Stereospecificity of the (*E*)- and (*Z*)-11 Myristoyl CoA Desaturases in the Biosynthesis of *Spodoptera littoralis* Sex Pheromone

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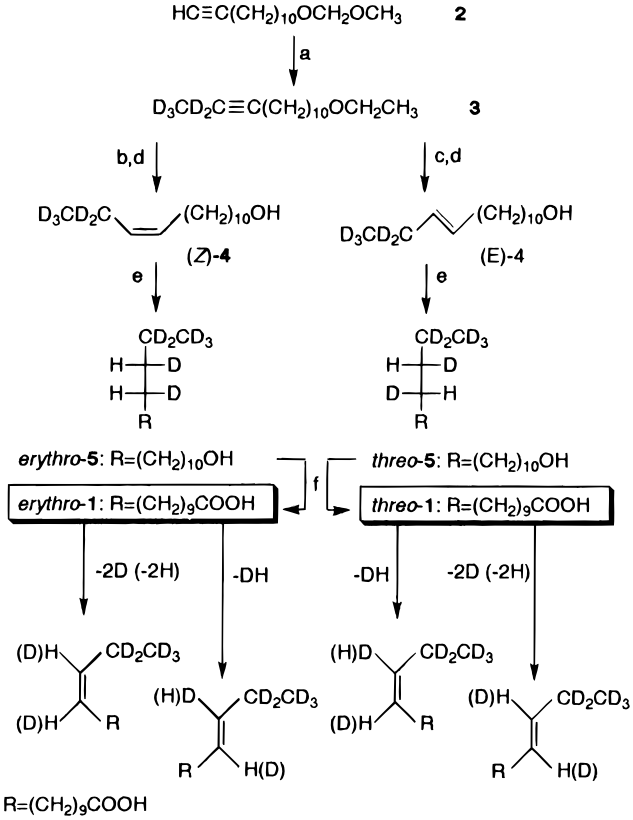
Unsaturated fatty acids are biosynthesized in nature from saturated fatty acids by the action of specific desaturases, among which (*Z*)-9 stearoyl CoA desaturase is the most ubiquitous one.[1−3] The biosynthetic pathways of lepidopteran sex pheromone blends involve the action of rather unusual desaturases, such as (*Z*)-11,[4] (*Z*)-10,[5] (*Z*)-13,[6] and (*Z*)-14[7] acyl CoA desaturases. Additionally, certain moth pheromone gland desaturases give rise to (*E*) fatty acids, also involved in the biosynthesis of the pheromone blend,[4] thus making these tissues excellent models to study these unique enzymes. The main body of literature concerning the biosynthetic pathways of (*E*) fatty acids deal with bacteria. In these prokaryotic cells, (*E*) unsaturated fatty acids are biosynthesized either by anaerobic pathways[8,9] or by isomerization of the corresponding (*Z*) compounds.[10] In moth pheromone glands, however, (*E*) fatty acids are biosynthesized by direct desaturation.[11] Our ongoing interest in desaturase enzymes involved in insect sex pheromone biosynthesis has led us to study the stereospecificity of the (*Z*)- and (*E*)-11 desaturases of myristic acid,[13] implied in the biosynthesis of *Spodoptera littoralis* sex pheromone.[14]

To carry out this research, we performed experiments with two different sets of probes: *erythro*- and *threo*-(11,12,13,13,14,14,14-$^2H_7$)myristic acids (*erythro*- and *threo*-**1**) and (12*R*)- and (12*S*)-(2,2,3,3,12-$^2H_5$)myristic acids ((12*R*)- and (12*S*)-**1**). The first two probes, *erythro*- and *threo*-**1**, would reveal the relative stereochemistry of hydrogens removed at C11 and C12, whereas enantiomerically pure (12*R*)- and (12*S*)-**1** would allow for assessing the stereospecificity of hydrogen removal at C12. Both *erythro*- and *threo*-**1** were synthesized from alkyne **2** by way of well-established reactions (Scheme 1). Deuteration of double bonds was carried out using the Wilkinson catalyst to ensure stereospecificity and to prevent deuterium scrambling.[15,16]

Scheme 1[a]



[a] Reagents: a, MeLi/THF, then ICD_2CD_3/HMPA (99%); b, H_2/Pd-$BaSO_4$/quinoline (96%); c, Na/NH_3 (86%); d, HCl/MeOH ((*Z*)-isomer, 83%; (*E*)-isomer, 74%); e, D_2/$RhCl(PPh_3)_3$/C_6H_6 (*erythro*-isomer, 94%; *threo*-isomer, 80%); f, CrO_3/H_2SO_4/H_2O (*erythro*-isomer, 96%; *threo*-isomer, 80%);

On the other hand, (12*R*)- and (12*S*)-**1** were prepared from alkyne **6** as shown in Scheme 2. Deuterium atoms at α and β positions of ester **8** were introduced by reaction of acetylenic ester **7** with magnesium in MeOD,[17] and the stereogenic center at C12 was generated by reaction of aldehyde **10** with the system Et_2Zn/Ti(O-*i*-Pr)$_4$/1,2-*N*,*N*′-bis(trifluoromethylsulfonylamino)-cyclohexane.[18] In this reaction, the use of the (1*R*,2*R*)-sulfonamide afforded the hydroxyester (12*S*)-**11**, whereas the (1*S*,2*S*)-sulfonamide furnished (12*R*)-**11**.[19] Reduction of tosyl-derivatives of (*R*)- and (*S*)-**11** with lithium aluminium deuteride followed by Jones oxidation gave the expected probes (12*R*)- and (12*S*)-**1**, respectively.[16] Probes were topically administered to the pheromone gland as dimethyl sulfoxide solutions (0.1 μL, 2.5 mg/mL).[14] A total dose of 2 μg was given in eight subsequent 1-h incubations with 0.25 μg. Fatty acid methyl esters were obtained by base methanolysis of pheromone gland lipidic extracts,[14] and analyses were performed by GC-MS as previously reported[14] using a polar SGE BP-20 capillary column (30 m × 0.20 mm) programmed from 60 °C to 150 °C at 2 °C/min and then to 260 °C at 7 °C/min after an initial delay of 2 min. The selected ion monitoring mode was used, and ions selected were the corresponding molecular ions for all isotopomers.

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(12) Topical application of (13,13,14,14,14-$^2H_5$)-(*Z*)-11-tetradecenoic acid to *S. littoralis* pheromone glands did not show any labeled methyl (*E*)-11-tetradecenoate in base methanolyzed pheromone gland lipidic extracts analyzed by gas chromatography coupled to mass spectrometry under selected ion monitoring mode.
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(19) Enantiomeric purity was >95% in both enantiomers, as determined by ^{19}F NMR analysis of the corresponding Mosher esters. The absolute configuration of hydroxyesters **11** is inferred from the known mechanism of the addition reaction of Et_2Zn to aldehydes in the presence of titanium complexes of chiral disulfonamides.[18]

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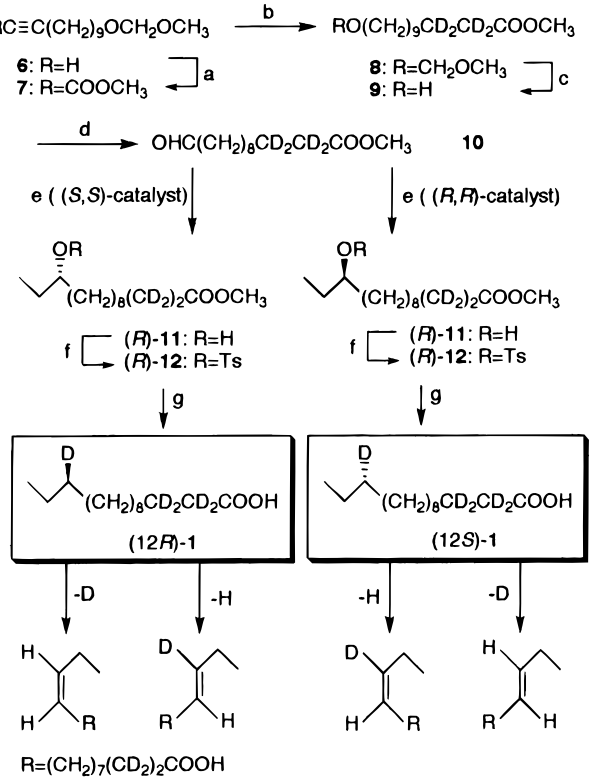
Scheme 2[a]



[a] Reagents: a, MeLi/THF, then ClCOOMe/THF (88%); b, Mg/MeOD (73%), see ref. 17; c, HCl/MeOH (83%); d, $(CO)_2Cl_2$/DMSO/Et_3N (75%); e, Et_2Zn/Ti(O-*i*-Pr)$_4$/1,2-*N*,*N*′-bis(trifluoromethylsulfonylamino)cyclohexane/toluene ((12*S*)-**11**, 66%; (12*R*)-**11**, 89%), see ref 18; f, TsCl/pyridine ((12*R*)-**12**, 72%; (12*S*)-**12**, 70%); g, $LiAlD_4/Et_2O$, then $CrO_3/H_2SO_4/H_2O$ ((12*R*)-**1**, 38%; (12*S*)-**1**, 43%).

Table 1. Relative Stereochemistry of Hydrogens Removed at C11 and C12 in Δ11 Desaturations of Myristic Acid

		isotopomer (mol %)[a]		
tracer	product[b]	d_5	d_6	d_7
erythro-**1**	E	6.1 ± 3.1	85.9 ± 16.3	8.0 ± 13.8
	Z	29.0 ± 1.9	3.4 ± 3.0	67.6 ± 1.9
threo-**1**	E	29.0 ± 3.0	5.8 ± 6.0	65.2 ± 3.4
	Z	0.5 ± 0.5	96.2 ± 3.3	3.3 ± 2.3

[a] Percentages of isotopomers correspond to the average ± standard deviation of three independent experiments performed with groups of four glands. Percentages were corrected for the abundances of $M^{\bullet+}$ + 1 and $M^{\bullet+}$ − 1 ions in the substrate probes. [b] Products refer to E, (*E*)-11-tetradecenoic acid and Z, (*Z*)-11-tetradecenoic acid, analyzed as methyl esters.

Table 2. Absolute Stereochemistry of Hydrogen Removed at C12 in Δ11 Desaturations of Myristic Acid

		isotopomer (mol %)[a]	
tracer	product[b]	d_4	d_5
(12*R*)-**1**	E	9.5 ± 7.0	90.5 ± 7.0
	Z	91.7 ± 6.2	8.2 ± 6.2
(12*S*)-**1**	E	91.7 ± 4.0	8.3 ± 4.0
	Z	12.0 ± 5.2	88.0 ± 5.2

[a] Percentages of isotopomers correspond to the average ± standard deviation of three independent experiments performed with groups of four glands. Percentages were corrected for the abundances of $M^{\bullet+}$ + 1 and $M^{\bullet+}$ − 1 ions in the substrate probes. [b] Products refer to E, (*E*)-11-tetradecenoic acid and Z, (*Z*)-11-tetradecenoic acid, analyzed as methyl esters.

The results obtained in the deuterium-labeling experiments are summarized in Tables 1 and 2. In these experiments, the stereochemistry of hydrogens removed at both C11 and C12 was deduced from the mass of the most abundant isotopomer of both (*Z*)- and (*E*)-11-tetradecenoic acid resulting from each probe, which indicated the number of deuterium atoms present in the unsaturated compounds and thus the number of deuterium atoms that had been lost from each substrate to form each isomer. The data obtained with *erythro*- and *threo*-**1** clearly showed that formation of (*E*)-11-tetradecenoic acid occurs by elimination of C11-H and C12-H of opposite stereochemistry, whereas (*Z*)-11-tetradecenoic acid is formed by removal of C11-H and C12-H of the same stereochemistry. In these experiments, *erythro*-**1** gave rise to two isotopomers, d_5 and d_7, of (*Z*)-11-tetradecenoic acid, arising from loss of either two deuterium or two hydrogen atoms from C11 and C12, respectively. The same result was obtained in the formation of (*E*)-11-tetradecenoic acid from *threo*-**1**. However, in both cases, the relative abundances of the d_5 isotopomers were lower than those of the d_7, probably due to the existence of kinetic isotope effects.[20] On the other hand, experiments with the enantiomerically pure substrates (12*R*)- and (12*S*)-**1** showed that in formation of the (*E*)-isomer the *pro*-(*S*) C12-H is specifically removed, whereas specific cleavage of the *pro*-(*R*) C12-H takes place to form the (*Z*)-isomer. In summary, the overall results demonstrate that formation of (*E*)-11-tetradecenoic acid takes place by stereospecific removal of the *pro*-(*R*) C11-H and the *pro*-(*S*) C12-H and desaturation to the (*Z*)-isomer occurs by stereospecific cleavage of *pro*-(*R*) C11-H and *pro*-(*R*) C12-H. Therefore, the results presented in this paper show opposite stereospecificities in removal of C12-H by both the (*E*)- and (*Z*)-11 myristoyl CoA desaturases. The results found for the (*Z*) enzyme are in agreement with those reported previously on the related (*Z*)-11 palmitoyl CoA desaturase[21] and (*Z*)-9 stearoyl CoA desaturase,[3,22,23] which specifically remove the *pro*-(*R*) hydrogen atoms at C11 and C12, and suggest a common steric course for all (*Z*) desaturase enzymes. On the other hand, the results reported in this Communication represent the first determination of the stereospecificity of a (*E*) fatty acyl desaturase in nature. Additional stereochemical studies on other (*E*) desaturase enzymes will establish if the steric course found here for the (*E*)-11 myristoyl CoA desaturase is common to all (*E*) desaturase enzymes. Biological desaturation of fatty acids to the corresponding (*Z*)-isomers is a *syn*-elimination reaction.[24] Assuming that desaturation to the (*E*)-isomers is also a *syn*-elimination process and that both (*Z*)- and (*E*)-desaturases are structurally related enzymes, the geometry of the resulting double bond would result from the different conformation adopted by the acyl substrate at the enzyme active site, which might be influenced by different amino acid sequence alignments in the enzyme active center of both (*Z*)- and (*E*)-enzymes.[25] Further work along these lines is in progress in our laboratories.

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Supporting Information Available: Experimental procedures, ^1H and ^{13}C NMR, and IR spectroscopic data for the products (9 pages). See any current masthead page for ordering and Internet access instructions.

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