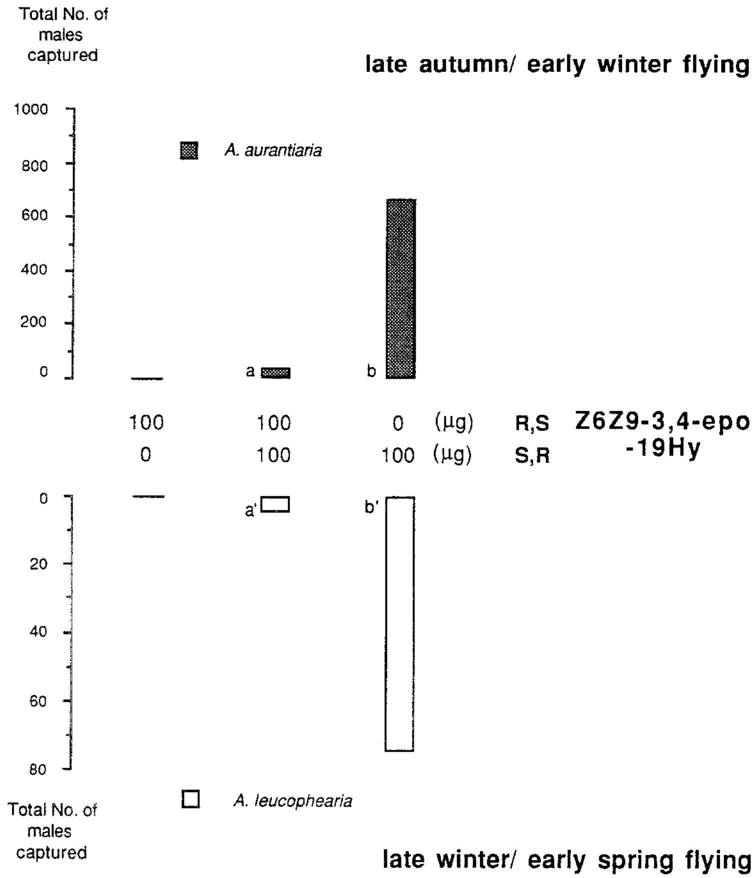

FIG. 6. Field captures of *A. aurantiaria* and *A. leucophearia* males at *S,R* and *R,S*-enantiomers of 6*Z*,9*Z*-*cis*-3,4-epo-19Hy. *A. aurantiaria*: Nagy-Hárshegy, Budapest, Hungary, November 11–15, 1991; 10 traps/bait combination *A. leucophearia*: Julianna-major, near Budapest, Hungary, March 2–13, 1992; 2 traps/bait combination. ANOVA—*A. aurantiaria*: $F = 457.7$, $P < 0.1\%$; *A. leucophearia*: $F = 7.34$, $P < 5\%$. For further details of statistical analysis, see Figure 4.

figuration of the natural products and revealed that the species under investigation produce these pheromone components in high enantiomeric purity. In this study, 3*Z*,9*Z*-6*R*,7*S*-epo-19Hy was identified as a female-produced pheromone component of *C. pennaria*, and it was shown to attract only conspecific males in the field. Its enantiomer was identified from *E. defoliaria* and found to attract males of this species. No interspecific attraction was observed: each enantiomer attracted only males of the respective species. When both enantiomers were present in a ratio of 1 : 1 (racemate), captures of both species were significantly lower as compared to captures at the corresponding pure enantiomers. In EAG studies, significantly higher responses were evoked in the two species by the corresponding naturally occurring enantiomers than by the ''nonnatural'' enantiomer. It can not be ruled out that responses attributed to the ''nonnatural'' enantiomers were actually evoked by the natural enantiomers that occurred in extremely low percentages in the synthetic samples. 3*Z*,6*Z*,9*Z*-19Hy was also identified from both *C. pennaria* and *E. defoliaria*; however its bis homologe, 3*Z*,6*Z*,9*Z*-21Hy was present only in the former species. The hydrocarbons per se were not attractive, but when added to the corresponding epoxide, showed a catch-increasing effect in both species. The ecology of these two species broadly overlaps: they live in the same habitats, their flight period partially overlaps (Escherich, 1931), and the diurnal rhythm of calling and mating also coincides (Szöcs & Tóth, unpublished). It seems that the absolute configuration of the epoxide pheromone component forms the basis for maintaining species-specificity in the pheromonal communication between *C. pennaria* and *E. defoliaria*.

Chirality seems to play an important role also in the pheromonal communication of all other *Erannis*/*Agriopis* species dealt with in the present study. 6*Z*,9*Z*-3*S*,4*R*-epo-19Hy was identified as a pheromone component of *A. aurantiaria*, and males of both the late autumn/early winter flying *A. aurantiaria* and the late winter/early spring flying *A. leucophearia* were trapped by using this enantiomer. Its antipode did not catch males of these species, and when added to 6*Z*,9*Z*-3*S*,4*R*-epo-19Hy, catches drastically decreased, indicating an antagonistic effect. Males of *A. marginaria* were better attracted to 3*Z*,9*Z*-6*S*,7*R*-epo-19Hy, showing a close relation to *E. defoliaria* with respect to males' attraction. Further investigation is needed, however, to reveal the chemical and enantiomeric composition of the pheromone produced by *A. marginaria* females. It is interesting to note that *S*,*R*-enantiomers of the 3,4- or 6,7-epoxynonadecadienes were found to be the pheromone components and/or attractants for males of all *Erannis*/*Agriopis* species investigated in this study, while an *R*,*S*-enantiomer was found the essential pheromone component in *C. pennaria*, a species only distantly related to *Erannis*/*Agriopis* spp.

This study casts some light on parallelisms in chirality-based separation of pheromonal channels between late autumn/early winter and late winter/early spring flying species. 3*Z*,9*Z*-6*S*,7*R*-epo-19Hy is an essential sex pheromone component for the late autumn/early winter flying *E. defoliaria* and a sex attractant for the late winter/early spring flying *A. marginaria*. However, the addition of the 6*R*,7*S*-enantiomer decreased captures of *E. defoliaria*, but did not affect captures of *A. marginaria*. The different influence of the enantiomer may reflect species specificity in the communication channel: during the flight period of *E. defoliaria*, the *R*,*S*-enantiomer is a key pheromonal component of *C. pennaria*, which flies at the same time. In contrast, no species was found that used this compound during the flight period of *A. marginaria*. As for 6*Z*,9*Z*-3*S*,4*R*-epo-19Hy, it is the sex pheromone of the late autumn/early winter flying *A. aurantiaria* and a sex attractant for the late winter/early spring flying *A. leucophearia*. There again, pheromonal communication channels are based on the same compounds, which are used by different species in late autumn/early winter or in late winter/early spring, respectively.

Based on the present study, the following attractant combinations are recommended for practical monitoring (numbers represent dosages in micrograms on rubber septa): for *C. pennaria*, a 50 : 50 : 15 blend of 3*Z*,9*Z*-6*R*,7*S*-epo-19Hy, 3*Z*,6*Z*,9*Z*-21Hy, and 3*Z*,6*Z*,9*Z*-19Hy; for *E. defoliaria*, a 50 : 50 blend of 3*Z*,9*Z*-6*S*,7*R*-epo-19Hy and 3*Z*,6*Z*,9*Z*-19Hy; for *A. aurantiaria* and for *A. leucophearia*, 100 μg of 6*Z*,9*Z*-3*S*,4*R*-epo-19Hy; for *A. marginaria*, a 50 : 15 blend of 3*Z*,9*Z*-6*S*,7*R*-epo-19Hy and 3*Z*,6*Z*,9*Z*-19Hy.

Among known chiral sex attractant or pheromone components of Lepidoptera, monoepoxide derivatives of homoconjugated polyenes represent the most frequent type, occurring exclusively in Geometridae, Arctiidae, and in some subfamilies of Noctuidae (Arn et al., 1992). Of the epoxide components identified in the present study, 3*Z*,9*Z*-6*S*,7*R*-epo-19Hy was reported by Millar et al. (1990b) as a pheromone component of another geometrid, *Eufidonia convergaria*. After assignment of the gross structure, field tests with optically active samples strongly indicated the natural product to show 6*R*,7*S* configuration. The same compound was also described as a sex attractant component for geometrids, *Anavitrinella pampinaria* (Millar et al., 1990a), *Caripeta angustiorata* (Millar et al., 1990b), *Hypagyrtis piniata* (Underhill, cited in Arn et al., 1992), as well as for the noctuid, *Rivula propinqualis* (Millar et al., 1990b). 3*Z*,9*Z*-6*R*,7*S*-epo-19Hy was reported as a sex attractant component for the geometrid, *Probole amicaria* (Millar et al., 1990a). 6*Z*,9*Z*-*cis*-3,4-epo-19Hy was identified as a sex pheromone component of *Ascotis selenaria* (Geometridae) (Becker et al., 1990, Cossé et al., 1992). The 3*S*,4*R* configuration showed high biological activity in both EAG studies and field tests, while the enantiomer proved to be much less active. It remains, however, unknown whether the natural pheromone is optically pure or represents a mixture of enantiomers. 6*Z*,9*Z*-(3*S*,4*R*)-epo-19Hy was also reported as a sex attractant for the two geometrids, *Probole americana* and *Sicya macularia* (Millar et al., 1990a). Our results represent the first unambiguous structure assignments of chiral epoxydiene pheromones by direct chemical method.

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