Sex Pheromone Biosynthesis in *Mamestra brassicae* L. (Lepidoptera: Noctuidae); Stereochemistry of the Δ11 Desaturation

Wilhelm Boland,*[a] Christian Frössl,[a] Michael Schöttler[a] and Miklós Tóth[b]

[a] *Institut für Organische Chemie der Universität Karlsruhe, Richard-Willstätter-Allee 2, D-76131 Karlsruhe, Germany*
[b] *Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, POB 102, Hungary*

(11S,12R)- and (11R,12S)-[^{2}H$_{14}$]palmitic acids 1 are converted in the pheromone gland of the moth *Mamestra brassicae* into the [^{2}H$_{11}$]- or [^{2}H$_{13}$]-hexadec-11-enyl acetates 2 by a formal *syn*-elimination of the C(11)-H$_R$ and C(12)-H$_R$ hydrogen atoms; this characterises the unique Δ11 desaturase from insects as closely related to the Δ9 desaturases from mammals, plants and bacteria.

The majority of the lepidopteran pheromones are monounsaturated acetate esters, alcohols or aldehydes with unbranched carbon backbones between 10 and 16 carbon atoms in length.[1] From biosynthetic studies using radio-labelled precursors or stable isotope techniques a unifying pattern has emerged according to which the chain length and the position of the double bond(s) is the result of two major enzymatic activities. There exist different terminal desaturases acting on CoA esters of saturated fatty acids which in combination with a highly selective chain shortening mechanism (β-oxidation) at various stages of the pheromone biosynthesis are responsible for the structural diversity within this class of compounds which is known today.[2] A unique Δ11 desaturase is involved in the biosynthesis of most pheromones, but Δ9 (ref. 3) and Δ10 (ref. 4) desaturases are also known. As outlined in Scheme 1, (*Z*)-hexadec-11-enyl acetate 2 (*Z*11-16 : OAc), the sex pheromone of *Mamestra brassicae*, is directly derived from palmitic acid by removal of two hydrogen atoms from the methylene groups at C(11) and C(12) followed by reduction and acetylation.[5,6] In contrast, the carbon chain of (*Z*)-tetradec-11-enyl acetate 3 (*Z*11-14 : OAc) in glands of the red-banded leaf roller *Argyrotaenia velutiana* arises from palmitic acid only after one β-oxidation cycle prior to the Δ11 desaturation and functional group manipulations[7] (Scheme 1).

While the mechanism(s) of the iron-containing, electron-transport-chain-linked, Δ9 desaturases from mammals, plants, bacteria or algae are well established,[8] only little is known about the Δ11 desaturases from lepidopterans.[9] In fact, the reaction appears to be similar to that observed from the Δ9 desaturase from mammals, except that NADH rather than NADPH is the preferred cofactor in certain insects (*e.g. Trichoplusia ni*).[10] Another promising attempt to gain more information about the Δ11 desaturase from insects could be
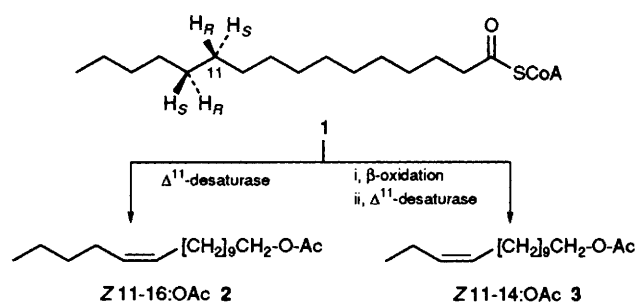
Scheme 1

seen in a stereochemical approach. The Δ^9 desaturases from animals or plants introduce a (Z)-double bond into the alkyl chain by syn-elimination of two hydrogen atoms from the vicinal carbon atoms C(9) and C(10) of the precursor acid.[8] If the Δ^{11} desaturase from insects manipulates the corresponding hydrogen atoms from the methylene groups at C(11) and C(12) in the same way, this finding could be indicative of mechanistic coincidences between both types of desaturases. According to Scheme 1, this approach requires chiral palmitic acids which are labelled at the carbon atoms C(11) and C(12), respectively. We report here that the topical administration of highly deuteriated (11S,12R)- and (11R,12S)-[2,2,3,4,5,5,6,6,7,8,9,9,11,12-^{2}H$_{14}$]palmitic acids[11] 1 (Fig. 1) to the pheromone gland of Mamestra brassicae can be successfully used to unravel the stereochemical course of a Δ^{11} desaturase from an insect. Owing to the high degree of deuterium labelling, the two acetates [^{1}H]-2 and [^{2}H$_n$]-2 exhibit base-line separation upon gas chromatographic analysis, and, hence, mass spectra of pure [^{2}H$_n$]metabolites are obtained (cf. Fig. 1).

Insects originated from a continuously maintained laboratory culture[12]. Emerging females of M. brassicae were kept at 25 °C under a regime of 18 h light–6 h dark cycles. 5–7 females (2–4 days old) were treated with CO$_2$ ca. 3 h before the end of the dark phase, when they normally start calling behaviour. The tip of the abdomen containing the pheromone gland of the anaesthetised female was everted by slightly squeezing the abdomen with the fingers, and 1 μl of a solution of (11S,12R)-[^{3}H$_{14}$]-1 or (11R,12S)-[^{2}H$_{14}$]-1 in dimethyl sulfoxide (DMSO) (0.1 mg ml^{-1}) was applied to the pheromone gland surface.[9] The females were kept for 2 h under normal rearing conditions. Then, the glands were excised and extracted for 10 min with hexane (3–5 μl/gland). Free fatty acids were converted into their methyl esters (diazomethane) prior to analysis by GC–MS. E4,Z7-13:OAc, adjusted to 10 ng μl^{-1}, was added to the combined extracts of 5–7 females as an internal standard. Although the production of natural [^{1}H]-2 is significantly reduced by the above treatment (ca. 10–50 ng [^{1}H]-2 instead of ca. 100 ng per untreated gland), there is a remarkable 10–40% de novo synthesis of deuterium-

labelled Z11-16:OAc 2 from the externally added, deuteriated palmitic acid (cf. Fig. 1, normalised to natural [^{1}H]-2).

Since the mass spectra of long chain acetates do not exhibit molecular ions under electron impact, the characteristic M$^{·+}$ −60 fragment ion (loss of CH$_3$COOH) was used for the analysis. Owing to the presence of two deuterium atoms at C(2) of both precursor acids 1, the loss of CH$_3$COOD (M$^{·+}$ −61) has to be considered in the case of deuteriated 2 derived from 1. As shown in Fig. 2 the corresponding fragment ion of the deuteriated metabolite Z11-16:OAc derived from (11S,12R)-[^{2}H$_{14}$]-1 is located at m/z 233 which is indicative of 11 deuterium atoms. The second experiment with (11R,12S)-[^{2}H$_{14}$]-1 leads to a fragment ion at m/z 235, in accord with the loss of only one deuterium atom due to the elimination of CH$_3$COOD.

The data clearly indicate that the Δ^{11} desaturase from M. brassicae removes the two vicinal hydrogen atoms from C(11) and C(12) of palmitic acid in a formal syn-fashion. A formal anti-elimination of two hydrogen atoms would result in two isotopomeric metabolites showing the fragment ions at m/z 234 due to the loss of H, D and CH$_3$COOD in both cases; this is obviously not the case (cf. Fig. 2). Instead, the isotopic pattern of the clusters of the fragment ions at m/z 233 or 235 is in perfect agreement with the isotopic labelling pattern of the individual precursors (11R,12S)- and (11S,12R)-[^{2}H$_{14}$]-1, respectively. It is therefore evident that the introduction of a cis-double bond by the Δ^{11} desaturase from M. brassicae exhibits extreme selectivity, specifically removing the C(11)-H$_R$ and C(12)-H$_R$ hydrogen atoms during double bond formation at C(11) and C(12), respectively. Interestingly, the absolute configuration of the removed hydrogen atoms is identical with that of the hydrogen atoms involved in desaturation reactions carried out by the Δ^9 desaturases from mammals, plants, bacteria or algae.[8] It remains to be clarified as to whether the other less common desaturases from insects,
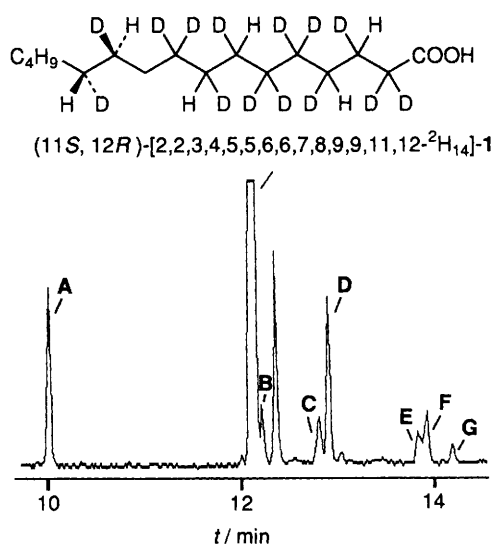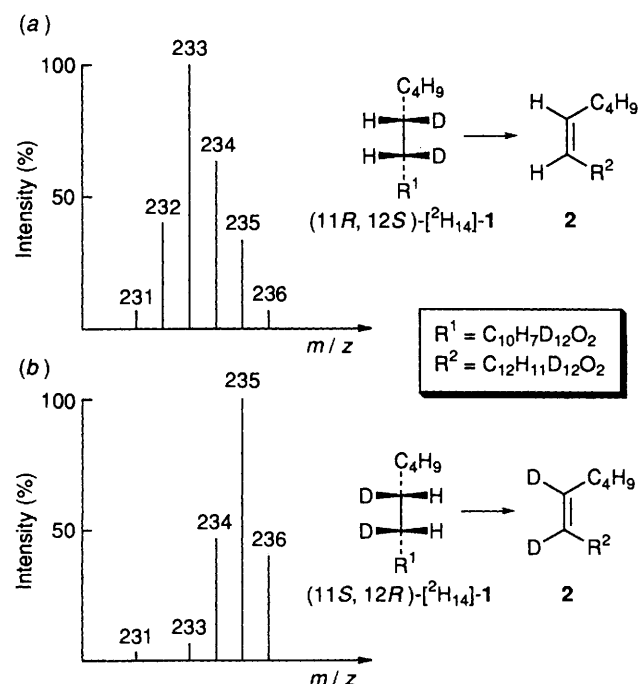

(11S, 12R)-[2,2,3,4,5,5,6,6,7,8,9,9,11,12-^{2}H$_{14}$]-1

Fig. 1 Gas chromatographic analysis of the volatile material from the pheromone gland of M. brassicae females. The sample was analysed on a 15 m × 0.25 mm fused silica column coated with SE 30 under programmed conditions: 80 °C for 4 min, then at 40 °C min^{-1} to 180 °C followed by 10 °C min^{-1} to 280 °C. Identification of the compounds: (A) internal standard (E4,Z7-13:OAc, 10 ng μl^{-1} of sample), (B) methyl [^{1}H]palmitate, (C) [^{2}H$_n$]-2, (D) [^{1}H]-2, (E) methyl [^{1}H]linoleate, (F) methyl [^{1}H]oleate, (G) methyl [^{1}H]stearate.

Fig. 2 Relative intensities of the relevant fragment ions (M$^{·+}$ −CH$_3$COOD) at m/z 233 and 235. The ion intensities are normalised to the most intense peak of each cluster. (a) Metabolite derived from administration of (11S,12R)-[^{2}H$_{14}$]-1; (b) metabolite derived from administration of (11R,12S)-[^{2}H$_{14}$]-1.

e.g. Δ^5, Δ^6, Δ^{10} and Δ^{12}, belong to the same category or exhibit different stereochemical aspects.

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