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IDENTIFICATION OF A NOVEL MOTH SEX PHEROMONE IN *Eriocrania cicatricella* (Zett.) (LEPIDOPTERA: ERIOCRANIIDAE) AND ITS PHYLOGENETIC IMPLICATIONS

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Abstract—Extracts from different body parts of adult female *Eriocrania cicatricella* (Zett.) were tested for electrophysiological activity on conspecific male antennae. Extracts from the Vth abdominal segment, containing a pair of exocrine glands, elicited the largest electroantennographic response when compared to extracts of other body parts. Female extracts were analyzed by gas chromatography with simultaneous flame ionization and electroantennographic detection (EAD). The EAD active peaks were identified as (*Z*)-4-hepten-2-one, (2*R*)-heptane-2-ol, and (2*R*)-(*Z*)-4-hepten-2-ol by coinjection on a gas chromatography and by comparison of mass spectra with those of synthetic standards. In field tests, a blend of these three pheromone components was highly attractive to conspecific males, and a subtractive assay confirmed that the unsaturated alcohol is the major pheromone component, whereas no definite behavioral activity could be assigned to the ketone or the saturated alcohol. A bait containing the two alcohols with *S*-configuration was attractive to male *E. sparrmannella* (Bosc), whereas no males of *E. cicatricella* were found in these traps. The sex pheromone compounds in *E. cicatricella* are

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chemically similar to pheromones reported in Trichoptera and they are produced in homologous glands.

Key Words—*Eriocrania cicatricella*, *Eriocrania sparrmannella*, Eriocraniidae, Lepidoptera, sex pheromone, EAG, GC-EAD, mass spectrometry, synthesis, evolution, (*Z*)-4-hepten-2-one, (2*R*)-heptan-2-ol, (2*R*)-(*Z*)-4-hepten-2-ol.

INTRODUCTION

Although sex pheromone communication systems in moths are generally well investigated, there is still a lack of data on the pheromones of the first evolutionary lineages of the order Lepidoptera. Absence of sex pheromones has been suggested for *Micropterix calthella* (L.) (Pirngruber, 1944). However, male attraction by synthetic lures and by conspecific females was reported for three eriocraniid species (Kozlov, 1985, 1988; Wagner and Rosovsky, 1991). The only examination of a nonditrysian species reported so far, *Lampronia capitella* (Clerck) (Prodoxidae), suggested that it may have the same type of sex pheromone as most of the ditrysian lepidopterans (Löfstedt, 1991).

Within the Trichoptera, the sister group of Lepidoptera, sex pheromone components are short-chain alcohols and ketones, which are different from the olefinic compounds that are widely used in Lepidoptera. Based on the accumulated data on the pheromones identified in Lepidoptera (Arn et al., 1992) and some previous studies on pheromone production in Trichoptera, independent evolution of sex pheromones of Trichoptera and Lepidoptera has been hypothesized (Löfstedt, 1991). An investigation of archaic Lepidoptera is needed to state whether the chemical structure of lepidopteran pheromone components (which generally include products of Δ11-desaturation of long-chain fatty acid precursors) has evolved within the Lepidoptera or whether it should be considered a synapomorphy[5] of the whole order.

Trichoptera differ from ditrysian Lepidoptera not only in the structure of the sex pheromones, but also in the location of the pheromone-producing glands, which are situated in the Vth abdominal segment (Nielsen, 1980). Similar glands have been described in some archaic Lepidoptera (for a list of taxa possessing these glands, see Davis, 1975), whereas most females of ditrysian moths possess specialized pheromone glands at the ovipositor (Percy-Cunningham and MacDonald, 1987). The function of the abdominal glands in the primitive Lepidoptera is still unclear, although it has been repeatedly hypothesized that they release some kind of pheromone (Razowski, 1975; Kristensen,

[5]Synapomorphy: a homologous character shared by two or more taxa and inferred to have been present in the nearest common ancestor but not in earlier ancestors nor in taxa outside this group.

1984a). Bioassay and chemical elucidation of components produced by abdominal glands is necessary to prove this hypothesis.

Since the presence of sex pheromone communication has already been demonstrated in Eriocraniidae (Kozlov, 1985, 1988; Wagner and Rosovsky, 1991), this family was chosen as a target group for a detailed investigation of pheromone communication in archaic Lepidoptera. Our aims in the present study were: (1) to associate the pheromone production with some morphological structures of the female's body, (2) to identify the sex pheromone components produced by female *Eriocrania cicatricella*, and (3) to contribute to an understanding of the evolutionary trend in location of pheromone-producing glands and pheromone composition in the Trichoptera and primitive Lepidoptera.

METHODS AND MATERIALS

Insects. Eriocranic cicatricella Zett. were collected from a young birch forest on May 1–6, 1992 (Turku, Finland) and on April 21–30, 1993 (Lund, Sweden). Adults were sexed immediately after capture, kept in plastic vials containing moist filter paper, and transported to the laboratory in Lund for electrophysiological tests and chemical analyses.

Preparation of extracts. Extracts of the whole abdomen, the Vth abdominal segment, and the ovipositor from adult females and the whole abdomen from adult males of *E. cicatricella* were used in electroantennogram (EAG) experiments. Extracts from the presumed pheromone gland located at the Vth abdominal segment of females were used for gas chromatography with electroantennographic detection (GC-EAD). The insects were anesthesized by cooling them to −20°C, and they were then dissected by use of different scalpels for different body parts to avoid contamination. The dissected segments were extracted for at least 30 min in pointed tubes containing 50 μl dichloromethane or hexane. These extracts were subsequently used for both electrophysiological tests and chemical identification.

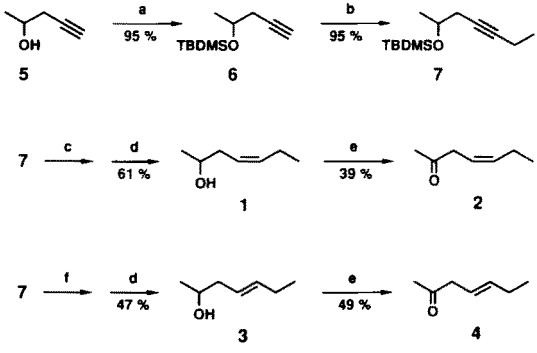
Electrophysiological and Chemical Analysis. To examine male antennal sensitivity to different body extracts of females, the tip of the male antenna of *E. cicatricella* was cut off and the antenna was mounted between two pipet electrodes containing Beadle-Ephrussi Ringer, and grounded via an Ag–AgCl wire. The tip electrode was connected to a high-impedance DC amplifier with automatic baseline drift compensation. One female equivalent of the different body extracts, deposited in the tip of a Pasteur pipet, was used as the odor source. One milliliter of air was puffed through the pipet and injected into the airstream flowing over the antenna (0.5 m/sec). The two different solvents used for the extraction, dichloromethane and hexane, were also tested for male EAG responses.

A Hewlett Packard 5830 GC equipped with a DB-wax column (30 m × 0.25 mm ID, J & W Scientific, Folsom, California) and an effluent split allowed simultaneous flame ionization (FID) and electroantennographic detection (EAD) of the separated pheromone components. Hydrogen was used as carrier gas, and the effluent split ratio was approximately 1:1. Samples were injected splitless. The injector temperature was 250°C and the split valve was opened 1 min after injection. The column temperature was maintained at 40°C for 3 min following the injection and then linearly increased to 230°C at a rate of 5°C/min. The outlet for the EAD was placed in a purified airstream flowing over the antennal preparation at a speed of 0.5 m/sec. Five microliters of extracts containing 5–10 female equivalents were injected into the column for the analysis. The equivalent chain lengths of active pheromone compounds relative to a homologous series of straight-chain acetates (7–22:OAc) were established.

Gas chromatographic–mass spectrometric analyses of GC-EAD active compounds were performed by using a fused-silica capillary (50 m × 0.32 mm ID) coated with FFAP, programmed from 60 to 220°C at a rate of 5°C/min. Mass spectra were recorded with a double focusing instrument VG 70-250 SE at 70 eV. Microhydrogenation was carried out according to Huwyler (1973). Enantiomeric separation was achieved by using a fused silica capillary (25 m × 0.32 mm ID) coated with a mixture of 6-*O*-methyl-2,3-di-*O*-pentyl-γ-cyclodextrin and OV-1701 (1:4) operated under the following conditions: 12 min at 60°C, then programmed to 120°C at a rate of 3°C/min. NMR spectra (400.1 MHz for ^{1}H and 100.6 for ^{13}C) of synthetic samples were run on a Bruker AMX 400; δ TMS = 0, δ $CDCl_3$ = 7.26 (s = singlet, bs = broad singlet, d = doublet, t = triplet, qu = quintet); attribution of ^{13}C signals was made according to DEPT spectroscopy (CH_3 and CH = +; CH_2 = −; quart C = 0).

Field Tests. Field-trapping was conducted in a young birch forest close to Lund (Sweden) and at a similar site in Turku (Finland). Synthetic blends were prepared in hexane. Red rubber septa (Arthur H. Thomas Co. Catalog No. 1780-J07) were used as dispensers, and the traps used were Lund II sticky traps (Anderbrant et al., 1989). All traps were hung on the branches of young birch trees 1–2 m above ground. Within a replicate (N = 5), each trap was set at least 5 m apart. The traps were checked every day, and trap positions within a series were randomized to minimize the effects of habitat heterogenities. Subtractive assays were first carried out in Lund, April 23–27, 1993, to determine the attractiveness to males of blends containing compounds isolated from the female pheromone gland extracts. The second set of field trapping tests was designed the same way and carried out in Turku, May 3–8, 1993. These experiments included a dose response test and an examination of the *R* and *S*-enantiomers of two alcohols.

Chemical Synthesis. The synthesis of the compounds used in the field tests is shown in Scheme 1 and is described as follows.



SCHEME 1. Syntheses of 4-hepten-2-ols (**1**) and (**3**) and 4-hepten-2-ones (**2**) and (**4**).

rac.-2-(t-Butyldimethylsiloxy)-4-heptyne **(7).** To a stirred suspension of 0.6 g (26 mmol) lithium amide in 30 ml liquid ammonia, 1.0 g (5.04 mmol) 3-(*t*-butyl-dimethylsiloxy)-1-pentyne **(6),** synthesized by alkynylation of methyloxirane according to Jacobson *et al.* (1982), followed by protection of the produced 4-pentyn-2-ol **(5),** was added during 15 min. Subsequently, 7.2 g (66 mmol) of ethylbromide was added, and the mixture was warmed to 0°C. After the addition of 50 ml ether and 20 ml saturated aqueous (aq.) ammonium chloride, the organic layer was separated, and the aqueous phase was extracted twice with 50 ml of ether. The combined organic solutions were dried over anhydrous magnesium sulfate and concentrated. The crude product (yield 1.05 g = 92%) was used for the next step without further purification.

rac.(Z)-4-Hepten-2-ol **(1).** According to the general procedure for the production of P-2 nickel (Brown and Ahuja, 1973), 260 mg (1.02 mmol) nickel acetate tetrahydrate dissolved in 10 ml of ethanol was reduced with 1.02 ml of a 1 M solution of sodium borohydride in ethanol. After hydrogen production ceased, 137 μl (2.04 mmol) ethylene diamine was added. To the black suspension 1.05 g (4.64 mmol) of **(7)** dissolved in 1 ml of ethanol was added. The stirred mixture was hydrogenated under standard conditions (1m H_2, room temperature). The mixture was filtered through a pad of celite, the solvent was evaporated, and then 10 ml of water and 10 ml ether were added. The organic layer was separated, the aqueous layer was extracted twice with 10 ml of ether, and the ethereal extracts were dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was dissolved in 15 ml of acetonitrile and stirred with 10 drops of 40% aq. hydrogen fluoride. After 1 hr, the mixture was poured onto brine and extracted twice with 20 ml of ether. The filtered organic

layer was washed with sodium bicarbonate, dried over magnesium sulfate, and concentrated. The residue was chromatographed on silica Merck 60/240–400 mesh impregnated with 10% $AgNO_3$ using a 2:1 mixture of pentane and diethylether, which, upon TLC, had been previously known to give an R_f value of 0.25, yielding 490 mg (61%) of **(1)**.

^{1}H NMR (400.1 MHz, $CDCl_3$, attribution of carbon atoms according to COSY experiment): δ = 5.57 (dtt, $J_{5,4}$ = 10.8 Hz, $J_{5,6}$ = 7.2 Hz, $J_{5,3}$ = 1.5 Hz, 1H, H-5), 5.32–5.41 (m, 1H, H-4), 2.77–3.82 (m, 1H, H-2), 2.14–2.29 (m, 2H, H-3), 2.08 (dqu, $J_{6,5}$ = $J_{6,7}$ = 7.4 Hz, 2H, H-6), 1.55 (bs, 1H, OH), 1.21 (d, $J_{1,2}$ = 6.2 Hz, 3H, H-1), 0.97 (t, 3H, H-7). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 135.12 (+, C 5), 124.46 (+, C 4), 67.70 (+, C 2), 37.04 (−, C 3), 22.71 (+, C 1), 20.70 (−, C 6), 14.25 (+, C 7).

rac.(E)-4-Hepten-2-ol **(3)**. To a solution of 1.9 g (8.4 mmol) of **(7)** in a mixture of 50 ml liquid ammonia and 20 ml tetrahydrofuran 0.6 g (25 mmol) sodium was added in small portions at −40°C. The dark blue solution was stirred for 1 h and subsequently warmed to room temperature. After the addition of 50 ml ether and 50 ml saturated aq. ammonium chloride, the organic layer was separated and the aqueous phase was extracted twice with 20 ml of ether. The combined organic solution was washed with 20 ml of water, dried with magnesium sulfate, and concentrated in vacuo. The crude product was diluted with 20 ml of acetonitrile. After the addition of 15 drops of 40% aq. hydrogen fluoride, the product was stirred for 1 hr, and worked up. The final purification was carried out by column chromatography (Merck 60 impregnated with 10% $AgNO_3$ using a 5:2 mixture of pentane and diethylether, which, upon TLC, had been previously known to give an R_f value of 0.22), yielding 460 mg (47%) of **(3)**.

^{1}H NMR (400, 1 MHz, $CDCl_3$): δ = 5.59 (dtt, $J_{5,4}$ = 15.2 Hz, $J_{5,6}$ = 6.4 Hz, $J_{5,3}$ = 1.3 Hz, 1H, H-5), 5.36–5.45 (m, 1H, H-4), 3.74–3.81 (m, 1H, H-2), 2.16–2.23 (m, 1H, H-3), 2.00–2.13 (m, 3H, H-3, H-6), 1.65 (bs, 1H, OH), 1.18 (d, $J_{1,2}$ = 6.1 Hz, 3H, H-1), 0.99(t, $J_{7,6}$ = 7.5 Hz, 3H, H-7). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 136.24 (+, C5), 124.82 (+, C4), 67.23 (+, C 2), 42.54 (−, C 3), 25.68 (−, C 6), 22.65 (+, C 1), 13.82 (+, C 7).

(Z)-4-Hepten-2-one **(2)**. To a solution of 0.4 g (3.6 mmol) of **(1)** in 20 ml dichloromethane, 1.4 g (3.6 mmol) pyridine dichromate and 1.4 g pulverized molsieve 4 Å (Herscovici et al., 1982) was added and vigorously stirred for 4 hr. The mixture was filtered through 10 g of silica (Merck 60), which was subsequently washed with 80 ml of ether. The organic solution was concentrated and chromatographed on silica (Merck 60 impregnated with 10% $AgNO_3$ using a 10:1 mixture of pentane and diethylether, which, upon TLC, had been previously known to give an R_f value of 0.25), yielding 150 mg (39%) of **(2)**.

^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 5.61 (dtt, $J_{5,4}$ = 10.7 Hz, $J_{5,6}$ = 7.1 Hz, $J_{5,3}$ = 1.5 Hz, 1H, H-5), 5.51 (dtt, $J_{4,3}$ = 7.1 Hz, $J_{4,6}$ = 1.4 Hz, 1H,

H-4), 3.17 (bd, 2H, H-3), 2.16 (s, 3H, H-1), 2.05 (qu, $J_{6,7}$ = 7.6 Hz, 2H, H-6), 0.99 (t, 3H, H-7). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 207.55 (o, C 2), 136.86 (+, C 5), 120.83 (+, C 4), 47.67 (−, C 3), 29.32 (+, C 1), 25.63 (−, C 6), 13.52 (+, C 7).

(E)-4-Hepten-2-one **(4).** The procedure was essentially the same as for the synthesis of **(2).** Purification was carried out by column chromatography on Merck 60 using a 8:1 mixture of pentane and diethylether as the eluent. The same yield was obtained as with (*Z*)-4-Hepten-2-one.

^{1}H NMR (400.1 MHz, $CDCl_3$): δ = 5.47–5.63 (m, 2H, H-4), 3.11 (bd, $J_{3,4}$ = 6.8 Hz, 2H, H-3), 2.14 (s, 3H, H-1), 2.02–2.10 (m, 2H, H-6), 0.99 (t, $J_{7,6}$ = 7.4 Hz, 3H, H-7). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 207.55 (o, C 2), 136.86 (+, C 5), 120.83 (+, C 4), 47.67 (−, C 3), 29.32 (+, C 1), 25.63 (−, C 6), 13.52 (+, C 7).

(2R)-(Z)-4-Hepten-2-ol **(1a).** Using (2*R*)-methyloxirane (Merck) in the synthesis of **(5),** the procedure was essentially the same as for the synthesis of **(1):** $[\alpha]_D^{22}$ = −4.9 (c = 2.2 CH_2Cl_2). The optical purity was 98% ee (enantiomeric excess) as determined by chiral gas chromatography.

(2S)-(Z)-4-Hepten-2-ol **(1b).** Using (2*S*)-methyloxirane in the synthesis of **(5),** the procedure was essentially the same as for the synthesis of 1: $[\alpha]_D^{22}$ = +4.8 (c = 1.8 CH_2Cl_2). The optical purity was 96% ee as determined by chiral gas chromatography.

Heptan-2-ol. While racemic 2-heptanol was commercially available (Merck), optically active products were synthesized from optically active methyloxirane by Grignard reaction (Gaylord and Becker, 1951).

RESULTS

Courtship Behavior. Eriocrania cicatricella is a day-flying insect. Males have an unusual type of search behavior. Starting from a height of 0.5–1.5 m, they fly slowly upwards, along a wave-form trajectory, up to 2–3 m height; then they fly downwards quickly. This cycle is usually repeated two to five times. Virgin females do not demonstrate any special pose when "calling." Males flew around the female (which usually was sitting on the thin branch of a birch), then landed 10–15 cm away from the female and ran to her displaying a typical wing-fanning behavior. If the population density was high, up to five males "dancing" around a female were observed. The moths took position with their heads in opposite directions 1–2 sec after copulation. During the first 20–40 sec, the male had his wings raised, then he folded them slowly. If more than one male was attracted, the other males tried to copulate with the pair during 1–2 min, and then flew away. Copulation usually took 10–30 min. Copulating pairs were most frequently observed from 14:00–19:00 hr, when the air temperature usually reached the daily maximum.

EAGs of Different Body Parts. Male EAG response to the extract from the Vth abdominal segment of female *E. cicatricella* was as high as to the extract of the whole abdomen (Figure 1), indicating that the gland contained in this segment produced the active compounds. The extract from female ovipositor elicited a low response compared to extracts from the rest of the abdomen. There were no significant male EAG responses to the two solvents, dichloromethane and hexane, used for the female extraction. The reaction of female antennae to the extracts of the abdomen of both sexes was also tested, but no responses were observed. Thus, we found no EAG evidence for a male-produced pheromone in this species.

GC-EAD of Female Extracts and Chemical Identification. Analysis of the extract from female Vth abdominal segment of *E. cicatricella* indicated that three compounds elicited strong responses from a conspecific male antenna (Figure 2). On a DB-wax column, the equivalent chain lengths of three pheromone candidates were 1236, 1310, and 1330, respectively. The mass spectra of these three GC-EAD active compounds suggested two methylcarbinols (*m/z* 45) and a methyl ketone (*m/z* 43), each having seven carbon atoms. According to its fragmentation pattern, one of them (II) could easily be identified as heptan-2-ol (McLafferty and Stauffer, 1989). A minor component (I) showed a mass spectrum almost identical to (*E*)-4-hepten-2-one (McLafferty and Stauffer, 1989). The mass spectrum of the main component (III) is shown in Figure 3. Upon microhydrogenation (Huwyler, 1973), the compound was converted to heptan-2-ol, proving the natural product to be an unbranched hepten-2-ol. Provided that this unsaturated methylcarbinol and the above mentioned methylketone, 4-hepten-2-one, are biogenetically related, the main component would most probably

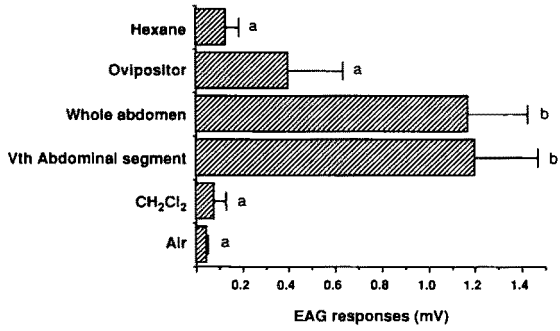


FIG. 1. EAG responses (mV ± SE) of male *Eriocrania cicatricella* to extracts of different body parts of conspecific females. Bars followed by the same letter are not statistically different according to a Kruskal-Wallis analysis of variance followed by pairwise comparisons using a Mann-Whitney U test ($P > 0.05$).

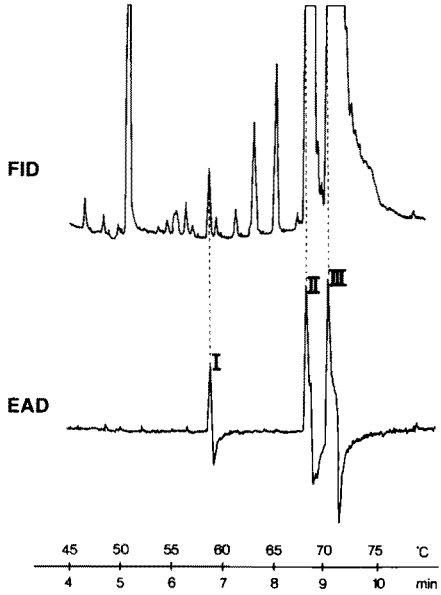


FIG. 2. Simultaneously recorded flame ionization detector (FID) and electroantennographic detector (EAD) responses using antennae of male *Eriocrania cicatricella* in response to pheromone gland extracts from conspecific females.

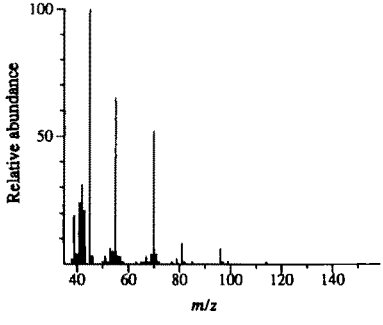


FIG. 3. Mass spectrum of the most abundant pheromone component, (2*R*)-(*Z*)-4-hepten-2-ol from female pheromone extracts of *Eriocrania cicatricella*.

show the double bond in position 4. Gas chromatographic investigations showed that the geometrical isomers of both 4-hepten-2-one and 4-hepten-2-ol could be very well separated on a FFAP-fused silica column under the conditions mentioned above. For the ketone, a separation value of $\alpha = 1.04$ [$R_t(Z)/R_t(E)$] was found, while the alcohol showed $\alpha = 1.10$ [$R_t(Z)/R_t(E)$]. Synthetic reference samples of (*Z*)-4-hepten-2-one and (*Z*)-4-hepten-2-ol coeluted with the natural products and showed the identical mass spectra. The absolute configuration of the natural alcohols could be determined by chiral gas chromatography on a cyclodextrin derivative. As shown with synthetic samples, the two racemates were well separated under the conditions mentioned above (Figure 4). The results revealed that this species produced pure enantiomers that coeluted with (2*R*)-(*Z*)-4-hepten-2-ol and (2*R*)-heptan-2-ol. The EAD active compounds in the extracts could thus be identified as (2*R*)-(*Z*)-4-hepten-2-ol, (2*R*)-heptan-2-ol, and (*Z*)-4-hepten-2-one in a ratio of 110:20:1, and an average female contained approximately 126 ng of (2*R*)-(*Z*)-4-hepten-2-ol, 23 ng of (2*R*)-heptan-2-ol and 1.1 ng of (*Z*)-4-hepten-2-one.

Field Tests. A synthetic mixture of these three compounds identified from *E. cicatricella* trapped large numbers of conspecific males in a field experiment (Table 1). Subtraction of either (*Z*)-4-hepten-2-one or (2*R*)-heptan-2-ol from the mixture did not significantly reduce attractiveness of the synthetic lure. Subtraction of (2*R*)-(*Z*)-4-hepten-2-ol caused complete loss of the attractivity. The importance of the enantiomeric composition of the two alcohols was elucidated

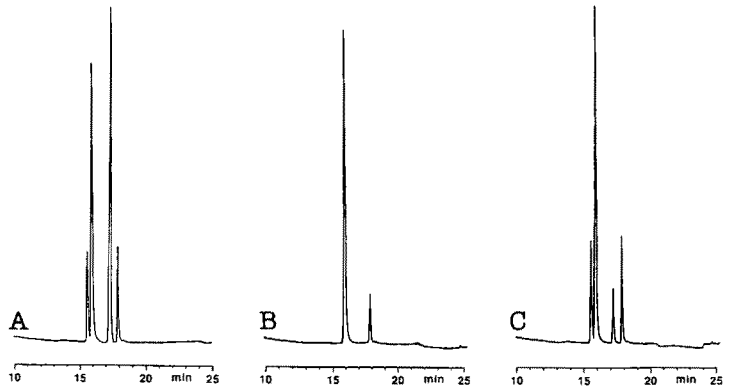


FIG. 4. Determination of the absolute configuration of the female sex pheromone components of *Eriocrania cicatricella* through chiral gas chromatography. Mixture of racemic (*Z*)-4-hepten-2-ol spiked with (2*R*)-(*Z*)-4-hepten-2-ol (first pair of peaks) and racemic 2-heptanol spiked with (2*S*)-heptanol (second pair of peaks) (A), natural gland extract (B), and mixture of racemic (*Z*)-4-hepten-2-ol, racemic 2-heptanol and natural gland extract (C).

in a field test in Turku, Finland (Table 2), which showed that the traps with baits containing the (*R*)-enantiomer of the major component, (*Z*)-4-hepten-2-ol, caught the highest number of males of *E. cicatricella*. Neither (2*R*)- nor (2*S*)-heptan-2-ol showed any effect on male attractivity. (2*S*)-(*Z*)-4-hepten-2-ol seemed to act as an inhibitor on the attraction of male *E. cicatricella*, but a large number of male *E. sparrmannella* (Bosc) were caught in the traps by the bait with this compound. When the dose of baits containing the two alcohols in (*R*)-configuration was decreased to half, the number of moths attracted to the

TABLE 1. SUBTRACTIVE ASSAY OF SYNTHETIC PHEROMONE CANDIDATES FOR *Eriocrania cicatricella* IN LUND, SWEDEN, APRIL 23–27, 1993

Treatment (μg)			Catch per trap (mean ± SE)[a]
(*Z*)-4-Hepten-2-one	(2*R*)-Heptan-2-ol	(2*R*)-(*Z*)-4-Hepten-2-ol	
0.5	15	100	33.4 ± 9.2 a
—	15	100	28.2 ± 8.3 a
0.5	15	—	0.6 ± 0.4 b
0.5	—	100	16.2 ± 5.0 a
—	—	—	0.2 ± 0.2 b

[a]Means followed by the same letter are not significantly different according to a Kruskal-Wallis analysis of variance followed by pairwise comparisons using the Fligner-Policello robust Mann-Whitney U test ($P > 0.05$), $N = 5$.

TABLE 2. FIELD TRAPPING OF MALE *Eriocrania cicatricella* WITH DIFFERENT ENANTIOMERS OF TWO PHEROMONE COMPOUNDS IN TURKU, FINLAND, MAY 3–8, 1993

Heptan-2-ol (μg)		(*Z*)-4-hepten-2-ol (μg)		Catch per trap (mean ± SE)[a]	
2*S*	2*R*	2*S*	2*R*	*E. cicatricella*	*E. sparrmannella*
—	15	—	100	131.0 ± 32.5 a	0 ± 0 b′
15	—	—	100	148.0 ± 30.6 a	0 ± 0 b′
—	15	100	—	0.2 ± 0.2 d	8.6 ± 1.6 a′
15	—	100	—	1.3 ± 0.5 d	13.3 ± 3.5 a′
—	—	—	100	102.8 ± 18.8 a	0 ± 0 b′
—	15	—	—	0.4 ± 0.5 d	0 ± 0 b′
7.5	7.5	50	50	13.8 ± 2.9 c	0 ± 0 b′
—	7.5	—	50	68.0 ± 16.7 b	0 ± 0 b′

[a]Means within each column followed by the same letter are not significantly different according to a Kruskal-Wallis analysis of variance followed by pairwise comparisons using the Fligner-Policello robust Mann-Whitney U test ($P > 0.05$), $N = 5$.

TABLE 3. FIELD TRAPPING OF MALE *Eriocrania cicatricella* WITH DIFFERENT DOSES OF PHEROMONE COMPOUNDS IN TURKU, FINLAND, MAY 3–8, 1993

Compounds (μg)			Catch per trap (mean ± SE)[a]
(*Z*)-4-hepten-2-one	(2*R*)-Heptan-2-ol	(2*R*)-(*Z*)-4-Hepten-2-ol	
0.05	1.5	10	6.2 ± 2.2 c
0.5	15	100	32.8 ± 14.3 b
5	150	1000	123.2 ± 31.1 a
—	1.5	10	4.4 ± 1.9 c
—	15	100	20.4 ± 7.5 b
—	150	1000	70.4 ± 31.8 ab

[a]Means followed by the same letter are not significantly different according to a Kruskal-Wallis analysis of variance followed by pairwise comparisons using the Fligner-Policello robust Mann-Whitney U test ($P > 0.05$), $N = 5$.

traps was significantly reduced (Table 2). A mixture of the two alcohols with (*R*)–configuration and this mixture plus (*Z*)-4-hepten-2-one were also tested in different doses. The highest dose of the three-component mixture was the most attractive. The attraction of males to the highest dose of the two-component mixture was not different from that of the medium dose of the three-component or the highest dose of the three-component mixture (Table 3).

DISCUSSION

Little is known about sex pheromones in archaic lepidopteran groups. The present investigation of the pheromone of *E. cicatricella* demonstrates that this primitive lineage of Lepidoptera (suborder Eriocraniina = Dacnonypha) is more similar to Trichoptera than to the well-investigated ditrysian lepidopterans, when it comes to pheromone communication. *E. cicatricella* produces short-chain ketones and alcohols as pheromone components, which are related to compounds earlier identified from Trichoptera; heptan-2-ol is a female-produced pheromone component in *Rhyacophila fasciata* (Mclachlan), and (*Z*)-6-nonen-2-one was identified in sternal gland extracts from male *Hydropsyche angustipennis* (Curtis) (Löfstedt et al., 1994). Our findings agree with the hypothesis of Wagner and Rosovsky (1991), which states that the presence of female-produced sex pheromones should be considered a synapomorphy of Trichoptera and Lepidoptera.

The pheromone components identified from *E. cicatricella*, (*Z*)-4-hepten-2-one, (2*R*)-(*Z*)-4-hepten-2-ol, and (2*R*)-heptan-2-ol are chemically very similar and most likely have a common biosynthetic origin. Within Lepidoptera, such

chemical structures have not been reported as pheromone components in any ditrysian family, but in the monotrysian family Nepticulidae similar chemical structures have been found (Tóth et al., 1995). Thus, the typical "lepidopteran" composition of sex pheromones has evolved within the order Lepidoptera. If we look at the phylogenetic tree of the order (Kristensen, 1984b), we can suppose that this event might be attributed a synapomorphy within nonditrysian Heteroneura, since one of the first lineages of the last taxa includes incurvarioid moths, which probably possess the "typical" lepidopteran pheromone (Löfstedt, 1991).

The site of pheromone synthesis and release may prove to be of special phylogenetic significance. A pair of glands opening on the Vth abdominal segment are considered an autapomorphy[6] of Amphiesmenoptera (Trichoptera + Lepidoptera), but these glands have been reduced independently in many lineages in both orders (Kristensen, 1984b). The ultrastructure of these glands (Kristensen, 1984a) is rather uniform within archaic Lepidoptera; the walls of the reservoirs are covered with secretory cells that belong to "type 3" according to the scheme by Noirot and Quennedy (1974). In most species in the family Eriocraniidae, the Vth abdominal segment is variously modified in association with the presence of the glands. The glands usually occur in both sexes (Davis, 1978), but in the genus *Eriocrania* we observed that the glands occur in the females only. In Trichoptera, female sex pheromones evidently are released from the Vth sternite (Wood and Resh, 1984; Löfstedt et al., 1994). The EAG test of different body parts showed that in Eriocraniidae, the pheromone production is associated with Vth abdominal segments. We were unable to isolate the gland specifically for EAG examination, but since no other secretory structures are known from this segment, we believe this is enough to associate the identified pheromones with the Vth abdominal glands. More advanced Lepidoptera, as summarized by Davis (1975), do not possess glands of the type mentioned above, and pheromone production, if present, is provided by other morphological structures. A large variety of pheromone-producing glands has been described, but generally females of ditrysian moths possess pheromone glands in the intersegmental region between abdominal segments VIII and XI (see references in Percy-Cunningham and McDonald, 1987).

Our field trapping experiments indicate that there is no significant reduction in the male attraction when (*Z*)-4-hepten-2-one or (2*R*)-heptan-2-ol was subtracted from the 3-component pheromone bait (Table 1). The chiral alcohols identified in *E. cicatricella* are pure (*R*)-enantiomers and (2*R*)-(*Z*)-4-hepten-2-ol is very attractive to conspecific males. When testing the attractivity of both (*R*)- and (*S*)-enantiomeric forms of these pheromone components in the field, we found that the *S* antipodes of the alcohols caught almost no *E. cicatricella*,

[6] Autapomorphy: The derived character state found in only one of two sister groups.

but large numbers of *E. sparrmannella*. Enantiomeric composition of the chiral components may contribute to the species specificity of this kind of pheromone. Geometrid moths have earlier been reported to use different combinations of chiral epoxides in their pheromone communication systems (Szöcs et al., 1993). Eriocranniidae provide another example within Lepidoptera of species where pheromone specificity is based on the chirality of the sex pheromone components.

Thus, both chemical and morphological data on the sex pheromone in this primitive moth, *E. cicatricella*, demonstrate the great similarities between the chemical communication system of the basic lineages of Trichoptera and Lepidoptera. This similarity can be explained by the common origin of these sister groups and therefore should be considered as a symplesiomorphy.[7] Sex pheromones used by most ditrysian species in Lepidoptera are derived from palmitic acid acted upon by various combinations of desaturases, β-oxidases, reductases, and acetyltransferases. The $\Delta 11$ desaturase seems to account for the largest number of unsaturated pheromone components, although several unusual desaturases such as $\Delta 10$, $\Delta 9$, and $\Delta 14$ are also involved in some species (see references in Löfstedt, 1991). Studies of pheromone biosynthesis in *E. cicatricella* will allow a comparison of biosynthetic routes to pheromones employed by primitive and more advanced lepidoptera.

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