Comparative Sex Pheromone Biosynthesis in the Obliquebanded Leafroller, *Choristoneura rosaceana*, and the Redbanded Leafroller, *Argyrotaenia velutinana*, Moths

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Biosynthesis of the major sex pheromone components of the obliquebanded leafroller, (*Z*)- and (*E*)-11-tetradecenyl acetates, is shown to proceed by Δ11 desaturation of myristate as in the related redbanded leafroller. A comparison between the amounts of deuterium label incorporated into the pheromone components from labelled myristic, palmitic and stearic acids gave a higher level of incorporation for the shorter chain acids, suggesting that Δ11 desaturation is a faster process than 2 carbon chain-shortening by β-oxidation.

Introduction

Research on the biosynthesis of sex pheromones of moths has shown that these chemicals are made from common fatty acids that are modified in the sex pheromone gland by several enzymatic processes [1].

In the redbanded leafroller moth, *Argyrotaenia velutinana* (Walker) (Tortricidae: Tortricinae), the major sex pheromone chemicals (*Z*)- and (*E*)-11-tetradecenyl acetates (Z- and E11-14:OAc) are biosynthesized by firstly 2 carbon chain-shortening through β-oxidation of palmitate to myristate and then Δ11 desaturation of myristate to the pheromone precursors (*Z*)- and (*E*)-11-tetradecenoates (Z- and E11-14:Acyl), [2, 3].

It is important to understand the mechanisms of sex pheromone biosynthesis in order to more fully understand the whole insect intraspecific communication system. Additionally a greater understanding of the biosynthetic pathways may offer an opportunity to develop new insect control methods, by for example, perturbing specific sequences of pheromone biosynthesis in the female's pheromone gland. However, if new control methods are to be developed using this principle, which are to be generally useful, it is essential that the mechanisms are more fully understood and to show that different species of moths use the same system to produce the same or similar chemicals.

The sex pheromone of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Tortricidae: Tortricinae) was originally identified as Z11-14:OAc [4], but was later shown to consist of small amounts of the additional chemicals, E11-14:OAc and (*Z*)-11-tetradecenol, [5]. In a comparative study on the fatty acyl moieties in *A. velutinana* and *C. rosaceana* (amongst other species), Wolf *et al.* [6] showed that these two species had approximately the same ratio of Z- and E11-14:Acyl precursors (40:60) in their gland and that it differed greatly from the final ratio of Z- and E11-14:OAcs (92:8 in *A. velutinana* and 97:3 in *C. rosaceana*).

During the course of developing techniques suitable for studying the biosynthesis of sex pheromones in other moths, we experimented on *C. rosaceana* and *A. velutinana* and report here the results of these studies that confirm that the biosynthesis of the sex pheromone chemicals of these species are by similar routes, *i.e.* Δ11 desaturation of myristate.

Materials and Methods

Insects

Both *C. rosaceana* and *A. velutinana* were reared in the laboratory on *semi*-synthetic diet [7]. Female moths were used 2–3 days after emergence, approxi-

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mately ½ h before the onset of the scotophase period.

Chemicals

Omega-labelled (14-D_3)-myristic acid (D_3-14:COOH) was purchased from ICON services, Summit, New Jersey. Omega-labelled (16-D_3)-palmitic (D_3-16:COOH) and (18-D_3)-stearic acids (D_3-18:COOH) were purchased from KOR Isotopes Inc., Cambridge, Massachusetts. All acids were greater than 98% isotopic purity. Synthetic reference chemicals of pheromone compounds and fatty acid methyl esters were available in the laboratory.

Analysis

The pheromone glands of 2–3 day old female moths were extruded using a small alligator clip applied to the end of the abdomen. The labelled fatty acids were applied as described [8], as DMSO solutions, to the gland using a 1.0 µl syringe under a binocular microscope. These were allowed to incubate for a total of 3–4 h. After the first hour of incubation the alligator clips were removed and the ovipositors of the insects allowed to return to their normal position. After incubation the glands were excised using fine forceps.

For analysis of pheromone chemicals, excised glands were extracted with distilled Skelly B for 16 h at ambient temperature. Excised glands were extracted in dichloromethane for 16 h at 3 °C for analysis of the fatty acyl compounds. Lipids extracted this way were converted to fatty acid methyl esters (FAME) by base methanolysis [9].

Analysis was by gas chromatography-mass spectrometry (GC-MS) as previously described [8]. Basically, the MS was used in the selected ion mode with chemical ionization using iso-butane as the reactant gas. A 30 m × 0.25 mm i.d. Supelcowax 10 capillary column programmed from 80–200 °C at 4 °C min^{-1}, following an initial delay of 3 min was used.

Results and Discussion

Base methanolysis of *C. rosaceana* pheromone glands revealed the FAME of the following common fatty acyl moieties: hexadecanoate (*Z*)-9-hexadecenoate, octadecanoate, (*Z*)-9-octadecenoate, (*Z,Z*)-9,12-octadecadienoate and (*Z,Z,Z*)-9,12,15-octadecatrienoate. In addition, the more specific methyl esters of dodecanoate (12:Acyl), two monounsaturated dodecenoates [tentatively identified as (*E*)- and (*Z*)-9-dodecenoates], tetradecanoate (14:Acyl) and E- and Z11-14:Acyls. The base methanolyzed glands of *A. velutinana* also contained those same methyl esters, previously reported [2].

The ratios of 14:Acyl:E11-14:Acyl:Z11-14:Acyl (see Table I) in the two species were virtually the same (26:43:31 in *C. rosaceana* and 24:43:33 in *A. velutinana*) as reported [6]. The average ratios of the corresponding pheromone acetates were 1.4:1.9:96.7 and 4.1:4.7:91.2 respectively.

C. rosaceana glands treated with D_3-14:COOH, D_3-16:COOH and D_3-18:COOH gave incorporation into both pheromone components, with increasing incorporation with decreasing precursor acid chain length (see Table II). In both the D_3-14:COOH and D_3-16:COOH runs, greater incorporation of label was observed in Z11-14:OAc. The D_3-18:COOH run showed a relatively higher proportion of incorporation of the label into E11-14:OAc, but the relatively small peak area of this component introduces considerable error into the determination. Saturated 14:OAc incorporated significantly less label than either of the two unsaturated acetates in the D_3-14:COOH run. It was not recorded in the other runs.

Table I. Relative intensities of ions from extracts of base methanolyzed pheromone glands of female obliquebanded and redbanded leafroller moths treated with deuterium-labelled palmitic acid.

Treatment (No. of females)	Relative intensity $(M+1)^+$	$(M+2)^+$	$(M+3)^+$	$(M+4)^+$
OBLR +D_3-16:COOH (5FE)				
12:Me	7.58	1.07	0.13	0.48
E9-12:Me	24.6	0.82	0.080	0.18
Z9-12:Me	6.70	3.60	0.70	0.42
14:Me	59.7	8.87	1.09	3.56
E11-14:Me	100.0	15.4	1.34	2.60
Z11-14:Me	72.4	11.7	0.98	2.95
RBLR +D_3-16:COOH (5FE)				
E11-14:Me	100.0	4.14	0.41	0.24
Z11-14:Me	71.3	2.71	0.26	0.30
+D_3-16:COOH (5FE)				
14:Me	56.8	11.6	1.12	0.79
E11-14:Me	100.0	16.8	1.64	0.86
Z11-14:Me	76.7	12.1	1.17	1.13
16:Me	352	73.7	7.93	5.30
Z9-18:Me	760	153	7.8	0.94

Table II. Relative intensities of ions from pheromone extracts from female obliquebanded and redbanded leafroller moths treated with deuterium-labelled saturated fatty acids.

Treatment (No. of females)	Relative intensity $(M+1)^+$	$(M+2)^+$	$(M+3)^+$	$(M+4)^+$
OBLR				
Control (5FE)				
14:OAc	0.40	0.073	ND	ND
E11-14:OAc	2.42	0.57	ND	ND
Z11-14:OAc	100.0	22.3	2.21	0.15
$+D_3$-16:COOH (5FE)				
E11-14:OAc	4.14	0.67	ND	0.12
Z11-14:OAc	100.0	15.5	2.70	7.34
$+D_3$-14:COOH (5FE)				
14:OAc	2.4	ND	ND	0.14
E11-14:OAc	1.5	0.26	0.08	0.70
Z11-14:OAc	100.0	15.5	8.27	56.2
$+D_3$-18:COOH (7FE)				
E11-14:OAc	2.38	0.37	ND	0.080
Z11-14:OAc	100.0	13.4	1.64	2.23
RBLR				
Control (5FE)				
12:OAc	12.3	1.61	0.20	ND
E9-12:OAc	1.42	0.27	ND	ND
Z9-12:OAc	2.72	0.71	ND	ND
14:OAc	5.22	1.01	0.11	ND
E11-14:OAc	7.06	1.04	0.16	ND
Z11-14:OAc	100.0	14.84	1.52	0.11
$+D_3$-16:COOH (5FE)				
12:OAc	4.95	0.82	0.07	3.14
14:OAc	3.93	1.42	0.15	0.20
E11-14:OAc	5.17	2.27	0.11	2.21
Z11-14:OAc	100.0	15.6	1.58	6.31
$+D_3$-16:COOH (5FE)				
14:OAc	4.48	0.87	0.19	0.079
E11-14:OAc	3.35	0.57	0.026	0.090
Z11-14:OAc	100.0	6.86	1.13	1.95

In both runs, *A. velutinana* incorporated more label from D_3-16:COOH into Z11-14:OAc than E11-14:OAc. Incorporation of label into dodecyl acetate (12:OAc), an additional pheromone component [10] was surprisingly high in one run.

Base methanolyzed glands of *C. rosaceana* treated with D_3-16:COOH incorporated label into methyl esters of 12:Acyl, the two unsaturated dodecenoates, 14:Acyl and E- and Z11-14:Acyls (Table I). If the tentative identification of E- and Z9-12:Acyls is correct, then incorporation of label into the *Z* isomer was greater, as it was for the Δ11-14:Acyls. Incorporation of the label into 14:Acyl was higher than into either pheromone precursor.

In *A. velutinana,* incorporation of the D_3-16:COOH label was also higher in the Z11-14:Acyl isomer than the E11-14:Acyl isomer.

The biosynthesis of the two major pheromone components in *C. rosaceana,* E- and Z11-14:OAc proceeds *via* Δ11 desaturation of myristate (see Fig. 1), as in the related species *A. velutinana* [2]. This adds further support to the hypothesis [11] that Δ11 desaturation as a generalized process is responsible for the biosynthesis of many pheromone compounds found in the family Tortricidae and probably other families of the Lepidoptera.

Labelled, D_3-18:COOH, D_3-16:COOH and D_3-14:COOH when applied to the pheromene glands of *C. rosaceana* gave increasing incorporation of the label into the pheromone components with decreasing chain length of the starting acid, with the greatest incorporation of 46.7% and 56.2% (into E- and Z11-14:OAc respectively) from D_3-14:COOH, further supporting biosynthesis from myristate. Additionally, this suggests that chain-shortening β-oxidation is a relatively slow process relative to Δ11 desaturation, at least in this insect.

In both *C. rosaceana* and *A. velutinana* glands the ratio of the two pheromone precursors E- and Z11-14:Acyl is approximately the same (ca. 43:32) and substantially different from the ratio of the two corresponding pheromone components. The consistently greater incorporation of label into the *Z* isomers tends to support the hypothesis that the higher *E*:*Z* ratio normally found in these glands is due to an accumulation of unused *E* isomer in the triacylglycerides [3].

We have shown the use of deuterium-labelling combined with GC-MS analysis to be a quick convenient tool for analysis of pheromone biosynthetic pathways. For the 2 species studied here, the labelled acids incorporated readily into the pheromone chemicals and fatty acyl intermediates. Using this method we have confirmed the more widespread use of a delta-11 desaturase for the biosynthesis of E- and Z11-14:OAcs in the Tortricidae.

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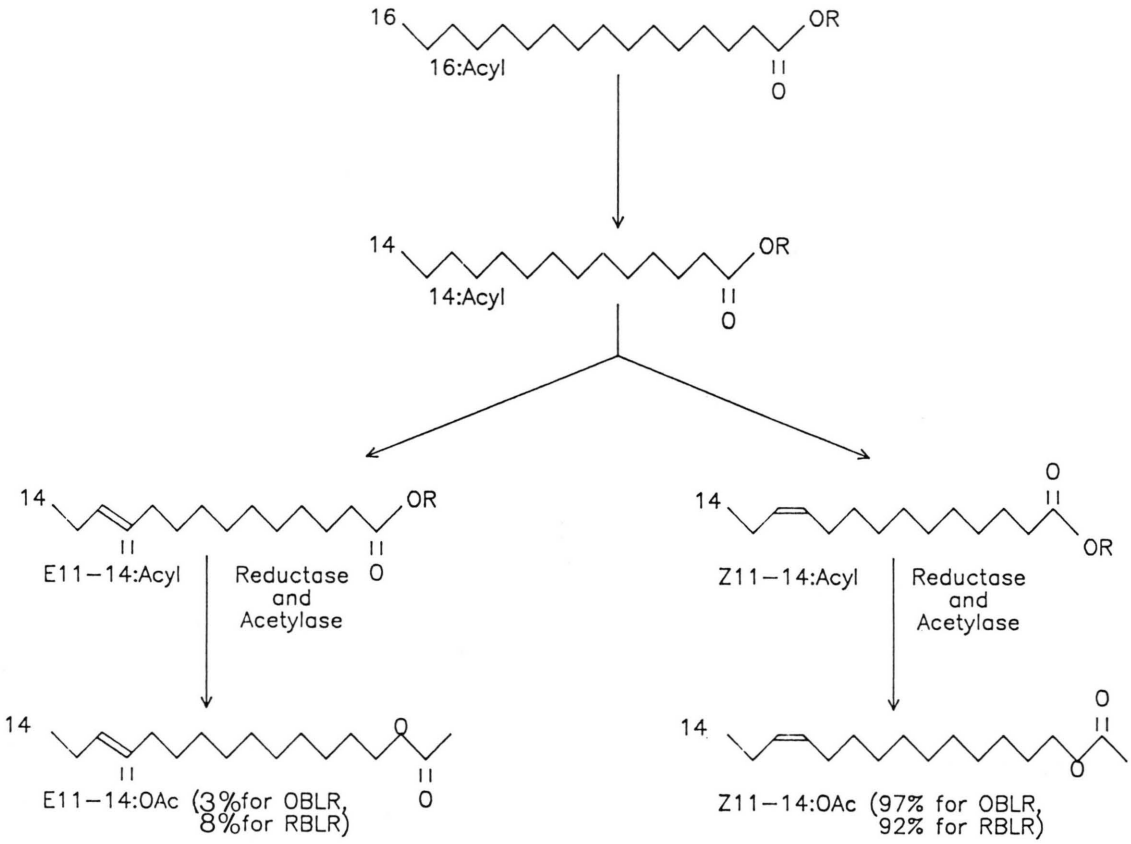

Fig. 1. Proposed scheme for biosyntheses of (*E*)- and (*Z*)-11-tetradecenyl acetates by Δ11 desaturation in the oblique-banded and redbanded leafroller moths.

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