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Sex pheromone biosynthesis in the pine caterpillar moth, *Dendrolimus punctatus* (Lepidoptera: Lasiocampidae): pathways leading to Z5-monoene and 5,7-conjugated diene components

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Abstract

Biosynthesis of the sex pheromone components (*Z*)-5-dodecenol and (*Z,E*)-5,7-dodecadienol in *Dendrolimus punctatus* was studied by topical application of deuterium-labeled fatty acids to pheromone glands and subsequent analysis of fatty acyl groups and pheromone components by gas chromatography-mass spectrometry. Our studies suggest that both (*Z*)-5-dodecenol and (*Z,E*)-5,7-dodecadienol can be biosynthetically derived from chain elongation of palmitate to stearate in the gland, and its subsequent Δ11 desaturation to produce (*Z*)-11-octadecenoate. After three cycles of 2-carbon chain-shortening, the pheromone glands produce (*Z*)-5-dodecenoate, which is then converted to (*Z*)-5-dodecenol by reduction. A second Δ11 desaturation of (*Z*)-9-hexadecenoate produces (*Z,E*)-9,11-hexadecadienoate, which is then chain shortened in two cycles of beta-oxidation and finally converted to (*Z,E*)-5,7-dodecadienol by reduction.

Keywords: 5,7-Conjugated diene; Sex pheromone biosynthesis; Deuterium label; *Dendrolimus punctatus*; Lasiocampidae

1. Introduction

Many sex pheromones in Lepidoptera are biosynthesized from CoA-derivatives of saturated FA[1] precursors by the combined action of several specific enzymes. The specific enzymes involved in this process are desaturases, chain-shortening enzymes, reductases, acetyltransferases, or oxidases (Bjostad et al., 1987; Morse and Meighen, 1987; Jurenka and Roelofs, 1993). The action of these enzymes in different combinations may produce the specific sex pheromones of most moth species.

The biosynthesis of conjugated diene sex pheromone compounds in moths has been studied in six species and the biosynthetic routes involved can be divided in two types. In the sex pheromone of *Bombyx mori*, the conjugated dienic bonds are formed by initial Δ11-desaturation of 16:Acyl, followed by the oxidative removal of two hydrogen atoms on the two carbons adjacent to the initial double bond (Yamaoka et al., 1984). Similar biosynthetic pathways were found in the biosynthesis of the sex pheromone of *Cydia pomonella* (Löfstedt and Bengtsson, 1988) and *Manduca sexta* (Fang et al., 1995). Another type of biosynthetic route, in which two sequential desaturation steps lead to the

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[1] Abbreviations used: Pheromone compounds and fatty acyl moieties are abbreviated in a standard way including (in order) geometry of the double bond, position of unsaturation, chain length follow by a colon and functionality. For example, Z5-12:OAc is (*Z*)-5-dodecenyl acetate, Z5-12:Ald is (Z)-5-dodecenal, Z5,E7-12:OH is (*Z,E*)-5,7-dodecadienol, Z5,E7-12:OPr is (*Z,E*)-5,7-dodecadienyl propionate, Z9,E11-16:Me is methyl (*Z,E*)-9,11-hexadecadienoate, Z7,E9-14:Acyl is (*Z,E*)-7,9-tetradecadienoate. FA, fatty acid; FAME, fatty acid methyl ester; Hez-PBAN, synthetic *Helicoverpa zea* pheromone biosynthesis-activating neuropeptide; GC-MS, gas chromatography-mass spectrometry; D_3-16:Acid, [16,16,16-$^2H_3$]-hexadecanoic acid; D_3-18:Acid, [18,18,18-$^2H_3$]-octadecanoic acid; D_9-Z11-16:Acid, [13,13,14,14,15,15,16,16,16-$^2H_9$]-(*Z*)-11-hexadecenoic acid; D_4-Z11-18:Acid, [15,15,16,16-$^2H_4$]-(*Z*)-11-octadecenoic acid; D_4-Z9-16:Acid, [13,13,14,14-$^2H_4$]-(*Z*)-9-hexadecenoic acid; D_4-Z7-14:Acid, [11,11,12,12-$^2H_4$]-(*Z*)-7- tetradecenoic acid.

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production of Z9,E11-14:OAc, was reported in *Spodoptera littoralis* (Martinez et al., 1990). This type of route to a sex pheromone component with conjugated double bonds was also found for the production of E9,E11-14:OAc in *Epiphyas postvittana* (Foster and Roelofs, 1990) and E5,Z7-12:OAc in *Thysanoplusia intermixta* (Ono et al., 2002). In *T. intermixta* Z11 desaturation produces Z11-16:Acyl from 16:Acyl and after chain-shortening E5 desaturation of Z7-12:Acyl yields the doubly unsaturated precursor.

The sex pheromone of *Dendrolimus punctatus* (Lepidoptera: Lasiocampidae) consists of a blend of Z5,E7-12:OH, Z5,E7-12:OAc and Z5,E7-12:OPr (Laboratory of Insect Pheromone, 1979). The monoenes, Z5-12:OH and Z5-12:OAc, have also been found as minor components of the sex pheromone (Zhao et al., 1993). Sex pheromones that have been identified in Lasiocampidae are all 5,7-dodecadienes with different terminal functional groups (alcohol, acetate, propionate and aldehyde) or different geometric isomers, except for the sex pheromone of *Gastropacha quercifolia* which was reported as a mixture of Z5-12:Ald and Z5-12:OH (Bestmann et al., 1993). The composition of the sex pheromone in *D. punctatus* is unique among the Lasiocampidae in that both (*Z,E*)-5,7-diene and (*Z*)-5-monoene compounds have been found in this species. The mixture of monoenic and dienic components with a 12-carbon skeleton is however similar to the pheromone of the previously mentioned noctuid *T. intermixta*, in which Z7-12:OAc was found in addition to E5,Z7-12:OAc (Ando et al., 1998). Although the biosynthetic route of E5,Z7-12:OAc in *T. intermixta* has been elucidated, the Z5,E7-12:OH (and its ester derivatives) in the Lasiocampidae do not have to be biosynthesized along the same route. From an evolutionary point of view it would be interesting to investigate whether these pheromone compounds with similar structures in different taxonomic groups are actually synthesized along similar or different biosynthetic routes. Are similar pheromone component structures evidence of common ancestry or an example of convergent evolution? Furthermore, accurate knowledge about the biosynthetic pathways leading to the production of moth sex pheromones is important to allow the prediction of minor pheromone components (Bjostad et al., 1984) and to define the range of potential pheromone components to be expected in closely related taxa.

In the present paper, the FA profile of the pheromone gland in *D. punctatus* is described, labeling experiments with different deuterium-labeled precursors are reported and hypothetical routes potentially leading to the production of the sex pheromone compounds, Z5,E7-12:OH and Z5-12:OH in *D. punctatus* are examined in the light of the available data.

2. Materials and methods

2.1. Chemicals

12:Me, 14:Me, 15:Me, 16:Me, *Z*9-16:Me, 17:Me, 18:Me, Z9-18:Me, Z9,Z12-18:Me, and Z9,Z12,Z15-18:Me were prepared from the corresponding FAs with diazomethane. Other FAMEs were prepared from the corresponding alcohols or their acetates, as described by Bjostad and Roelofs (1984).

Synthetic Hez-PBAN was purchased from Peninsula Laboratories (Belmont, CA). D_3-16:Acid and D_3-18:Acid were purchased from ICN Biomedicals, (Cambridge, MA) and Larodan Fine Chemicals, (Malmö, Sweden), respectively. The deuterium enrichment was 98% for D_3-16:Acid and 99% for D_3-18:Acid. D_9-Z11-16:Acid was a gift from M. Bengtsson (Department of Plant Protection Sciences, Swedish University of Agricultural Sciences). D_4-Z11-18:Acid (93.5% $^2H_4$) and D_4-Z9-16:Acid (95.3% $^2H_4$) were prepared as described previously (DeJarlais and Emken, 1978; Jurenka et al., 1994). The purity of both FAs was >99%. D_4-Z7-14:Acid was prepared as described by Adlof et al. (1978) for the 18-carbon analogue. The D_4-Z7-14:Me (80% Z-/20% E-isomer) was obtained from the Wittig coupling of heptyltriphenylphosphonium iodide (4,4,5,5-d4) and methyl 7-oxoheptanoate. To prepare the phosphonium iodide, 4-heptyn-1-ol was reduced with deuterium gas/Wilkinson's catalyst to yield 1-heptanol-4,4,5,5-d4. The alcohol was converted to the iodide with phosphorous pentoxide/phosphoric acid/potassium iodide and then converted to the phosphonium salt. The aldehyde ester was prepared by the reductive ozonization of cycloheptene (Siclari, 1980). The D_4-Z7-14:Me was separated from its E-isomer by silver resin chromatography (DeJarlais et al., 1983) and treated with alcoholic potassium hydroxide to yield the D_4-Z7-14:Acid (Chemical purity >98%; deuterium distribution: 0.6% d0, 0.6% d1, 0.9% d2, 3.5% d3, 90.9% d4, 2.3% d5, 0.5% d6, 0.7% d8).

2.2. Insects and application of labeled precursors to pheromone glands

Cocoons containing pupae of *D. punctatus* were collected in pine forests of Jiangxia Province, China, and transported to the Institute of Zoology, Beijing. They were kept at 22–25 °C in a 16–8 h light–dark cycle. Virgin female moths were collected just after eclosion. Twenty four to 48 h old female moths were used for analyses of FAs in the pheromone glands and for the labeling experiments. After identification of FAs in the pheromone glands, solutions of selected labeled FAs in DMSO were prepared just prior to each experiment. Pressure was applied to the abdomen of a female moth

by a plastic clip so that the pheromone gland was everted.

A given labeled FA (20 μg/μl) in 0.2–0.4 μl DMSO was topically applied to a pheromone gland and, 30 min later, the clip was removed and the female was released into a cage. When the pheromone glands were incubated with D_3-16:Acid or D_3-18:Acid for a normal incubation time (1.5 h), only a low incorporation into some of the potential pheromone intermediates, but not into the pheromone component Z5,E7-12:OH was observed. Since pheromone production is regulated by a PBAN-like factor, the females were injected with Hez-PBAN or head extracts and then the glands were incubated in vivo with D_3-16:Acid for 1.5 h. Under these conditions the label was incorporated into methyl esters of all potential pheromone intermediates, but not into Z5,E7-12:OH. Similar treatment of glands with D_3-18:Acid resulted in a low incorporation of the label into most of potential pheromone intermediates, but not into any pheromone component.

In order to obtain labeled pheromone components in measurable quantities, different incubation times (4, 8, 18 and 24 h) were tested with D_3-16:Acid, D_4-Z11-18:Acid and D_4-Z9-16:Acid. Finally 8 h was chosen as a proper incubation time for D_4-Z11-18:Acid and 18 h for D_3-16:Acid, D_4-Z9-16:Acid, D_4-Z7-14:Acid and control (the glands were incubated with 0.2 μl DMSO only). To examine the involvement of chain elongation in pheromone biosynthesis from D_4-Z7-14:Acid a chain elongation inhibitor, 2-octynoic acid in DMSO, was topically applied to pheromone gland (3.5 μg/female) 30 min before application of D_4-Z7-14:Acid. In all labeling experiments, the time for application of labeled compounds was adjusted to allow extraction of pheromone glands 6–7 h after darkness.

2.3. Extracts and methanolysis

For one replicate in a treatment, eight to 20 glands were dissected 6–7 h after darkness and extracted in 20 to 30 μl of 2:1 chloroform:methanol (v/v) for 24 h. Base methanolysis was utilized to convert fatty acyl moieties to the corresponding methyl esters and pheromone esters to alcohols for analysis. The extract was evaporated to apparent dryness in a microvial with a stream of nitrogen. A 0.5 M KOH-methanol solution (20–30 μl) was added, and allowed to react for 1 h at room temperature. A 1.0 M solution of HCl in water (25 μl) and 25 μl of hexane were added and the sample was shaken for 1 min. The hexane layer containing the methyl esters was then recovered and concentrated to 2–3 μl for GC or GC-MS analysis.

2.4. Dimethyl disulfide (DMDS) derivatization

Reference monounsaturated methyl esters and methanolyzed gland extracts were converted into DMDS adducts as described by Dunkelblum et al. (1985). After reaction with DMDS monounsaturated methyl esters $RCH{=}CHR'COOCH_3$ are converted to $RCH(SMe)\text{-}CH(SMe)R'COOCH_3$. Thus the double bond positions were determined by the occurrence of the diagnostic ions M^+, $[RCH(SMe)]^+$, $[R'CH(SMe)\text{-}COOCH_3]^+$ and $[R'CH(SMe)COOCH_3]^+$-32 after GC-MS analysis of derivatives. Reducing the amounts of all reagents to one-tenth of those used by Dunkelblum et al. (1985) largely decreased the contamination of the DMDS derivatives.

2.5. Analyses

Capillary GC analyses were performed on a Pye Unicam 204 GC equipped with a splitless capillary injector, a flame ionization detector, and a HP 3394 integrator. A polar CBWX column (30 × 0.25 mm i.d., J&W Scientific Inc.) was maintained at 80 °C for 1 min following injection, and then programmed to 160 °C at 4 °C/min. Hydrogen was used as carrier gas at 50 cm/s linear velocity. A nonpolar methyl silicone column (50 m × 0.2 mm i.d., Hewlett Packard) was maintained at 80 °C for 1 min following injection and then programmed to 220 °C at 4 °C/min. The linear velocity of the hydrogen carrier gas was in this case 42 cm/s. The injector was operated in the splitless mode and the purge valve was opened 0.25 min after injection.

GC-MS analyses were conducted on a Finnigan MAT ITD 810 mass detector coupled with a HP 5890 GC. A BP-20 capillary column (25 m × 0.25 mm i.d., SGE) was maintained at 80 °C for 1 min after injection and then programmed to 190 °C at 4 °C for analyses of FAMEs in the EI mode. A non-polar BP-1 column (25 m × 0.25 mm i.d.) was programmed from 80 °C to 250 °C at 30 °C/min for analyses of DMDS derivatives. Both columns were operated in the splitless mode and the purge valve was opened 0.75 min after injection. Monitoring of potential pheromone precursors in trace amounts was performed by GC-MS analysis with chemical ionization, using isobutane as reagent gas. In this analysis, a mass range of 150 to 320 was scanned for monitoring methyl esters of C14, C16 and C18 acids with a conjugated diene. Mass chromatograms of the following diagnostic ions of the monitored components were extracted from the total ion chromatogram and displayed: m/z of $(M+1)^+$, $(M\text{-}32+1)^+$ and $(M\text{-}74+1)^+$ for Z7,E9-14:Me, Z9,E11-16:Me and Z11, E13-18:Me. Further confirmation of the identity of a

certain peak was obtained by careful comparison of its retention time with that of reference compounds.

To determine abundance of isotopomers of endogenous and labeled FAMEs and alcohols, samples were separated on a BP-20 column under the same temperature program and analyzed by mass spectrometry in the CI mode. Mass chromatograms of the following diagnostic ions of the monitored components were extracted from the total ion chromatogram and displayed: m/z of $(M+1)^+$ for unlabeled FAMEs and Z5-12:OH in all of labeling experiments; $(M+4)^+$ for labeled FAMEs and Z5-12:OH in labeling experiments with D_3-16:Acid and D_3-18:Acid; $(M+5)^+$ for labeled unsaturated FAMEs and Z5-12:OH in labeling experiments with D_4-Z11-18:Acid, D_4-Z9-16:Acid and D_4-Z7-14:Acid. Because the most abundant diagnostic ion is $(M-18+1)^+$ for Z5,E7-12:OH in our GC-MS system, this ion was used for monitoring native Z5,E7-12:OH and the corresponding ions, $(M-18+4)^+$ and $(M-18+5)^+$ for the incorporation of the three and four deuterium atom-labeled FAs into Z5,E7-12:OH. Relative incorporation of a certain labeled compound was calculated as:

$$\text{Relative incorporation} = \left(\frac{\text{abundance of labeled compound}}{\text{abundance of its native compound}}\right) \times 100.$$

3. Results

3.1. Identification of potential pheromone precursors

A satisfactory separation of all FAMEs was obtained when the methanolysed samples of pheromone gland extracts of *D. punctatus* were analyzed on the CBWX or BP-20 capillary columns for GC and GC-MS, respectively. GC traces from the CBWX and BP-20 column were very similar. A total ion chromatogram of the methanolysed pheromone gland extract analyzed on the BP-20 capillary is shown in Fig. 1. The components of the methanolysed gland extracts were tentatively identified by comparing GC retention times with those of reference methyl esters on the polar BP-20 and the nonpolar BP-1 columns. In addition to the common fatty methyl esters 16:Me, Z9-16:Me, 18:Me, Z9-18:Me, Z9,Z12-18:Me and Z9,Z12,Z15-18:Me, the esters identified included large amounts of Z5-12:Me, Z5,E7-12:Me, Z11-16:Me, Z11-18:Me, as well as smaller amounts of 12:Me, Z7-12:Me, E5,Z7-12:Me, 14:Me, Z5-14:Me, Z7-14:Me, Z9-14:Me, 15:Me, 17:Me and Z13-18:Me. During base methanolysis, all acetates and propionates are converted to the corresponding alcohols. Z5,E7-12:OH and Z5-12:OH were found on the BP-20 column, whereas on the BP-1 column, only Z5-12:OH was apparent (Z5,E7-12:OH coeluted with Z5-12:Me).

GC identification of FAMEs was confirmed by GC-MS analyses. All saturated methyl esters had diagnostic peaks at m/z of M^+ and m/z 74 (base peak). The mass spectra of all monounsaturated methyl esters had diagnostic peaks at M^+, M^+-31, M^+-32, M^+-74, 74

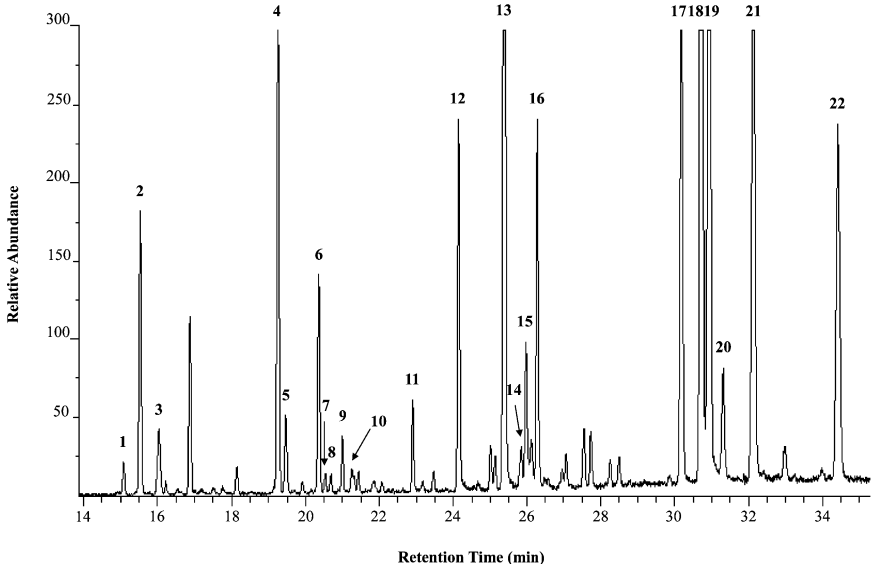

Fig. 1. Total ion chromatogram (25 m × 0.25 mm i.d. BP-20 column) of a base methanolysed pheromone gland extract from female *Dendrolimus punctatus*. See text for abbreviation of compound names: 1, 12:Me; 2, Z5-12:Me; 3, Z7-12:Me; 4, Z5,E7-12:Me; 5, E5,Z7-12:Me; 6, 14:Me; 7, Z5-12:OH; 8, Z5-14:Me; 9, Z7-14:Me; 10, Z9-14:Me; 11, 15:Me; 12, Z5,E7-12:OH; 13, 16:Me; 14, Z7-16:Me; 15, Z9-16:Me; 16, Z11-16:Me; 17, 18:Me; 18, Z9-18:Me; 19, Z11-18:Me; 20, Z13-18:Me; 21, Z9,Z12-18:Me; 22, Z9,Z12,Z15-18:Me.

and a base peak at 55. The mass spectra of the conjugated Z5,E7-12:Me and E5,Z7-12:Me had the same diagnostic peaks at m/z of 210 (M^+), 178 (M^+-32), 136 (M^+-74) and a base peak of 79. On the BP-20 capillary column, which was used in our GC-MS system, all four stereoisomers of methyl 5,7-dodecadienoate could be separated. The Z5,E7-12:Me and E5,Z7-12:Me that were identified tentatively in methanolysed gland extracts had identical mass spectra and the same retention times as those of corresponding synthetic reference compounds.

GC-MS analysis of DMDS derivatives of a methanolysed sample further confirmed the identity of the monounsaturated methyl esters, especially for the double bond location in these esters. However, the diagnostic ions of the DMDS derivatives of the presumed Z5-14:Me, Z9-14:Me and Z7-16:Me were below our limit of detection. The DMDS derivatives of polyunsaturated esters including Z5,E7-12:Me and E5,Z7-12:Me were not found by their extracted diagnostic ions in the GC-MS analysis. Similar results have been obtained in other studies on dienic compounds suggesting that these esters might polymerize or their derivatives are not sufficiently volatile to elute under the conditions used for GC analysis (Dunkelblum et al., 1985).

Only two conjugated diene methyl esters, Z5,E7-12:Me and E5,Z7-12:Me, were found by GC and GC-MS analyses in the methanolysed sample of the gland extract. The longer homologues Z7,E9-14:Me, Z9,E11-16:Me and Z11,E13-18:Me that could be expected were subsequently monitored by mass chromatogram analysis (see Materials and methods). Even with this sensitive analytical method, no diagnostic ion of Z11,E13-18:Me was found. Neither was another dienic fatty acid methyl ester, Z9,E11-16:Me, found by the same method. In contrast, the diagnostic ions of Z7,E9-14:Me were found in the same ratio and with the same retention time as for the reference compound. With the same approach, we could not find any trace of some other unusual FAMEs (Z6-12:Me, E7-12:Me and E9-14:Me), potential intermediates in other hypothetical biosynthetic pathways leading to the *Dendrolimus* pheromone components.

3.2. Labeling experiment with D_3-16:Acid

When the glands were incubated with D_3-16:Acid for 18 h, the label was significantly incorporated into all potential biosynthetic pheromone intermediates, Z5-12:Acyl, Z5,E7-12:Acyl, Z7-14:Acyl, Z7,E9-14:Acyl (only detected once), Z9-16:Acyl, 18:Acyl, Z11-18:Acyl, as well as other unusual fatty acyls, Z7-12:Acyl, Z9-14:Acyl, Z11-16:Acyl, Z13-18:Acyl (Table 1 and Fig. 2). D_3-16:Acid was also incorporated into the pheromone components, Z5,E7-12:OH and Z5-12:OH, in low but significant amounts (Table 1).

Table 1
Incorporation of deuterium-labeled FAs into pheromone components and fatty acyls including pheromone intermediates (analyzed as methyl esters) in the sex pheromone glands of *D. punctatus*

Incorporated compounds	Relative incorporation from different labeled FAs[a]				
	M + 4		M + 5		
	Control	D_3-16:Acid	Control	D_4-Z11-18:Acid	D_4-Z9-16:Acid
Z5-12:Me	0	$13.4 \pm 8.6^{*}$	0	$17.0 \pm 8.2^{**}$	$7.4 \pm 3.5^{**}$
Z7-12:Me	0	$13.8 \pm 4.7^{***}$	0	0	0
Z5,E7-12:Me	0	$2.9 \pm 1.8^{*}$	0	$5.0 \pm 4.0^{*}$	$2.6 \pm 1.0^{**}$
E5,Z7-12:Me	0	2.3[c]	0	0	0
Z5-12:OH	0	3.2[b]	0	$19.3 \pm 10.0^{**}$	7.6[c]
Z7-14:Me	0	$9.3 \pm 4.6^{*}$	0	$31.4 \pm 8.9^{***}$	$23.4 \pm 17.1^{*}$
Z9-14:Me	0	$15.2 \pm 2.5^{***}$	0	0	0
Z5,E7-12:OH	0	$6.6 \pm 4.0^{*}$	0	$6.0 \pm 3.0^{**}$	$5.4 \pm 3.7^{*}$
Z7,E9-14:Me	0	6.1[b]	0	$28.2 \pm 18.3^{*}$[d]	0
16:Me	0	$13.9 \pm 8.0^{*}$	0	0	0
Z9-16:Me	0	$7.2 \pm 4.5^{*}$	0	$8.5 \pm 4.0^{**}$	$386 \pm 280^{*}$
Z11-16:Me	0.1 ± 0.2	$22.5 \pm 8.4^{**}$	0	0	0
18:Me	0	$9.8 \pm 3.7^{*}$	0	0	0
Z11-18:Me	0	$5.6 \pm 1.6^{***}$	0.1 ± 0.2	136.9 ± 195.6	1.4 ± 1.3[d]
Z13-18:Me	0	$8.8 \pm 3.1^{**}$	0	0	0

[a] Relative incorporations are the mean calculated from following replicates: 4 for control; 6 for D_3-16:Acid; 7 for D_4-Z11-18:Acid; 6 for D_4-Z9-16:Acid. Asterisk indicates that the mean is significantly different from that in control at various levels ($^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; *t*-test).

[b] Calculated from one replicate.

[c] Calculated from two replicates.

[d] Calculated from three replicates.

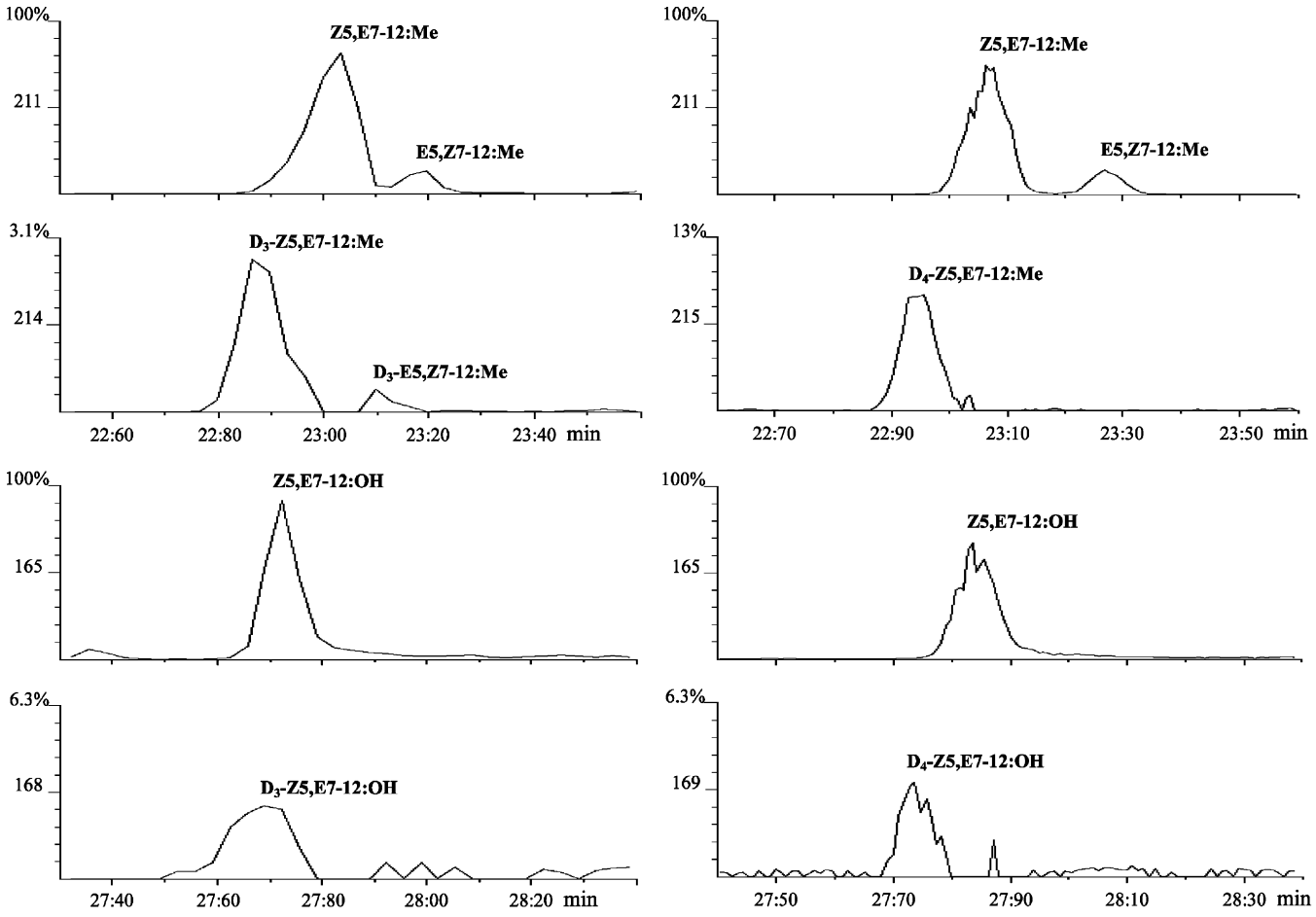

Fig. 2. GC-MS analyses (CI, isobutane) of Z5,E7-12:Me and Z5,E7-12:OH from glands incubated with D_3-16:Acid (left) and D_4-Z11-18:Acid (right). Upper mass chromatograms showed unlabeled and D_3-labeled Z5,E7-12:Me and E5,Z7-12:Me (left); unlabeled and D_4-labeled Z5,E7-12:Me (right). Lower mass chromatograms showed unlabeled and D_3-labeled Z5,E7-12:OH (left); unlabeled and D_4-labeled Z5,E7-12:OH (right).

3.3. Labeling experiment with D_4-Z11-18:Acid

Incubation of the glands with D_4-Z11-18:Acid for 8 h resulted in a significant incorporation of the label into the putative pheromone intermediates, Z9-16:Acyl, Z7-14:Acyl, Z5-12:Acyl, Z7,E9-14:Acyl, and Z5,E7-12:Acyl and pheromone components Z5-12:OH and Z5,E7-12:OH (Table 1 and Fig. 2).

3.4. Labeling experiment with D_4-Z9-16:Acid

When the pheromone glands were incubated with D_4-Z9-16:Acid for 18 h, the label was again incorporated into the putative pheromone precursors Z7-14:Acyl, Z5-12:Acyl and Z5,E7-12:Acyl, as well as the pheromone components, Z5,E7-12:OH and Z5-12:OH. Very small relative incorporation was found in Z11-18:Acyl, but not in Z7,E9-14:Acyl (Table 1).

3.5. Labeling experiment with D_4-Z7-14:Acid

In order to determine whether Z5,E7-12:OH could be biosynthesized by Δ9 desaturation of Z7-14:Acyl, application of D_4-Z7-14:Acid with or without previous application of 2-octynoic acid to the pheromone glands was performed. Three experiments incubating glands with D_4-Z7-14:Acid showed that the label not only was incorporated into the putative pheromone intermediates, Z5-12:Acyl and Z5,E7-12:Acyl, but also into Z9-16:Acyl with a relative incorporation from 11.0% to 44.1% (control in Table 2). Trace amounts of labeled Z11-18:Acyl were also found in two experiments but no labeled Z5-12:OH, Z5,E7-12:OH and Z7,E9-14:Acyl were found in any experiment. The analysis of the glands that were treated with the chain-elongation inhibitor 2-octynoic acid first and then incubated with D_4-Z7-14:Acid, showed that incorporation into Z9-16:Acyl decreased significantly to 0.1-5.1% in three experiments (Table 2). In the same treatment, relative incorporation of Z5,E7-12:Acyl and Z5-12:Acyl was

Table 2
Incorporation of D_4-Z7-14:Acid into pheromone intermediates (analyzed as methyl esters) of *D. punctatus* without or with application of the chain elongation inhibitor, 2-octynoic acid

Incorporated compound	Control (without 2-octynoic acid)[a]			Treatment (with 2-octynoic acid)[b]		
	test 1	test 2	test 3	test 4	test 5	test 6
Z5-12:Me	5.0	8.8	13.0	3.2	0	0.2
Z5,E7-12:Me	1.8	1.6	0.8	0.2	0	0
Z5 -12:OH	0	0	0	0	0	0
Z5,E7-12:OH	0	0	0	0	0	0
Z7-14:Me	1188.0	479.6	595.2	170.9	683.0	86.1
Z7,E9-14:Me	0	0	0	0	0	0
Z9-16:Me	19.3	11.0	44.1	5.1	0.1	1.7
Z11-18:Me	trace	0	trace	0	0	0

[a] 20 μg D_4-Z7-14:Acid was applied to the gland then incubated for 18 h. 15 females were used for one test.
[b] 3.5 μg 2-octynoic acid was first applied to the gland, 30 min later, 20 μg D_4-Z7-14:Acid was applied and incubated for 18 h. 2–4 females were used for one test.

much smaller than in the group that was treated only with D_4-Z7-14:Acid.

4. Discussion

4.1. Biosynthetic pathway of Z5-12:OH

A series of homologous monounsaturated fatty acyls, i.e. Z11-18:Acyl, Z9-16:Acyl, Z7-14:Acyl and Z5-12:Acyl, was found in the pheromone gland. This suggests that the pheromone component Z5-12:OH is produced by interaction of a Δ11 desaturase with 18:Acyl, forming Z11-18:Acyl which following three cycles of 2-carbon chain-shortening (β-oxidation) forms Z5-12:Acyl. This putative precursor is finally reduced to Z5-12:OH (Fig. 3). This proposed biosynthetic pathway was confirmed by subsequent labeling experiments using D_3-16:Acid, D_4-Z11-18:Acid and D_4-Z9-16:Acid. All labeled compounds were incorporated into the proposed pheromone intermediates and D_4-Z11-18:Acid was significantly incorporated into Z5-12:OH (Table 1).

This biosynthetic pathway leading to Z5-12:OH is the same as the one described for *Trichoplusia ni* (Bjostad and Roelofs, 1984), in which Z5-12:OAc is a minor component of the sex pheromone. Z5-12:Acyl has also been found in *Gastropacha quercifolia*, a species in the same family as *D. punctatus,* and has been proposed as the immediate precursor of the pheromone components Z5-12:OH and Z5-12:Ald (Bestmann et al., 1993). However, in *G. quercifolia*, the longer homologues were not reported to support the proposed pathway.

4.2. Biosynthetic pathway of Z5,E7-12:OH

The biosynthetic pathway of the pheromone component, Z5,E7-12:OH, is more involved. If the biosynthesis of the pheromone component Z5,E7-12:OH in *D. punctatus* took place as in *B. mori* (Yamaoka et al., 1984; Ando et al., 1988), i.e. formation of the second double bond by removal of hydrogen atoms on the two carbons adjacent to the initial double bond, this would imply monounsaturated intermediates with a double bond in even position, like Δ6-12:Acyl, Δ8-14:Acyl or Δ10-16:Acyl that could be converted to Z5,E7-12:Acyl, Z7,E9-14:Acyl or Z9,E11-16:Acyl. However, no such monounsaturated fatty acyl moieties were found in the pheromone gland of *D. punctatus.* Thus it is more likely that the conjugated double bonds in

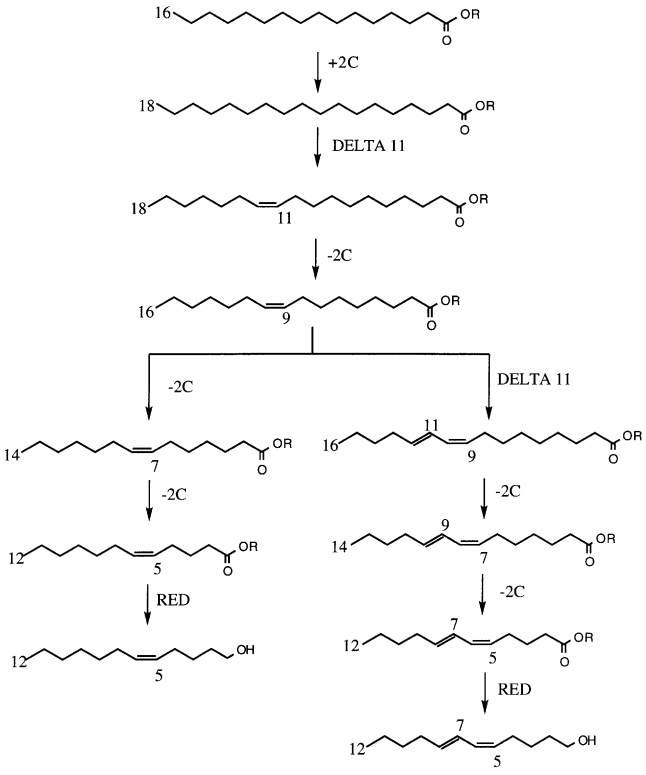

Fig. 3. Proposed biosynthetic pathways for the sex pheromone components of *Dendrolimus punctatus*.

Z5,E7-12:OH are produced by two consecutive steps of desaturation, similar to what has been reported in *S. littoralis* (Martinez et al., 1990). The labeling experiments showed that the immediate pheromone precursor, Z5,E7-12:Acyl is produced after initial chain elongation of 16:Acyl to 18:Acyl, which is then acted on by a Δ11 desaturase to form Z11-18:Acyl, and a second desaturation taking place on Z11-18:Acyl or one of its chain-shortened homologues, i.e. Z9-16:Acyl, Z7-14:Acyl or Z5-12:Acyl. Large amounts of Z11-18:Acyl were found in the gland (Fig. 1) and the fact that labeled 16:Acid was significantly incorporated into 18:Acyl, Z11-18:Acyl and all of its shorter homologues, as well as Z5,E7-12:Acyl and Z5,E7-12:OH (Table 1) supports this pathway. This hypothesis was further confirmed by labeling experiment with D_4-Z11-18:Acid. This label was not only incorporated into its homologues Z9-16:Acyl, Z7-14:Acyl and Z5-12:Acyl, but also into Z7,E9-14:Acyl, Z5,E7-12:Acyl and Z5,E7-12:OH (Table 1). Labeled 16:Acid was also incorporated into other uncommon fatty acyl moieties, including Z11-16:Acyl, Z9-14:Acyl, Z7-12:Acyl and Z13-18:Acyl, but these fatty acyls could be byproducts in pheromone biosynthesis. This suggestion was supported by a labeling experiment with D_9-Z11-16:Acid, in which the label was incorporated into Z11-16:Acyl, Z9-14:Acyl, Z7-12:Acyl and Z13-18:Acyl in large relative amounts, but no labeled Z5,E7-12:Acyl or Z5,E7-12:OH were found (data not shown). At this point we conclude that the major question remaining to be resolved is: Which monounsaturated fatty acyl is the substrate of the second desaturation step producing the E double bond?

4.2.1. The first possible biosynthetic route to Z5,E7-12:OH: Δ13 desaturation of Z11-18:Acyl

A first possibility would be that the second desaturation involves a E13 desaturase acting on Z11-18:Acyl to form Z11,E13-18:Acyl, followed by three cycles of 2-carbon chain-shortening to produce the immediate pheromone precursor Z5,E7-12:Acyl. Although large amounts of Z11-18:Acyl were found in the pheromone glands, neither the two intermediates Z11,E13-18:Acyl and Z9,E11-16:Acyl implied in this putative pathway, nor any E13-18:Acyl was found in any extract. If Z5,E7-12:Acyl were produced by this biosynthetic route, the production of deuterium-labeled Z5,E7-12:Acyl and Z5,E7-12:OH in gland incubations with D_4-Z9-16:Acid would require initial chain elongation of the monounsaturated precursor. Incubation with D_4-Z11-16:Acid and the resulting formation of D_4-Z13-18:Acyl, as well as formation of a small amount of D_4-labeled Z11-18:Acyl from D_4-Z9-16:Acid confirmed that chain-elongation can indeed take place, but much more incorporation was found in the shorter homologues Z7-14:Acyl and Z5-12:Acyl than in Z11-18:Acyl. This suggests to us that chain-shortening rather than chain-elongation is the predominant reaction in support of the alternative hypotheses that labeled Z5,E7-12:Acyl and Z5,E7-12:OH are formed from D_4-Z9-16:Acid by interaction of a second desaturase with Z9-16:Acyl or its shorter homologue Z7-14:Acyl, possibilities that will be examined below.

4.2.2. The second possible biosynthetic route to Z5,E7-12:OH: Δ11 desaturation of Z9-16:Acyl

A second possibility for production of Z5,E7-12:Acyl is thus that the 2-carbon chain-shortening of Z11-18:Acyl produces Z9-16:Acyl, in which another Δ11 desaturation occurs to form Z9,E11-16:Acyl, which would then be converted to Z5,E7-12:Acyl by two cycles of 2-carbon chain-shortening (Fig. 3). Although Z9,E11-16:Acyl was not found in the glands, the results from labeling experiments with D_3-18:Acid (data not shown), D_3-16:Acid, D_4-Z11-18:Acid and D_4-Z9-16:Acid (Table 1) are all in agreement with this proposed biosynthetic pathway. In particular, D_3-16:Acid and D_4-Z11-18:Acid were incorporated into all identified pheromone intermediates including trace amounts of the intermediate Z7,E9-14:Acyl, as well as the actual pheromone component, Z5,E7-12:OH. The fact that treatment of the glands with D_3-16:Acid resulted in a considerable amount of labeled Z11-16:Acyl, demonstrated that Δ11 desaturation does occur in C16 fatty acyls. The reason why Z9,E11-16:Acyl was not found in the glands may be that the conversion from Z9,E11-16:Acyl to Z5,E7-12:Acyl is very fast and thus no detectable amounts of the first intermediate would be accumulated during the pheromone biosynthesis. The same reasoning may explain why only trace quantities of Z7,E9-14:Acyl were found in the pheromone gland.

4.2.3. The third possible biosynthetic route to Z5,E7-12:OH: Δ9 desaturation of Z7-14:Acyl

A third possibility would be that Z5,E7-12:Acyl might be produced by Δ9 desaturation of Z7-14:Acyl to form Z7,E9-14:Acyl followed by 2-carbon chain-shortening. Whereas labeling experiments with D_4-Z11-18:Acid or D_4-Z9-16:Acid resulted in large incorporation into the doubly unsaturated alcohol and some incorporation into its fatty acyl precursor, incubation of the glands with D_4-Z7-14:Acid resulted in low incorporation of the label into Z5,E7-12:Acyl and no incorporation into Z5,E7-12:OH (Table 2). D_4-Z7-14:Acid was, however, incorporated into Z5-12:Acyl and to a somewhat lower extent also into Z9-16:Acyl. This suggests that the small amount of labeled Z5,E7-12:Acyl observed after incubation with D_4-Z7-14:Acid was produced by chain elongation of labeled Z7-14:Acyl to form labeled Z9-16:Acyl, followed by Δ11 desaturation and two cycles of 2-carbon chain-shortening. The application of the chain elongation inhibitor, 2-octynoic acid, before incubation of the glands with D_4-Z7-14:Acid resulted

in a large decrease of labeled Z9-16:Acyl, as well as Z5,E7-12:Acyl, which is in agreement with this suggestion. 2-Alkynoic acid was used as an inhibitor of lipid synthesis in animals (Robinson et al., 1963), and also used as an inhibitor of the chain elongation in the housefly (Renobales et al., 1986). The results are, however, not fully conclusive. The production of labeled Z5-12:Acyl from D_4-Z7-14:Acid also decreased, which suggests a side inhibitory effect on chain-shortening (G. Blomquist, personal communication). Thus the decrease of labeled Z5,E7-12:Acyl as a result of treatment with the chain-elongation inhibitor could also be explained as a side effect on chain-shortening and not due to an inhibition of chain elongation to Z9-16:Acyl. The low overall production of labeled Z5,E7-12:Acyl and Z5,E7-12:OH from D_4-Z7-14:Acid speaks against E9-desaturation of Z7-14:Acyl being the mechanism responsible for the formation of the second double bond.

4.2.4. *The fourth possible biosynthetic route of Z5,E7-12:OH: Δ7 desaturation of Z5-12:Acyl*

A final possibility comprises the formation of Z5,E7-12:Acyl by E7 desaturation of Z5-12:Acyl. A relatively large amount of Z5-12:Acyl occurs in the gland and could be the substrate for the second desaturation reaction, similar to what was reported in *T. intermixta* (Ono et al., 2002). A Δ7 desaturase has to our knowledge never been reported in any insect species or eukaryotic system so far. If Z5,E7-12:OH was produced in the pheromone glands involving E7 desaturation of Z5-12:Acyl, then D_4-Z7-14:Acid should be as easily incorporated into Z5,E7-12:Acyl and Z5,E7-12:OH as the other labeled pheromone precursors D_3-16:Acid, D_4-Z11-18:Acid or D_4-Z9-16:Acid. However, the data from the experiment with D_4-Z7-14:Acid indicated that no label was incorporated into Z5,E7-12:OH and the relative incorporation of the label into Z5,E7-12:Acyl was lower than that from D_3-16:Acid, D_4-Z11-18:Acid or D_4-Z9-16:Acid.

4.2.5. *Z11 and E11 desaturase in D. punctatus*

While labeled Z5,E7-12:Acyl and E5,Z7-12:Acyl were produced from labeled D_3-16:Acid, incubations of the glands with D_4-Z11-18:Acid or D_4-Z9-16:Acid resulted in label incorporation only into Z5,E7-12:Acyl. This indicated that no isomerization occurs in monoene or diene compound during the process of pheromone biosynthesis in *D. punctatus* and this is similar to what was reported in *Manduca sexta* (Fang et al., 1995). Thus two specific desaturases, a Z11 and a E11 desaturase, may act on 18:Acyl to produce Z11 and E11-18:Acyl. Alternatively one desaturase may interact with several substrates and produce different products depending on the substrate. The Δ11 desaturase in the European corn borer *Ostrinia nubilalis* interacts with both 16:Actyl and 14:Acyl and produces a mixture of E- and Z11-14:Acyl but pure Z11-16:Acyl (less than 1% E) (Wolf and Roelofs, 1987; Roelofs et al., 2002). Furthermore it was shown that the E11 desaturase in the light brown apple moth *Epiphyas postvittana* may interact with E9-14:Acyl, as well as the saturated analogues 14:Acyl and 16:Acyl (Liu et al., 2002). Thus, in *D. punctatus* one desaturase may produce a strongly Z-biased mixture of Z11- and E11-18:Acyl. After formation of Z9 and E9-16:Acyl by 2-carbon chain-shortening, the same desaturase may again act on both Z9 and E9-16:Acyl to produce a mixture of Z9,E11, E9,Z11, Z9,Z11 and E9,E11-16:Acyl. Interaction of the desaturase with a saturated substrate would produce mainly the Z isomer whereas interaction with a monoene would yield predominantly a second double bond with opposite geometry. Because of Z9-16:Acyl being by far the most abundant monounsaturated substrate the Z9,E11-isomer will be the most abundant product. Finally Z5,E7, E5,Z7, Z5,Z7 and E5,E7-12:Acyl could be produced by two cycles of 2-carbon chain-shortening from the above C16 dienic acyls. In fact, in addition to Z5,E7- and E5,Z7-isomer (Fig. 1), sometimes trace amounts of Z5,Z7 and E5,E7-isomer were all found in the pheromone glands. E11-18:Acyl and E9-16:Acyl implied as intermediates in the production of these minor isomers were not found in the pheromone glands, but this could be because of their quantity being too small to be detected or due to the difficulty to separate them from other more abundant fatty acyl methyl esters. For example, E11-18:Me could co-elute with Z9-18:Me and E9-16:Me with Z7-16:Me.

Labeling experiments indicated that pheromone biosynthesis in the gland proceeds very fast in many moth species. For example, in *Ostrinia furnacalis*, 10 min after application of labeled 16:Acid, the labeled pheromone components Z and E12-14:OAc could be detected (Zhao et al., 1995). However, much longer incubation time was needed in the pheromone glands of *D. punctatus* to incorporate deuterium-labeled 16:Acid, Z11-18:Acid or Z9-16:Acid into pheromone compounds. A similar phenomenon was found in *Manduca sexta* (Fang et al., 1995). In this species, labeled pheromone compounds, conjugated diene and triene aldehydes, were produced only after 24 h incubation time. Why such a long incubation time is needed for pheromone production in some moth species remains to be elucidated.

5. Conclusion

Our study conclusively demonstrates that Z5-12:OH is biosynthesized by interaction of a Δ11-desaturase with 18:Acyl, followed by three cycles of beta-oxidation and subsequent reduction. Furthermore, all results from

our study, including identification of fatty acyls in the pheromone gland and labeling experiments with a variety of potential precursors, are consistent with the hypothesis that after initial chain elongation of 16:Acyl to form 18:Acyl the biosynthetic pathway leading to the pheromone component Z5,E7-12:OH in *D. punctatus,* starts with Δ11 desaturation to form Z11-18:Acyl, which is then chain shortened to Z9-16:Acyl. A second Δ11 desaturation may then produce Z9,E11-16:Acyl. Finally Z5,E7-12:OH is produced from Z9,E11-16:Acyl by two cycles of chain-shortening and reduction (Fig. 3). Once Z5,E7-12:OH and Z5-12:OH are produced, the corresponding acetates may be produced by acetylation as in many other insect species (Jurenka and Roelofs, 1989). The unique pheromone component Z5,E7-12:OPr could be formed by propionylation.

Whether one or several Δ11 desaturases with different specificity with respect to chain length of the substrate and geometry of the product are involved in sex pheromone biosynthesis in *D. punctatus* may eventually be elucidated by cloning of the gene(s) and their expression in bacterial or yeast systems (Liu et al., 2002).

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References

Adlof, R.O., Miller, W.R., Emken, E.A., 1978. Synthesis Of *cis* and *trans* methyl 8- and 13-octadecenoate-d2 and d4 isomers. J. Label. Compd. Radiopharm. 15, 625–636.

Ando, T., Hase, T., Arima, R., Uchiayma, M., 1988. Biosynthetic pathway of bombykol, the sex pheromone of the silkworm moth. Agric. Biol. Chem. 52, 473–478.

Ando, T., Inomata, S., Shimada, R., Nomura, M., Uehara, S., Pu, G.-Q., 1998. Sex pheromones of *Thysanoplusia intermixta* and *T. orichalcea*: Identification and field tests. J. Chem. Ecol. 24, 1105–1116.

Bestmann, H.J., Attygalle, B.A., Garbe, W., Kern, F., Martichonok, V., Schafer, D., Vostrowsky, O., Hasenfu, I., 1993. Chemical structure and final steps of biosynthesis of the female sex pheromone of *Gastropacha quercifolia* (Lepidoptera: Lasiocampidae). Insect Biochem. Molec. Biol. 23, 793–799.

Bjostad, L.B., Roelofs, W.L., 1984. Sex pheromone biosynthetic precursors in *Bombyx mori*. Insect Biochem. 14, 275–278.

Bjostad, L.B., Linn, C.E., Du, J.-W., Roelofs, W.L., 1984. Identification of new sex pheromone components in *Trichoplusia ni* predicted from biosynthetic precursors. J. Chem. Ecol. 10, 1309–1324.

Bjostad, L.B., Wolf, W.A., Roelofs, W.L., 1987. Pheromone biosynthesis in Lepidoptera: desaturation and chain-shortening. In: Prestwich, G.D., Blomquist, G.J. (Eds.), Pheromone Biochemistry. Academic Press, Orlando, pp. 77–120.

DeJarlais, W.J., Emken, E.A., 1978. Synthesis of di-, tetra- and hexadeuterated octadecenoates. J. Label. Comp. Radiopharm. 15, 451–460.

DeJarlais, W.J., Adlof, R.O., Emken, E.A., 1983. Acetonitrile as eluent in silver resin column chromatography. J. Am. Oil Chem. Soc. 60, 975–979.

Dunkelblum, E., Tan, S.H., Silk, P.J., 1985. Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry: Application to analysis of fatty acids in pheromone glands of four Lepidoptera. J. Chem. Ecol. 11, 265–277.

Fang, N., Teal, P.E.A., Doolittle, R.E., Tumlinson, J.H., 1995. Biosynthesis of conjugated olefinic systems in the sex pheromone gland of female tobacco hornworm moths, *Manduca sexta* (L.). Insect Biochem. Molec. Biol. 25, 39–48.

Foster, S.P., Roelofs, W.L., 1990. Biosynthesis of a monoene and a conjugated diene sex pheromone component of the lightbrown apple moth by Δ11 desaturation. Experientia 46, 269–273.

Jurenka, R.A., Roelofs, W.L., 1989. Characterization of the acetyltransferase used in pheromone biosynthesis in moths: specificity for the Z isomer in Tortricidae. Insect Biochem. 19, 639–644.

Jurenka, R.A., Roelofs, W.L., 1993. Biosynthesis and endocrine regulation of fatty acid derived sex pheromones in moths. In: Stanley-Samuelson, D.W., Nelson, D.R. (Eds.), Insect Lipids: Chemistry, Biochemistry and Biology. University of Nebraska Press, Lincoln, pp. 353–388.

Jurenka, R.A., Haynes, K.F., Adlof, R.O., Bengtsson, M., Roelofs, W.R., 1994. Sex pheromone component ratio in the cabbage looper moth altered by a mutation effecting the fatty acid chain-shortening reactions in the pheromone biosynthetic pathway. Insect Biochem. Mol. Biol. 24, 373–381.

Laboratory of Insect Pheromone, 1979. Isolation, identification and synthesis of EAG-active components of the pine-caterpillar moth sex pheromone (Chinese). Kexue Tongbao (Chinese Sci. Bull.) 24, 1004–1008.

Liu, W., Jiao, H., Murray, N.C., O'Connor, M., Roelofs, W.L., 2002. Gene characterized for membrane desaturase that produces (*E*)-11 isomers of mono- and diunsaturated fatty acids. Proc. Natl. Acad. Sci. USA 99, 620–624.

Löfstedt, C., Bengtsson, M., 1988. Sex pheromone biosynthesis of (*E,E*)-8,10-dodecadienol in codling moth *Cydia pomonella* involves *E*9 desaturation. J. Chem. Ecol. 14, 903–915.

Martinez, T., Fabrias, G., Camps, F., 1990. Sex pheromone bio synthetic pathway in *Spodoptera littoralis* and its activation by a neurohormone. J. Biol. Chem. 265, 1381–1387.

Morse, D., Meighen, E., 1987. Pheromone biosynthesis: enzymatic studies in Lepidoptera. In: Prestwich, G.D., Blomquist, G.J. (Eds.), Pheromone Biochemistry. Academic Press, Orlando, pp. 121–158.

Ono, A., Imai, S., Inomata, A., Watanabe, T., Ando, T., 2002. Biosynthetic pathway for production of a conjugated dienyl sex pheromone of a Plusiinae moth, *Thysanoplusia intermixta*. Insect Biochem. Mol. Biol. 32, 701–708.

Renobales, M.De., Wakayama, E.J., Halarnkar, P.P., Reitz, R.C., Pomonis, J.G., Blomquist, G.J., 1986. Inhibition of hydrocarbon

biosynthesis in the housefly by 2-octadecynoate. Arch. Insect Biochem. Physiol. 3, 75–86.

Robinson, J.D., Brady, R.O., Bradley, R.M., 1963. Biosynthesis of fatty acids: IV Studies with inhibitors. J. Lipid Res. 4, 144–150.

Roelofs, W.L., Liu, W., Hao, G., Jiao, H., Rooney, A.P., Linn, Jr. C.E., 2002. Evolution of moth sex pheromones via ancestral genes. Proc. Natl. Acad. Sci. USA 99, 13621–13626.

Siclari, F. Omega-hydroxy alkenoic acid. 1980 Mar 11. U.S. PATENT 4, 192, 813:1–8

Wolf, W.A., Roelofs, W.L., 1987. Reinvestigation confirms action of Δ11-desaturases in spruce budworm moth sex pheromone biosynthesis. J. Chem. Ecol. 13, 1019–1027.

Yamaoka, R., Taniguchi, Y., Hayashiya, K., 1984. Bombykol biosynthesis from deuterium-labeled (Z)-11-hexadecenoic acid. Experentia 40, 255–271.

Zhao, C.H., Li, Q., Guo, X.Y., Wang, X.Y., 1993. New components of sex pheromone in the pine caterpillar moth, *Dendrolimus punctatus*: identification of chemical structures and field tests. (Chinese) Acta Entomol. Sin. 36, 247–250.

Zhao, C.H., Fang, L., Bengtsson, M., Löfstedt, C., 1995. Substrate specificity of acetyltransferase and reductase enzyme systems used in pheromone biosynthesis by Asian corn borer, *Ostrinia furnacalis*. J. Chem. Ecol. 21, 1495–1510.