Direct inhibition of (*Z*)-9 desaturation of (*E*)-11-tetradecenoic acid by methylenehexadecenoic acids in the biosynthesis of *Spodoptera littoralis* sex pheromone

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Abstract Analysis by gas chromatography coupled to mass spectrometry demonstrated that 11,12-methylenehexadec-11-enoic acid and 12,13-methylenehexadec-12-enoic acid are β-oxidized in *Spodoptera littoralis* sex pheromone gland to 9,10-methylenetetradec-10-enoic acid and 10,11-methylenetetradec-10-enoic acid, respectively. This result supported our previous hypothesis that inhibition of the (*Z*)-9 desaturation of (*E*)-11-tetradecenoic acid by those C-16 fatty acids is actually caused by their corresponding β-oxidation products. However, although (2,2,3,3-$^{2}H_4$)-11,12-methylenehexadec-11-enoic acid was not chain-shortened to the C-14 derivative, its activity as inhibitor of the (*Z*)-9 desaturation reaction was similar to that exhibited by 11,12-methylenehexadec-11-enoic acid. Therefore, the C-16 cyclopropene fatty acids may inhibit the (*Z*)-9 desaturation enzyme by themselves, probably through interaction of the cyclopropene ring with a binding site of the enzyme with the substrate double bond.—**Fabrias, G., L. Gosalbo, J. Quintana, and F. Camps.** Direct inhibition of (*Z*)-9 desaturation of (*E*)-11-tetradecenoic acid by methylenehexadecenoic acids in the biosynthesis of *Spodoptera littoralis* sex pheromone. *J. Lipid Res.* 1996. **37:** 1503–1509.

Supplementary key words inhibition • desaturase • sex pheromone • cyclopropene fatty acid • biosynthesis • *Spodoptera littoralis* • lepidoptera • insect

Evidence that two naturally occurring cyclopropene fatty acids (CPFA) inhibited the desaturation of stearic to oleic acid was first presented in vertebrate systems by Reiser and Raju (1). These compounds, named sterculic and malvalic acids, have 18 and 17 carbon atoms, respectively, with the cyclopropene ring at positions 9,10 (sterculic acid) and 8,9 (malvalic acid). It was later shown that both acids inhibited the 9,10-desaturation of different C-12 to C-20 aliphatic acids, regardless of the chain length (2). The importance of the ring position upon the effectiveness of the 9,10-desaturation inhibition was studied by Fogerty, Johnson and Pearson (3), who found that only when the ring was included in the C-9 and/or C-10 carbon atom was the cyclopropene fatty acid biologically active.

Many lepidopteran sex pheromones are biosynthesized from fatty acids by limited β-oxidation steps in conjunction with specific desaturases, some of them unique in eucaryotic cells (4). Our interest in this topic led us to study the action of CPFA on those desaturase enzymes. In our model insect, *Spodoptera littoralis* (Lepidoptera: Noctuidae), a complex system of desaturases is involved in the biosynthesis of the female sex pheromone (5): *a)* a (*Z*)-11 desaturase of hexadecanoic acid (16:Acid); *b)* two specific (*Z*) and (*E*)-11 desaturases of tetradecanoic acid (14:Acid); and *c)* a specific (*Z*)-9 desaturase of (*E*)-11-tetradecenoic acid (E11-14:Acid).

Abbreviations: CPFA, cyclopropene fatty acid; DMSO, dimethyl sulfoxide; FAME, fatty acid methyl ester; GC, gas–liquid chromatography; GC–MS, gas–liquid chromatography coupled to mass spectrometry; MHA, C-16 cyclopropene fatty acid or methylenehexadecenoic acid; MTA, C-14 cyclopropene fatty acid or methylenetetradecenoic acid; SIM, selected ion monitoring. Compounds are abbreviated: 16:Me, methyl hexadecanoate; d_3 16:Me, methyl (16,16,16-$^{2}H_3$) hexadecanoate; 11-MHA:Me, methyl 11,12-methylenehexadec-11-enoate; $d_4$11-MHA:Me, methyl (2,2,3,3-$^{2}H_4$) 11,12-methylenehexadec-11-enoate; 12-MHA:Me, methyl 12,13-methylenehexadec-12-enoate; 9-MTA:Me, methyl 9,10-methylene tetradec-9-enoate; 10-MTA:Me, methyl 10,11-methylenetetradec-10-enoate; d_5Z9,E11-14:Me, methyl (13,13,14,14,14-$^{2}H_5$) (Z,E)-9, 11-tetradecadienoate; $d_3$14:Me, methyl (14,14,14-$^{2}H_3$) tetradecanoate; d_5E11-14:Me, methyl (13,13,14,14,14-$^{2}H_5$) (E)-11 tetradecanoate; 16:Acid, hexadecanoic acid; $d_3$16:Acid, (16,16,16-$^{2}H_3$) hexadecanoic acid; $d_7$16:Acid, (2,2,3,3,16,16,16-$^{2}H_7$) hexadecanoic acid; 11-MHA, 11,12-methylenehexadec-11-enoic acid; $d_4$11-MHA, (2,2,3,3-$^{2}H_4$) 11,12-methylenehexadec-11-enoic acid; 12-MHA, 12,13-methylene hexadec-12-enoic acid; 10-MTA, 10,11- methylenetetradec-10-enoic acid; 14:Acid, tetradecanoic acid; $d_3$14:Acid, (14,14,14-$^{2}H_3$) tetradecanoic acid; E11-14:Acid, (*E*)-11 tetradecenoic acid; d_5E11-14:Acid, (13,13,14,14,14-$^{2}H_5$) (*E*)-11 tetradecenoic acid.

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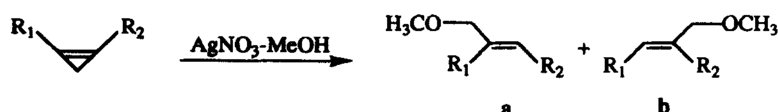

	$M^{+\cdot}$	$M^{+\cdot}$- 31	$M^{+\cdot}$- R_2
10-MTA: R_1=C_3H_7, R_2=$(CH_2)_8COOMe$	284	251	127
9-MTA: R_1=C_4H_9, R_2=$(CH_2)_7COOMe$	284	251	113
12-MHA: R_1=C_3H_7, R_2=$(CH_2)_{10}COOMe$	312	281	127
11-MHA: R_1=C_4H_9, R_2=$(CH_2)_9COOMe$	312	281	113
$d_4$11-MHA: R_1=C_4H_9, R_2=$(CH_2)_7(CD_2)_2COOMe$	316	285	113

Fig. 1. Structure of the allyl ethers resulting from treatment of methyl esters of cyclopropene fatty acids with $AgNO_3$-MeOH. Two different regioisomers are to be expected when $R_1 \neq R_2$. The mass spectra of regioisomers **a**, with a GC longer retention time, have been used throughout this study for structural characterization of MHAs and MTAs. The characteristic ions formed from each compound are also shown.

In previous articles (6, 7) we reported on the inhibitory effect of several C-16 cyclopropene fatty acids (MHAs, see **Fig. 1**) with the ring at positions 10,11 (10-MHA), 11,12 (11-MHA), or 12,13 (12-MHA) on both the (*Z*)-11 desaturase of 16:Acid and the (*Z*)-9 desaturase of E11–14:Acid. On the basis of Fogerty et al. (3) structure–activity relationship studies, the latter effect of the above 11,12-methylenehexadec-11-enoic acid (11-MHA) and 12,13-methylenehexadec-12-enoic acid (12-MHA) was unexpected, as they did not fulfill one of the critical structural requirements to inhibit the (*Z*)-9 desaturase enzyme, namely neither C-9 nor C-10, which are the positions desaturated in E11–14:Acid, were included in the cyclopropene ring. This requirement would be present in the corresponding C-14 cyclopropene fatty acids (MTAs, see Fig. 1) that would arise from chain-shortening of those MHAs. Therefore, we suggested that both 11-MHA and 12-MHA could be β-oxidized in the insect pheromone gland to give the corresponding MTAs, which would be the real active compounds on the (*Z*)-9 desaturase enzyme. In agreement with this assumption, synthetic 10,11-methylenetetradec-10-enoic acid (10-MTA), resulting from a putative β-oxidation of 12-MHA in the gland, inhibited the above (*Z*)-9 desaturation reaction (8). In this article we demonstrate that β-oxidation of the above mentioned MHAs does occur in the pheromone gland. However, we present additional evidence that both 11-MHA and 12-MHA can also inhibit the (*Z*)-9 desaturation of E11–14:Acid by themselves.

MATERIALS AND METHODS

Insects

S. littoralis were reared in our laboratory under a 16-h light/8-h dark photoregime at 25 ± 2°C. Larvae were fed an artificial diet (9). One week before adult emergence, pupae were sexed and transferred to a reverse photoperiod chamber. Only 1- to 2-day-old virgin females were used in the experiments.

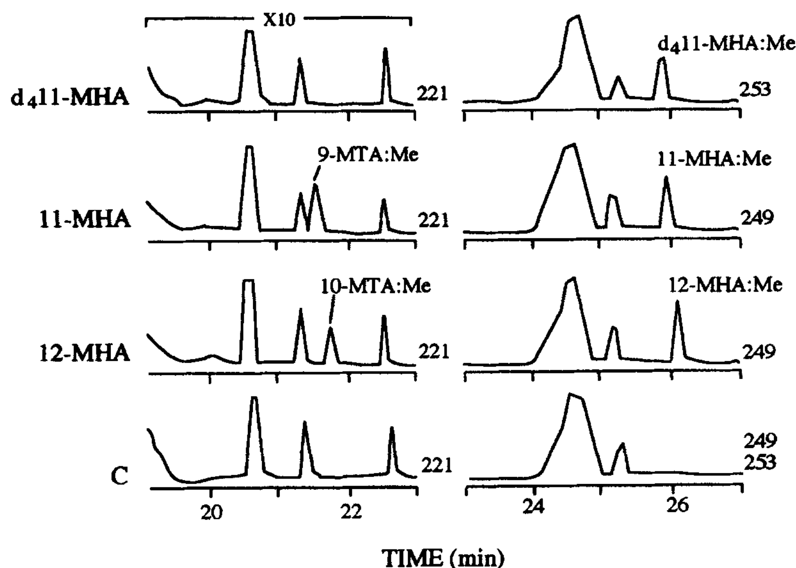

Fig. 2. GC–MS traces from methanolyzed *S. littoralis* sex pheromone glands after treatment with several MHAs. FAME from control (C) glands and glands treated with 12-MHA, 11-MHA, and $d_4$11-MHA were analyzed in each experiment for the presence of the corresponding MTA, arising from putative β-oxidation of each precursor. GC retention times of synthetic standards were: 10-MTA:Me, 21.73; $d_4$11-MHA:Me, 25.85; 11-MHA:Me, 25.92; and 12-MHA:Me, 26.12 min. The ions monitored in each analysis are given next to the traces and correspond to: 249 (12-MHA:Me and 11-MHA:Me), 253 ($d_4$11-MHA:Me), and 221 (10-MTA:Me and 9-MTA:Me). Analyses were carried out with groups of three glands.

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Chemicals

Dimethylsulfoxide (DMSO) was obtained from Sigma (St. Louis, MO) and (16,16,16-$^2H_3$)hexadecanoic acid ($d_3$16:Acid) was from IC Chemikalien (Munich, Germany). Cyclopropene fatty acids 11-MHA, (2,2,3,3-$^2H_4$)11,12-methylenehexadec-11-enoic acid ($d_4$11-MHA), 12-MHA, 10-MTA (8), and labeled compounds (13,13,14,14,14-$^2H_5$)(*E*)-11-tetradecenoic acid (d_5E11-14:Acid) (5) and (2,2,3,3,16,16,16-$^2H_7$)hexadecanoic acid ($d_7$16:Acid) (10) were synthesized in our laboratories.

Treatments

Inhibition of (Z)-9 desaturation of E11-14:Acid. These bioassays were performed as described previously (7), using 10^{-4} to 1 μg of inhibitor dissolved in 0.1 μL of DMSO. Controls received DMSO only. After 30 min of incubation, a DMSO solution of d_5E11-14:Acid (0.1 μL, 10 μg/μL) was topically applied and glands were dissected 2 h later. Individual glands were extracted and processed as described below.

Chain-shortening of MHAs and $d_7$16:Acid. The same procedure described above was followed. Insects were treated with 1 μg of the cyclopropene fatty acid (12-MHA, 11-MHA or $d_4$11-MHA) or 4 μg of $d_3$16:Acid or $d_7$16:Acid. Controls received 0.1 μL of DMSO. Glands were excised after 2.5 h of incubation and processed for fatty acid methyl ester (FAME) analysis.

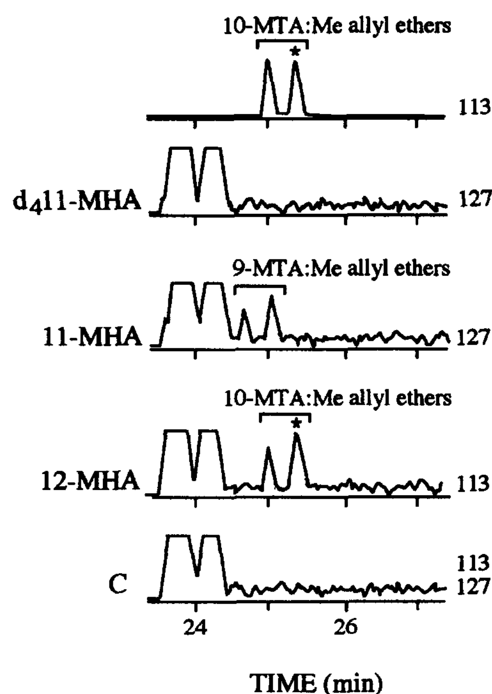

Fig. 3. GC–MS traces of methanolyzed and further $AgNO_3$-MeOH derivatized *S. littoralis* sex pheromone gland lipidic extracts after treatment with several MHAs. FAME from control (C) glands and glands treated with 12-MHA, 11-MHA, and $d_4$11-MHA were analyzed in each experiment for the presence of the allyl ethers derived from the corresponding MTAs. The top trace corresponds to a synthetic sample of 10-MTA:Me after reaction with $AgNO_3$-MeOH. The selected ions monitored are given next to the traces and correspond to: 127, 9-MTA:Me allyl ethers and 113, 10-MTA:Me allyl ethers. Mass spectra of peaks marked with an asterisk are given in Fig. 4. Analyses were performed with groups of five glands.

Preparation of fatty acid methyl esters

Glands were extracted with chloroform–methanol 2:1 and further submitted to base methanolysis as described elsewhere (11). In some analyses, methyl tridecanoate (10 ng/gland) was included in the hexane for extraction to allow quantification. Extracts were stored at -20°C until analysis.

Derivatization of CPFA with $AgNO_3$-MeOH

The procedure of Schneider, Loke, and Hopkins (12) was followed. Methyl esters of the cyclopropene fatty acids (100 μg) were reacted with 200 μL of a saturated solution of $AgNO_3$ in anhydrous MeOH for 2 h at 30°C. After this time, water (500 μL) was added and the solution was extracted with hexane (3 × 200 μL). The organic layer was dried over anhydrous $MgSO_4$, filtered with a plug of cotton, and concentrated to a suitable volume for gas chromatography coupled to mass spectrometry (GC–MS) analysis. For derivatization of natural extracts, the FAME solutions obtained by base methanolysis of the lipidic extracts were concentrated to dryness and submitted to the same treatment.

Analytical methods

Analyses were carried out by GC–MS, using a Fisons gas chromatrograph (8000 series) coupled to a Fisons MD800 mass selective detector. A Hewlett-Packard (HP-1) fused silica capillary column (30 m × 0.20 mm) programmed from 80°C to 250°C at 5°C/min was used. Helium was used as carrier gas at a pressure of 15 Psi. Source temperature was 200°C and injector temperature was 250°C. The electron impact mode was used under selected ion monitoring (SIM) or SCAN conditions. Molecular ions ($M^{\bullet +}$) were selected for all the methyl esters, except for those of cyclopropene fatty acids, in which the ion $M^{\bullet +}$-31 was monitored. For allyl ethers, the ions monitored were $M^{\bullet +}$, $M^{\bullet +}$-31, and the ion arising from loss of R_2 from the molecular ion (see Fig. 1).

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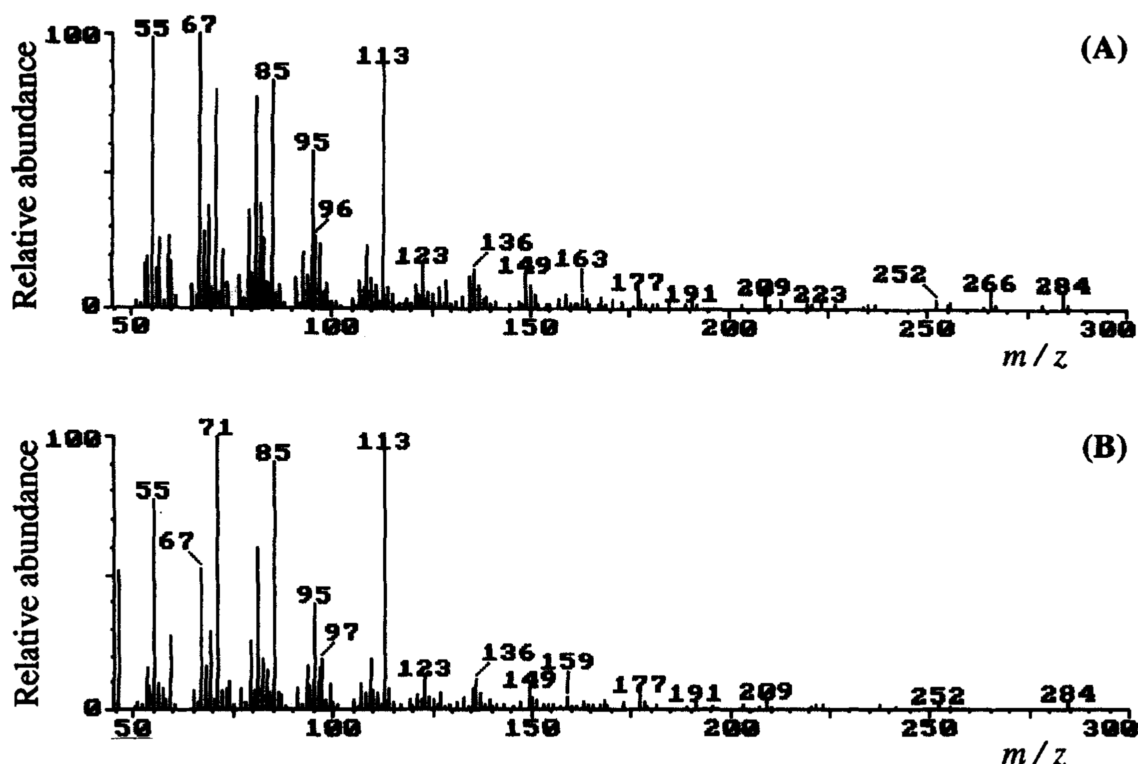

Fig. 4. Mass spectra of allyl ethers formed from natural and synthetic 10-MTA:Me. The mass spectra correspond to the peaks labeled with an asterisk in Fig. 3: (A), natural 10-MTA:Me allyl ether; (B), synthetic 10-MTA:Me allyl ether. The analysis was performed by GC–MS under SCAN mode, with a pool of 10 glands.

RESULTS

Chain shortening of MHAs and $d_7$16:Acid

As shown in **Fig. 2**, GC–MS traces of methanolyzed extracts from pheromone glands previously treated with 12-MHA exhibited two peaks, which were absent in the control run, at *m/z* 249 and 221, with retention times identical to those of synthetic samples of methyl 12,13-methylenehexadec-12-enoate (12-MHA:Me) and methyl 10,11-methylenetetradec-10-enoate (10-MTA:Me), respectively. Likewise, when glands were incubated with 11-MHA, GC–MS traces of methanolyzed extracts showed also peaks at *m/z* 249 and 221, which on the basis of their retention times were assigned to methyl 11,12-methylenehexadec-11-enoate (11-MHA:Me) and methyl 9,10-methylenetetradec-9-enoate (9-MTA:Me). In another group of experiments, glands were incubated with $d_4$11-MHA. In the analysis of the resulting FAME extracts, (2,2,3,3-$^2H_4$)methyl 11,12-methylenehexadec-11-enoate ($d_4$11-MHA:Me) was present when monitoring the ion at *m/z* 253. However, the peak corresponding to 9-MTA:Me, which was observed in extracts from glands treated with 11-MHA, was absent in the chromatograms obtained when monitoring the ion at *m/z* 221. In the experiments with 12-MHA, quantitative analyses indicated that the amounts of 12-MHA and 10-MTA recovered were, respectively, 25.6 ± 7.0 and 3.6 ± 1.1 ng per female (mean ± SE, n = 5). Likewise, 23 ± 9.2 and 3.2 ± 2.1 ng per female of 11-MHA and 9-MTA were found in the treatments with 11-MHA.

To unambiguously confirm the structures of the above resulting MTAs, pheromone glands were incubated with either 12-MHA, 11-MHA or $d_4$11-MHA and the resulting FAME extracts were treated with $AgNO_3$-MeOH before GC–MS analyses. Kircher (13) reported that cyclopropenes react with $AgNO_3$-MeOH to form stable allyl ethers, whose mass spectra exhibit fragmentation patterns that are very useful for the structural characterization of the parent CPFA (14). Thus, besides ions at $M^{\bullet+}$ and $M^{\bullet+}$-31, which allowed us to ascertain the molecular weight of the parent cyclopropene, the ion at $M^{\bullet+}$-R_2 (see Fig. 1) indicates the cyclopropene ring position in the chain. When this procedure was first applied to analytical samples of standard 11-MHA, 12-MHA, and 10-MTA, the mass spectra of the derived allyl ethers exhibited all the diagnostic ions expected in all cases (see Fig. 1). In the light of these results, pheromone

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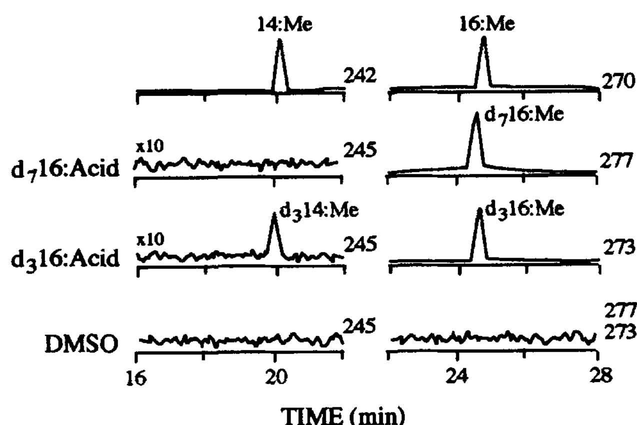

Fig. 5. Chain-shortening of labeled palmitic acids in *S. littoralis* pheromone glands. FAME from glands incubated with d3 16:Acid, d7 16:Acid, and DMSO were analyzed in each experiment for the presence of d3 14:Me. The top trace corresponds to natural 14:Me and 16:Me. The selected ions monitored in the analyses are given near to the traces and are: 270 (16:Me); 273 (d3 16:Me); 277 (d7 16:Me); 242 (14:Me); and 245 (d3 14:Me).

glands were incubated with either 12-MHA or 11-MHA and the resulting FAME extracts were treated with $AgNO_3$-MeOH and analyzed by GC–MS, under both SIM and SCAN modes. As shown in **Fig. 3,** the chromatograms obtained under SIM mode by monitoring the diagnostic ion at *m/z* 113 in a derivatized extract from glands treated with 12-MHA exhibited two peaks, which were identified as the allyl ethers derived from 10-MTA:Me, as they eluted at the same retention time as those present in the chromatrograms corresponding to standard 10-MTA:Me after derivatization. Additionally, an extract corresponding to a pool of 10 glands treated with 12-MHA was analyzed by GC–MS under SCAN mode. As shown in **Fig. 4,** the mass spectrum of the natural derivative was identical to that of an authentic standard sample. A similar result was obtained when glands were incubated with 11-MHA. Also in this case, two peaks, corresponding to the allyl ethers from 9-MTA:Me, were observed when monitoring the diagnostic ion at *m/z* 127 (Fig. 3). Both the order of elution and the difference in retention times existing between the allyl ethers from both standard 10-MTA:Me and 9-MTA:Me were similar to those between the allyl ethers from 11-MHA:Me and 12-MHA:Me. In other experiments, glands were treated with d4 11-MHA. In this case, analysis of $AgNO_3$-MeOH derivatized FAME extracts revealed that the two allyl ethers derived from 9-MTA:Me, which were observed in extracts from glands treated with 11-MHA, were absent in the chromatrogram obtained when monitoring the ion at *m/z* 127 (Fig. 3).

Previous to the desaturase inhibition experiments with d4 11-MHA, we secured that chain shortening of fatty acids was precluded in our model system by tetradeuteration at C-2 and C-3 of the aliphatic chain. Groups of glands were incubated with d3 16:Acid and d7 16:Acid and the corresponding FAME solutions were analyzed by GC–MS (**Fig. 5**). When the ion at *m/z* 245 was monitored, the peak corresponding to (14,14,14-$^2H_3$)methyl tetradecanoate (d3 14:Me) was present in extracts from glands treated with d3 16:Acid; however, this peak was absent in extracts from glands that had been incubated with d7 16:Acid in simultaneous experiments.

Desaturase inhibition

Pheromone glands of *S. littoralis* that had been incubated with either 11-MHA or d4 11-MHA exhibited a reduced ability to desaturate d5 E11-14:Acid to (*Z,E*)-9,11-tetradecadienoic acid. In these bioassays, the ratios between the abundances of ions 243 and 245, corresponding to methyl (13,13,14,14,14-$^2H_5$)(*Z,E*)-9,11-tetradecadienoate (d5 Z9,E11-14:Me) and methyl (13,13,14,14,14-$^2H_5$)(*E*)-11 tetradecenoate (d5 E11-14:Me), respectively, were calculated from the GC–MS analyses and used to monitor this desaturation step. As shown in **Fig. 6,** the dose-effect curves obtained followed similar patterns in both 11-MHA and d4 11-MHA and no significant differences were found at any dose between the two inhibitors (unpaired, two tailed *t* test; $P > 0.05$).

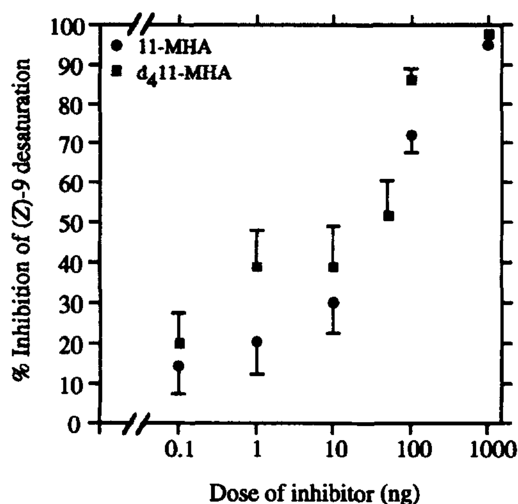

Fig. 6. Effect of different doses of 11-MHA and d4 11-MHA on (*Z*)-9 desaturation of d5 E11-14:Acid. Determinations were performed as indicated in Materials and Methods. Abcissa, the amount of inhibitor administered. Ordinate, percentage of inhibition. The ratios between the abundances of ions at *m/z* 243 (d5 Z9,E11–14:Me) and *m/z* 245 (d5 E11–14:Me) were determined in controls (C) and treatments (T) from the GC–MS traces and the percentage of inhibition was calculated as 100-(T/C × 100). Each point represents mean ± SE of 8–12 determinations.

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DISCUSSION

The first objective of this work was to experimentally prove that chain-shortening of MHAs to the corresponding MTAs occurred in the pheromone gland. Although cyclopropenes are thermally unstable, they can be directly analyzed by GC under special conditions (15), in which decomposition is negligible and quantification can be reliably performed. Thus, we first undertook the monitorization of MHAs β-oxidation in vivo using GC–MS as analytical tool. The results obtained in these studies indicated that β-oxidation of both 11-MHA and 12-MHA to the corresponding 9-MTA and 10-MTA, respectively, does occur in the pheromone gland. Furthermore, to obtain structural information about the formed MTAs, we carried out a series of experiments in which GC–MS analysis of the C-14 CPFA present in the extracts was performed after derivatization with $AgNO_3$-MeOH. In these experiments, the unambiguous identification of the expected MTA in each case confirmed that both 12-MHA and 11-MHA are chain-shortened in the pheromone gland.

However, quantification of the β-oxidation process by direct GC–MS analysis indicated that only a small part, ca. 10%, of the administered MHA was chain-shortened to the corresponding C-14 derivative. In contrast, the activity of 11-MHA as inhibitor of the (*Z*)-9 desaturation reaction was similar to that of 10-MTA previously reported (8). One possible explanation is that only the fraction of CPFA bound through the carboxylate group, which is cleavable under the base methanolysis conditions, was considered in the quantification experiments. However, the MHAs administered might also have reacted with nucleophilic groups through the highly reactive cyclopropene ring, giving rise to derivatives stable to basic conditions. This fraction was not taken into account in the quantification analyses and a higher percentage of β-oxidation might actually have occurred. To clarify this point, an analog of 11-MHA stable towards β-oxidation was synthesized and bioassayed, anticipating that if the effect of the MHAs on the Z9 desaturation reaction is actually caused by their β-oxidation derivatives, the stable analog would not inhibit this transformation. According to Pohl, Raichle, and Ghisla (16), β-oxidation of α,β-tetradeuterated fatty acids occurs at a very low rate in mammalian systems, because of a very high primary isotope effect on the acylCoA dehydrogenase enzyme. Thus, we expected that introduction of deuterium atoms at C-2 and C-3 of 11-MHA would greatly increase its stability towards β-oxidation. To assess the efficacy of this modification in our model system, and given the difficulty encountered in the synthesis of cyclopropenes, we carried out a series of experiments with $d_7$16:Acid, whose preparation in the laboratory was less arduous. Furthermore, the absence of reactive sites other than the carboxylate group in $d_7$16:Acid would allow a reliable assessment of the β-oxidation process. In the experiments with this model tracer we confirmed that whereas $d_3$16:Acid was normally β-oxidized to the corresponding (14,14,14-$^2H_3$)tetradecanoic acid ($d_3$14:Acid), $d_7$16:Acid did not give rise to the chain-shortened derivative under the same experimental conditions.

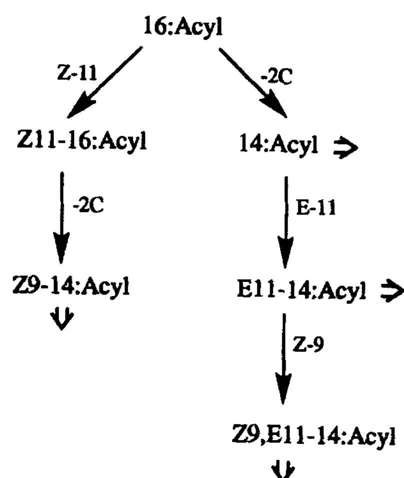

Fig. 7. Biosynthetic pathway of *S. littoralis* sex pheromone blend. The reactions involved are: (*Z*)-11 desaturation (Z11); (*E*)-11 desaturation (E11); (*Z*)-9 desaturation (Z9), and β-oxidation (-2C). Open arrows indicate reduction and acetylation.

In the light of this result, indicating that deuteration at C-2 and C-3 was a suitable approach to increase stability of 11-MHA towards β-oxidation, $d_4$11-MHA was synthesized and glands were incubated with this putative inhibitor. GC–MS analyses of FAME extracts confirmed that β-oxidation of $d_4$11-MHA after the total incubation time of the inhibition bioassays was negligible.

However, as indicated in the above results section, both 11-MHA and $d_4$11-MHA had similar potencies as inhibitors of the (*Z*)-9 desaturase, thereby indicating that the effect of either 11-MHA or 12-MHA on the (*Z*)-9 desaturation of E11–14:Acid is not caused, at least exclusively, by the corresponding MTA arising from their chain-shortening.

In the biosynthetic pathway of *S. littoralis* sex pheromone (**Fig. 7**), (*Z*)-9-tetradecenoic acid (Z9–14:Acid) is exclusively biosynthesized by β-oxidation of (*Z*)-11-hexadecenoic acid (Z11–16:Acid), whereas no Z9–14:Acid is formed from tetradecanoic acid (14:Acid) (5). These results indicate that the presence of a double bond at C-11 is required for the (*Z*)-9 desaturase enzyme action. As 12-MHA, 11-MHA and $d_4$11-MHA have the cyclopropene ring at or next to this position, we hypothesize that these compounds may inhibit the (*Z*)-9 desaturase enzyme by interfering with the binding region of the enzyme with the substrate double bond. A

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similar mechanism has been recently proposed by Cao, Blond, and Bézard (17) to explain the inhibitory effect of both sterculic and malvalic acids on the delta-5 and delta-6 desaturation of fatty acids with double bonds at either C-8 or C-9. Thus, whereas the MHAs would inhibit the (*Z*)-11 desaturase of 16:Acid by reaction of the cyclopropene ring with the enzyme active center (18, 19), they would inhibit the (*Z*)-9 desaturase of E11-14:Acid by interfering with the binding site of this enzyme with the substrate double bond. Further work on this topic is being conducted in our laboratories with radiolabeled cyclopropene fatty acids to evaluate their fate after its application on the insect pheromone gland.

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