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SEX PHEROMONE BIOSYNTHESIS OF (*E,E*)-8,10-DODECADIENOL IN CODLING MOTH *Cydia pomonella* INVOLVES *E*9 DESATURATION

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Abstract—Sex pheromone biosynthesis in the codling moth *Cydia pomonella* (Lepidoptera; Tortricidae) was studied by topical application of deuterated fatty acids in DMSO to pheromone glands. The incorporation of deuterium label into fatty acids and alcohols in the pheromone gland was monitored by gas chromatography with flame ionization detection and mass spectrometry in the selected ion monitoring mode. Dodecanol, (*E*)-9-dodecenol, (*E,E*)-8,10-dodecadienol, tetradecanol, and hexadecanol were found in gland extracts. The application of [12,12,12-$^2H_3$]dodecanoic acid resulted in labeled dodecanol, (*E*)-9-dodecenol, and (*E,E*)-8,10-dodecadienol, as well as the corresponding labeled acids. No label was incorporated into tetradecanol or hexadecanol or any acid with more than 12 carbon atoms. The application of labeled tetradecanoic or hexadecanoic acid introduced label not only into the 12-carbon alcohols, but also into tetradecanol, or tetradecanol and hexadecanol, respectively. The application of (*E*)-[11,11,12,12,12,-$^2H_5$]9-dodecenoic acid, whose facile synthesis is described, resulted in labeled (*E*)-9-dodecenol and (*E,E*)-8,10-dodecadienol. The (*E,E*)-8,10-dodecadienol so produced was characterized by an ion at *m/z* 186, equivalent to $[M]^+$ of a dienol labeled with four deuterons. Thus, one deuterium label is lost when the labeled (*E*)-9-monoene is converted to the (*E,E*)-8,10-diene. We conclude that (*E,E*)-8,10-dodecadienol is synthesized by chain shortening (β-oxidation) of palmitic acid to dodecanoic acid, followed by an unusual *E*9 desaturation and subsequent conversion of this intermediate into the conjugated precursor, which is finally reduced to the pheromone alcohol. The evolutionary significance of *E*9 desaturation being responsible for pheromone production in an Olethreutinae species is discussed.

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Key Words—pheromone, Lepidoptera, Tortricidae, *Cydia pomonella*, biosynthesis, palmitic acid, (*E*)-9-dodecenoic acid, (*E,E*)-8,10-dodecadienol, *E*9 desaturation, capillary gas chromatography, deuterium, labeled precursors.

INTRODUCTION

Available information concerning pheromones in many lepidopteran families indicates that most of the pheromone components can be biosynthetically derived from hexadecanoic (palmitic) acid, by chain shortening through β-oxidation and desaturation, involving a Δ 11-desaturase (Roelofs and Bjostad, 1981, 1984). Z11 desaturation of hexadecanoic acid followed by chain shortening and subsequent reduction and acetylation can produce the whole homologous series (Z)-11-hexadecenyl, (Z)-9-tetradecenyl, (Z)-7-dodecenyl, and (Z)-5-decenyl acetate, as was shown in the turnip moth (Löfstedt et al., 1986a). If chain shortening precedes desaturation, Δ11-unsaturated 14-carbon compounds and their homologous chain shortened Δ 9-unsaturated 12-carbon compounds can be produced. Such compounds are the predominant pheromone components within the Tortricidae (Roelofs and Brown, 1982).

Interestingly, many tortricids within the Olethreutinae subfamily have Δ 8- and Δ 10-unsaturated, or Δ 8,10 doubly unsaturated pheromone components. Analysis of pheromone precursors in two primitive New Zealand tortricids supported the idea of Δ 10 desaturation being responsible for the production of Δ 8- and Δ 10-unsaturated compounds (Löfstedt and Roelofs, 1985). Roelofs and Brown (1982) suggested that the Δ 8,10 doubly unsaturated pheromone components could be produced by Δ 10 desaturation, followed by chain shortening and a new Δ 10 desaturation. Alternatively, they suggested that the production of these pheromone components could be accounted for by a Δ 11-desaturase producing 11-tetradecenoate moieties. The Δ 11-tetradecenoate could then be biosynthetically converted into a Δ 10,12 conjugated system and subsequently chain shortened to the Δ 8,10-12 carbon compounds. Such a pathway was confirmed in the silkworm moth *Bombyx mori*, which stores large amounts of (Z)-11-hexadecenoate (Z11–16:acyl) (Bjostad and Roelofs, 1984) that are converted to (*E*, Z)-10,12-hexadecenoate (*E*,Z10,12–16:acyl), the immediate fatty acid precursor of bombykol (*E*,Z)-10,12-hexadecadienol (Yamaoka et al., 1984).

The major pheromone component of the codling moth was identified as (*E*,*E*)-8,10-dodecadienol (*E*,*E*8,10–12:OH) (Roelofs et al., 1971). In addition to other geometric isomers and the corresponding aldehyde and acetate, the codling moth pheromone gland was also found to contain dodecanol (12:OH), tetradecanol (14:OH), hexadecanol (16:OH), and (*E*)-9-dodecanol (*E*9–12:OH) (Arn et al., 1985; Einhorn et al., 1984). The occurrence of *E*9–12:OH

led Arn et al. (1985) to suggest that (*E*)-9-dodecenoate (*E*9–12 : acyl) could be a monounsaturated intermediate in biosynthesis of the pheromone. In fact *E*9–12 : acyl was the only unusual monounsaturated fatty acyl moiety found by us in a preliminary precursor analysis, indicating its potential role in pheromone biosynthesis.

In an earlier study on pheromone biosynthesis in the turnip moth, we showed that deuterium-labeled fatty acids, as well as their corresponding acetates, could be separated from unlabeled analogs by gas chromatography on polyethylene glycol-type stationary phases (Löfstedt et al., 1986a. In the present paper pheromone biosynthesis in the codling moth is investigated by the same technique. Deuterium labeled fatty acids are applied topically to the pheromone gland and their incorporation into pheromone alcohols is studied.

METHODS AND MATERIALS

Insect Sources and Topical Application of Labeled Fatty Acids to Glands. Codling moths for the experiments with saturated fatty acids were obtained as pupae from Drs. H. Arn and E. Mani, Wädenswil, Switzerland. Moths for the experiment with labeled (*E*)-9-dodecanoic acid (*E*9–12 : acid) were obtained from a culture in our laboratory, initiated from eggs obtained from Dr. R. Bues, Avignon, France. The pupae and emerged insects were kept at a 16 : 8 hr light–dark cycle. Emerged insects were fed a 5% sucrose solution.

Labeled fatty acids in DMSO (4 μg in 0.2 μl) were applied topically to the pheromone glands of 2- to 4-day-old *Cydia pomonella* females as described earlier by Bjostad and Roelofs (1983) for other lepidoptera. After the application at the beginning of the dark period, the glands were held everted for 1 hr until they were dissected from the ovipositors and extracted for analysis.

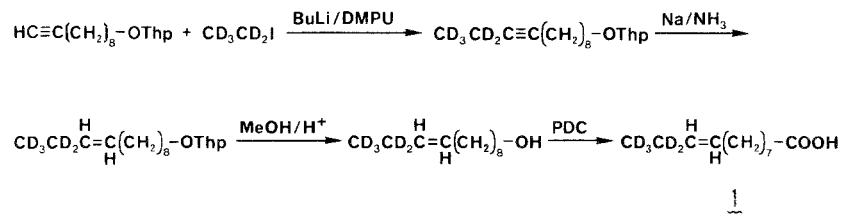
Pheromone Gland Extraction and Methylation. The pheromone glands were dissected from the ovipositors with a pair of fine forceps and then extracted with 10 μl of redistilled hexane for analysis of pheromone alcohols in the gland. After 10–30 min, the hexane extract was recovered and 10 μl of a 2 : 1 (v/v) mixture of chloroform and methanol was added for total lipid extraction (Folch et al., 1957). Under these conditions more than 90% of the acyl moieties were contained in the chloroform–methanol extract. Fatty acyl moieties in this extract were converted to methyl esters by base-catalyzed methanolysis as described in detail by Löfstedt et al. (1986b). The samples usually were analyzed immediately on the gas chromatograph or occasionally stored in the freezer at $-20°C$ until analysis.

Gas Chromatographic Analysis with Flame Ionization Detection and Selected Ion Monitoring. Capillary gas chromatography with flame ionization

detection (GC-FID) was performed on a Hewlett Packard model 5880 GC equipped with a 30-m × 0.25-mm-id DB-wax column (cross-linked polyethylene glycol) (J&W Scientific Inc., Rancho Cordova, California). Conditions of chromatography were: hydrogen carrier gas velocity 40 cm/sec at 80°C; split valve opened 1 min after injection; temperature maintained at 80°C for 2 min following injection and then programmed at 5°C/min to 230°C. Under these conditions omega-labeled ($^2H_3$) fatty acid methyl esters and acetates are resolved from the corresponding nonlabeled compounds, so that the labeled compounds elute slightly earlier (Löfstedt et al., 1986a). Synthetic samples of the deuterium-labeled alcohols [12,12,12-$^2H_3$]dodecanol, [14,14,14-$^2H_3$]tetradecanol, [16,16,16-$^2H_3$]hexadecanol, and (*E*)-[11,11,12,12,12-$^2H_5$]9-dodecenol were analyzed together with a series of homologous unlabeled straight-chain alcohols, and their equivalent chain lengths (ECLs) were calculated. ECL values of the saturated alcohols were 1197, 1396, and 1597, respectively. ECL of the labeled (*E*)-9-dodecenol was 1236 compared to 1242 for unlabeled. Mass spectrometry with electron impact ionization was performed on a Hewlett Packard model 5970B GC-MS system equipped with a 59970B computer system, and interfaced with a Hewlett Packard 5890 GC (courtesy of Dr. J. Eyem, Hewlett Packard, Sweden). The molecular ion of *E,E8, 10–12:OH* amounts to about 10% of the base peak in EI spectra of this compound and is suitable for sensitive and selective analysis by selected ion monitoring (SIM) in the EI mode (Löfstedt and Odham, 1984). An acquisition program was designed to monitor the incorporation of (*E*)- [11,11,12,12,12-$^2H_5$]9-dodecanoic acid into *E,E*8,10–12:OH. The ions *m/z* 181.20, 182.20, 185.20, 186.20, and 187.20 were monitored, being equal to $[M\text{-}1]^+$, and $[M]^+$ for the native *E,E*8,10–12:OH, and to $[M]^{+\cdot}$ for the corresponding alcohols with three, four, and five deuterium labels, respectively.

Chemicals. Deuterium-labeled saturated fatty acids were purchased from Larodan Fine Chemicals, Malmö, Sweden. The deuterium enrichment of these omega-labeled acids was 99%. The acids are abbreviated: [12,12,12-$^2H_3$]dodecanoic acid, [12-D_3]12:acid, etc.

(*E*)-[11,11,12,12,12-$^2H_5$]9-dodecenoic acid (1) [11,12-D_5]*E*9–12:acid) was prepared from 1-(2-tetrahydropyranyloxy)-decyne (1.4 g, 6 mmol), *n*-butyllithium (4.4 ml, 1.44 M in hexane) in dry THF and [$^2H_5$]ethyl iodide in 10 ml freshly distilled DMPU (1,3-dimethyloxohexahydropyrimidine) (Bengtsson and Liljefors, 1988). Reduction with sodium in liquid ammonia (Warthen and Jacobson, 1973) gave the pure *E* monoene, which was treated with *p*-toluene sulfonic acid in methanol to give the corresponding alcohol. Oxidation with pyridinium dichromate (PDC) in dry dimethylformamide (DMF) (Corey and Smidt, 1979) gave the final product (0.52 g, 40% overall yield) (Scheme 1).



SCHEME 1.

The product was purified by TLC mesh column chromatography (Taber, 1982) and argentation liquid chromatography (Houx et al., 1974). An EI mass spectrum of the acid was recorded on a Finnigan 4021 mass spectrometer and [^{1}H]NMR on a Varian XL-300 spectrometer in $CDCl_3$ solutions with Me_4Si as internal reference: *m/z* 203 (M^+, 2%), 185(2), 143(2), 123(3), 110(4), 97(15), 87(12), 83(24), 74(42), 69(45), 60(42), 55(78), 43(100), 33(6). [^{1}H]NMR ($CDCl_3$) (300 MHz); δ 1.30 (m, 8 H, CH_2CH_2), 1.56–1.67 (m, 2H, CH_2—C—COOH), 1.93–1.99 (m, 2H, CH_2—C=C), 2.34 (t, 2H, CH_2—COOH), 5.32–5.46 (m, 2H, CH=CH)

The deuterium enrichment of the acid was >95% according to FAB high-resolution mass spectroscopy on a ZAB-HF, VG Analytical, 11-250, mass spectrometer: $[M\text{-}H]^-_{calc}$ = 202.185, $[M\text{-}H]^-_{obs}$ = 202.178. [12,12,12-$^2H_3$]-Dodecanol, [14,14,14-$^2H_3$]tetradecanol, [16,16,16-$^2H_3$]hexadecanol, and (*E*)-[11,11,12,12,12-$^2H_5$]9-dodecenol were prepared from the corresponding acids by reduction with lithium aluminum hydride in dry diethyl ether.

RESULTS

In a first set of experiments, sex pheromone glands were incubated with [12-D_3]12:acid. The glands incorporated the labeled acid into the alcohols 12:OH, *E*9–12:OH, and *E*,*E*8,10–12:OH (Table 1, Figure 1A), as well as into the corresponding fatty acyl moieties 12:acyl, *E*9–12:acyl, and *E*,*E*8,10–12:acyl (Table 1, Figure 2A). The peaks, indicating 12-D_3-labeled pheromone component and pheromone precursor analogs, were all absent in chromatograms of unlabeled glands (Figures 1B, 2B). The relatively low amount of labeled methyl dodecanoate (12:Me) recovered indicates that only a fraction of the 4 μg of topically applied [12-D_3]12:acid is incorporated into the pheromone gland fatty acid metabolism. No labeled 14- and 16-carbon alcohols or acyl moieties were produced.

In a second set of experiments, pheromone glands were incubated with [14-D_3]14:acid and [16-D_3]16:acid (Table 1). The relative amounts of labeled

TABLE 1. INCORPORATION OF DEUTERIUM-LABELED FATTY ACIDS INTO SEX PHEROMONE OF CODLING MOTH, *Cydia pomonella*.[a]

Labeled precursor	No. of ♀	Native *E*,*E*8, 10–12:OH (ng/♀)	Amount of labeled compound relative to native (=100)									
			Alcohols					Acyl moieties as methyl esters				
			12:OH	*E*9–12:OH	*EE*8, 10–12:OH	14:OH	16:OH	12:Me	*E*9–12:Me	*EE*8, 10–12:Me	14:Me	16:Me
[12-D_3] 12:acid	3	0.1	58	*[b]	257	0	0	—	—	—	—	—
	7	0.1	58	30	144	0	0	95	30	140	0	0
	8	0.5	108	74	103	0	0	65	49	134	0	0
[14-D_3] 14:acid	4	2	2	0	4	10	0	—	—	—	—	—
	4	1.5	8	—[c]	8	19	0	0	0	0	25	0
[16-D_3] 16:acid	4	0.5	32	30	65	49	25	2	0	36	1	1
[11,12-D_5]-	7	0.1	0	1000	287	0	0	0	302	236	0	0
*E*9–12:acid	5	0.7	0	664	76	0	0	0	567	120	0	0

[a] List of experiments including replicates.
[b] contaminated.
[c] —not analyzed.

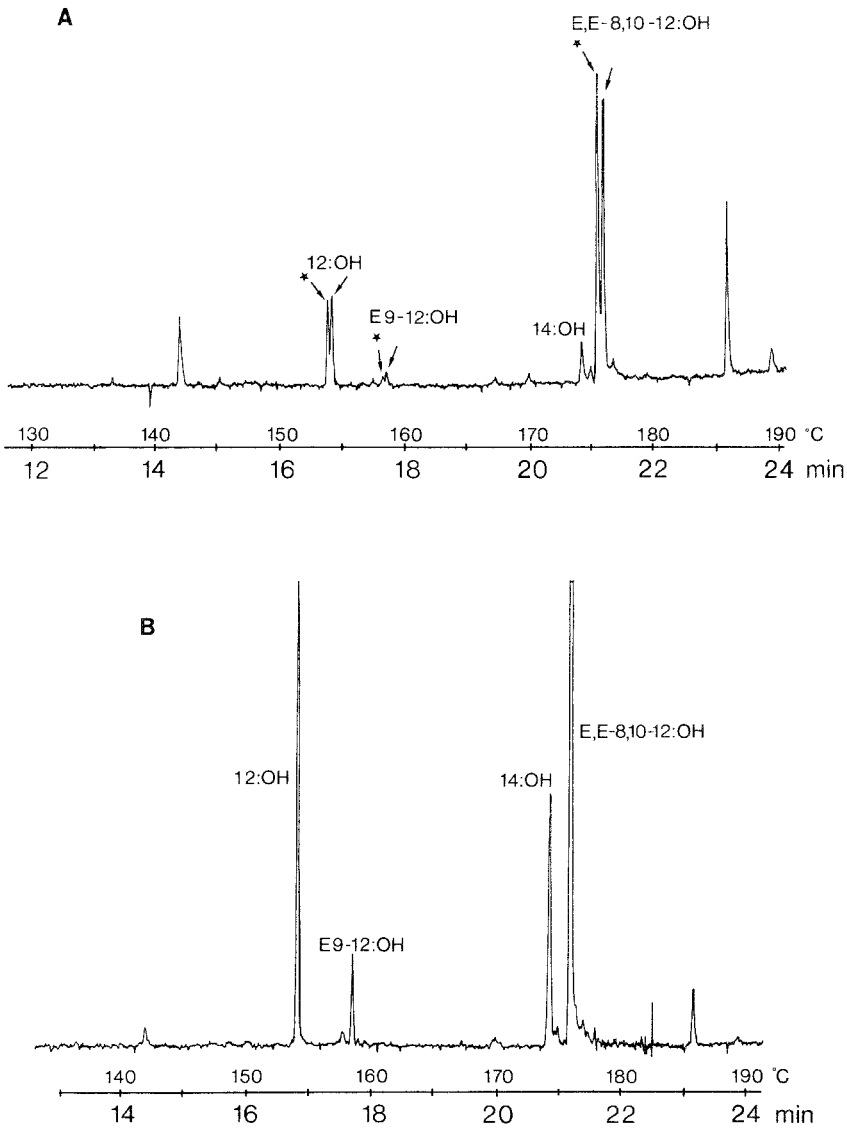


FIG. 1. Incorporation of deuterium label from topically applied [12,12,12-$^2H_3$]dodecanoic acid into pheromone components in glands of 8 female *Cydia pomonella*, monitored by capillary gas chromatography with flame ionization detection (A) and a control of two unlabeled glands (B). Peaks with (*) have the predicted retention times of omega-labeled ($^2H_3$) analogs of the pheromone components. See text for abbreviations.

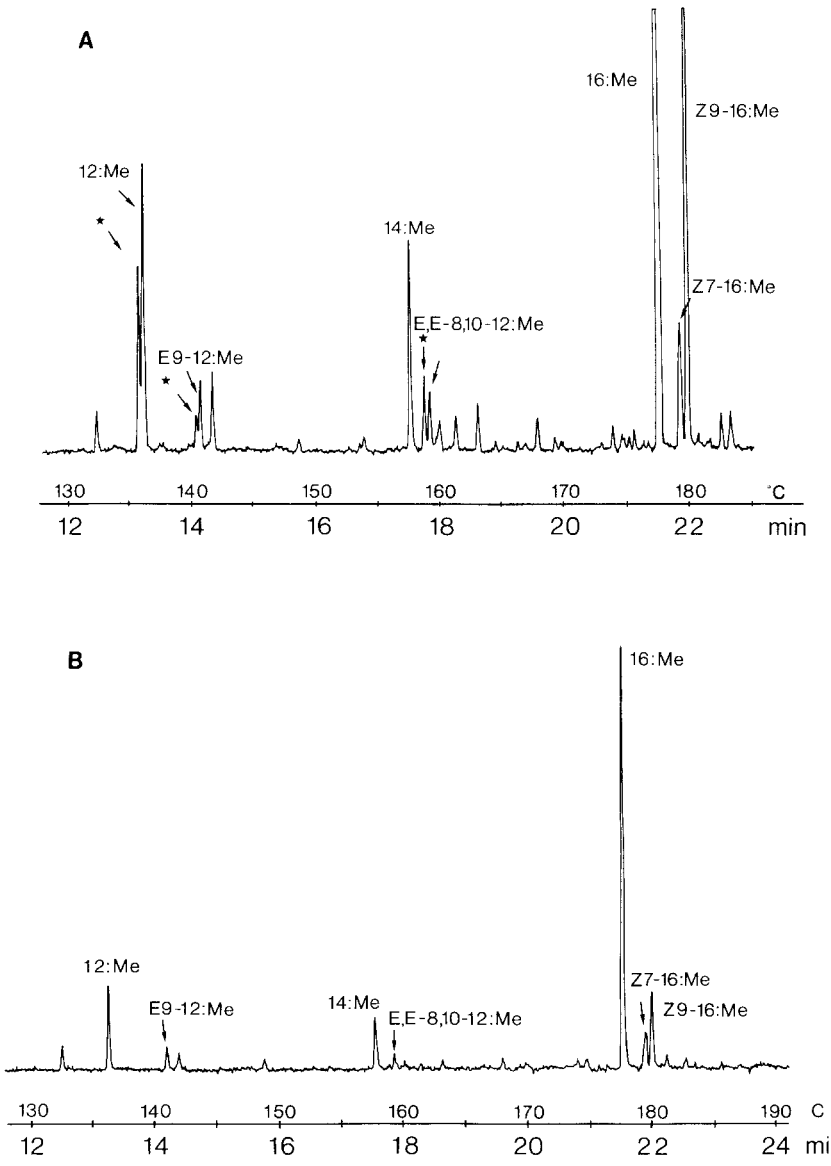


FIG. 2. Incorporation of deuterium label from topically applied [12,12,12-$^2H_3$]-dodecanoic acid into fatty acids in glands of eight female *Cydia pomonella*, monitored by capillary gas chromatography with flame ionization detection (A) and a control of two unlabeled glands (B). The first peak (*) in a pair has the predicted retention time of the omega-labeled ($^2H_3$) analog of the respective fatty acids. See text for abbreviations.

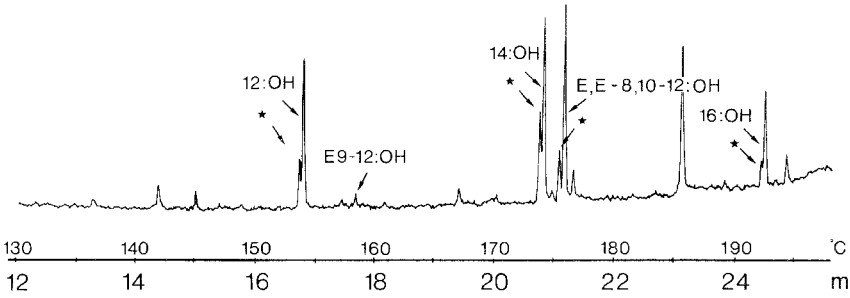


FIG. 3. Incorporation of deuterium label from topically applied [16,16,16-$^2H_3$]-hexadecanoic acid into alcohols in glands of four female *Cydia pomonella*, monitored by gas chromatography with flame ionization detection. The first peak (*) in a pair has the predicted retention time of the omega-labeled ($^2H_3$) analog of the respective alcohol. See text for abbreviations.

alcohols and acids produced with the [14-D_3]14:acid precursor were low, but still 4–8% of labeled *E,E*8,10–12:OH was obtained. This time, the label was found also in 14:OH and 14:Me. When glands were incubated with [16-D_3]16:COOH, the label was found in all the pheromone gland alcohols, including 16:OH (Figure 3).

Finally, pheromone glands were incubated with [11,12-D_5]*E*9–12:acid. These incubations produced large relative amounts of *E*9–12:OH and *E,E*8,10–12:OH, as well as their corresponding fatty acyl groups (Table 1). No label was incorporated into any of the saturated alcohols or acids studied. The incorporation of [11,12-D_5]*E*9–12:acid into *E,E*8,12:OH was further studied by mass spectrometric analysis of the products of an incubation. We found that incubation of glands with acid labeled with five deuterium atoms produced *E,E*8,10–12:OH labeled with four rather than five deuterons (Figure 4). The signal *m/z* 187 indicating [D_5]*E,E*8,10–12:OH was only 13% of *m/z* 186, indicating [D_4]*E,E*8,10–12:OH. The production of *E,E*8, 10–12:OH labeled with four deuterons should be due to metabolic (as opposed to mass spectrometric) loss of one deuterium atom as the mass spectrum of unlabeled *E,E*8,10–12:OH contained no detectable amounts of $[M-1]^+$.

DISCUSSION

We conclude that the major pheromone component of the codling moth *E,E*8,10–12:OH is biosynthesized from palmitic acid by two cycles of β-oxidation, followed by *E*9 desaturation of dodecanoic acid and a subsequent trans-

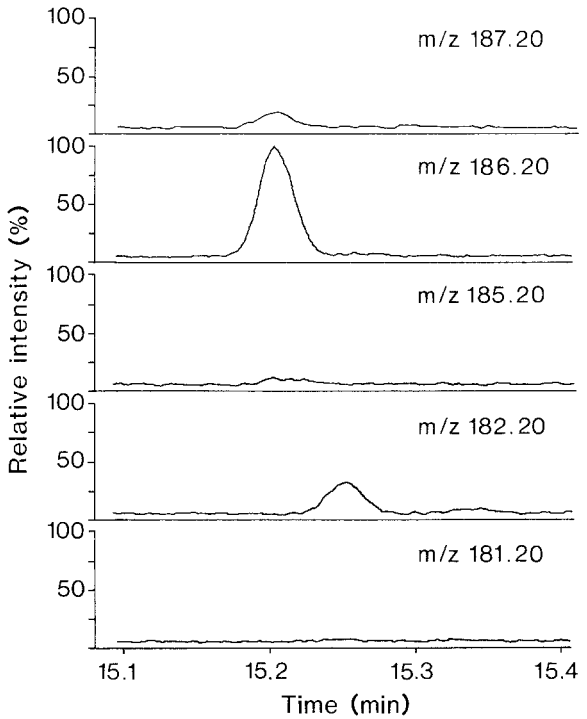


FIG. 4. Incorporation of deuterium label from topically applied (*E*)-[11,11,12,12,12-$^2H_5$]9-dodecenoic acid into (*E*,*E*)-8,10-dodecadienol in glands of seven female *Cydia pomonella*, analyzed by selected ion monitoring of ions *m/z* 181.20, 182.20, 185.20, 186.20, and 187.20. See text for details.

formation of the *E*9 double bond into the conjugated (*E*,*E*)-8,10-diene. The acid is finally reduced to produce the corresponding alcohol (Figure 5). The production of the conjugated diene before the reduction of acid to alcohol is supported by the presence of labeled *E*,*E*8,10–12 : acyl.

The fact that the unusual *E*9 desaturation of dodecanoic acid is a key step is supported by the absence of labeled chain-elongation products in the incubations with [12-D_3]12 : acid. An alternative route to the monoene could have been Δ 11 desaturation of tetradecanoic acid, a reaction widely distributed in tortricids (Roelofs and Brown, 1982), followed by chain shortening to give *E*9–12 : acyl. However, in this case [12-D_3]12 : acid should first have been elongated to labeled tetradecanoic acid (which we could not detect) and then desaturated and chain-shortened to produce the labeled *E*9–12 and *E*,*E*8,10–12 moieties.

We found that the incorporation of labeled precursors, as well as the amount of pheromone components in the glands, were quite variable from one experi-

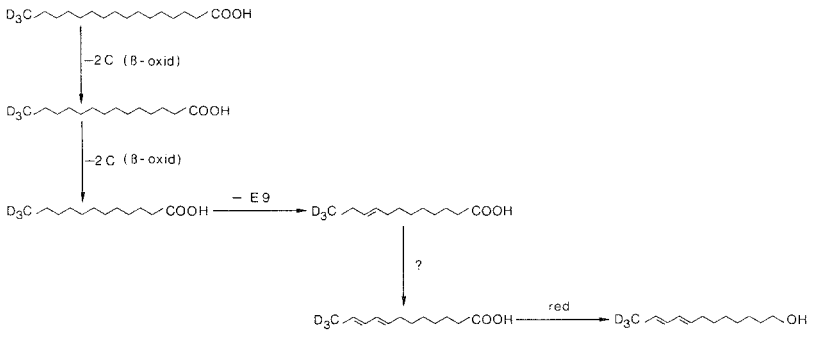
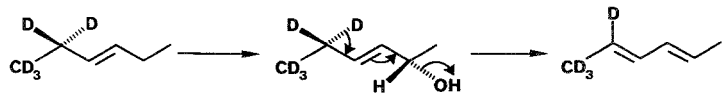


FIG. 5. Biosynthesis of sex pheromone gland constituents from hexadecanoic acid in the codling moth, *Cydia pomonella*. The proposed reactions involved are chain shortening (β-oxidation), *E*9 desaturation, conversion of the monoene into a conjugated diene, and reduction of the acids to alcohols.

ment to another (Table 1), even though usually four or more females were included in one batch. This problem is not easily overcome by merely increasing the number of females in each batch, as Bjostad and Roelofs (1986) noticed the same phenomenon with as many as 20 glands used for each extraction. For this reason, we preferred to estimate the relative, rather than absolute, amounts of labeled compounds produced. Ideally, incorporation of each compound should have been measured relative to the incorporation of an internal standard, a compound with known metabolic activity, applied simultaneously.

The similarity of pheromone biosynthesis in the codling moth with that of *E*,*Z*10,12-16:OH in the silkworm moth is interesting, as the two species are representatives of two quite different lepidopteran families, Tortricidae and Lymantriidae, respectively. The loss of one deuteron, when the dienic pheromone precursor in *C. pomonella* is formed from [11,12-D_5]*E*9-12:acid, was not investigated in detail, but we suggest that the key steps involve oxidation of one of the α positions surrounding the double bond, followed by a 1,4 elimination of water (Scheme 2). Such a reaction mechanism accounts also for the



SCHEME 2.

biosynthesis of bombykol. Yamaoka et al. (1984) report 15% of $[M]^+$ for bombykol labeled with two deuterons relative to $[M]^+$ for the native bombykol when (*Z*)-[11,12-$^2H_2$]11-hexadecenoic acid is metabolized to bombykol. There is no indication of a product with one deuterium label only. This demonstrates that

both labeled atoms are retained when [11,12-$^2H_2$]Z11-16:acyl is converted to bombykol in *B. mori*.

*E*9 desaturation has not been described before in moth pheromone biosynthesis. Roelofs and Brown (1982) used information available on pheromone production in the Tortricidae to discuss taxonomy and the possible phylogeny of this group of Lepidoptera. Our finding of an *E*9-desaturase in *C. pomonella* does not resolve the classic controversy over the phylogenetic relationship among different tortricid subfamilies or tribes. However, the novel enzyme inferred tells us that there is no close biosynthetic relationship between the production of Δ 8 and Δ 10 monounsaturated compounds by a Δ 10-desaturase, and the production of Δ 8,10 doubly unsaturated compounds involving *E*9 desaturation. Whether the *E*9-desaturase is more closely related to the *E*11-, the Z10-, or the Z11-desaturase remains an open question, as does the phylogenetic position of the Olethreutinae relative to the Tortricinae.

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REFERENCES

ARN, H., GUERIN, P.M., BUSER, H.R., RAUSCHER, S., and MANI, E. 1985. Sex pheromone blend of the codling moth, *Cydia pomonella:* Evidence for a behavioral role of dodecan-1-ol. *Experientia* 41:1482–1484.

BENGTSSON, M. and LILJEFORS, T. 1988. DMPU—An alternative to HMPT in moth sex pheromone synthesis. *Synthesis* (in press).

BJOSTAD, L.B., and ROELOFS, W.L. 1981. Sex pheromone biosynthesis from radiolabeled fatty acids in the redbanded leafroller moth. *J. Biol. Chem.* 256:7936–7940.

BJOSTAD, L.B., and ROELOFS, W.L. 1983. Sex pheromone biosynthesis in *Trichoplusia ni*: Key steps involve delta-11 desaturation and chain shortening. *Science* 220:1387–1389.

BJOSTAD, L.B., and ROELOFS, W.L. 1984. Sex pheromone biosynthetic precursors in *Bombyx mori*. *Insect Biochem.* 14:275–278.

BJOSTAD, L.B., and ROELOFS, W.L. 1986. Sex pheromone biosynthesis in the red-banded leafroller moth, studied by mass labeling with stable isotopes and analysis with mass spectrometry. *J. Chem. Ecol.* 12:431–450.

COREY, E.J., and SMIDT, G. 1979. Useful procedures for the oxidation of alcohols involving pyridinium dichromate in aprotic media. *Tetrahedron Lett.* 5:399–402.

EINHORN, J., BEAUEVAIS, F., GALLOIS, M., DESCOINS, C., and CAUSSE, R. 1984. Constituants secondaires de la phéromone sexuelle du Carpocapse des Pommes, *Cydia pomonella* L. (Lepidoptera, Tortricidae). *C.R. Acad. Sci. Paris Ser. III* 299:773–778.

FOLCH, J., LEES, M., and SLOANE, S.G.H. 1957. A simple method for the isolation and purification of total lipides from animal tissue. *J. Biol. Chem.* 226:497–509.

HOUX, N.W.H., VOERMAN, S., and JONGEN, W.M.F. 1974. Purification and analysis of synthetic

insect sex attractants by liquid chromatography on a silver-loaded resin. *J. Chromatogr.* 96:25–32.

LÖFSTEDT, C., and ODHAM, G. 1984. Analysis of moth pheromone acetates by selected ion monitoring using electron impact and chemical ionization mass spectrometry. *Biomed. Mass Spectrom.* 11:106–113.

LÖFSTEDT, C., and ROELOFS, W.L. 1985. Sex pheromone precursors in two primitive New Zealand tortricid moth species. *Insect Biochem.* 15:729–734.

LÖFSTEDT, C., ELMFORS, A., SJÖGREN, M., and WIJK, E. 1986a. Confirmation of sex pheromone biosynthesis from [16-D_3]palmitic acid in the turnip moth. *Experientia* 42:1059–1061.

LÖFSTEDT, C., LÖFQVIST, J., LANNE, B.S., VAN DER PERS, J.N.C., and HANSSON, B.S. 1986b. Pheromone dialects in European turnip moths *Agrotis segetum. Oikos* 46:250–257.

ROELOFS, W.L., and BJOSTAD, L.B. 1984. Biosynthesis of lepidopteran pheromones. *Bioorg. Chem.* 12:279–298.

ROELOFS, W.L., and BROWN, R.L. 1982. Pheromones and evolutionary relationships of Tortricidae. 1983. *Annu. Rev. Ecol. Syst.* 13:395–422.

ROELOFS, W.L., COMEAU, A., HILL, A., and MILICEVIC, G. 1971. Sex attractant of the codling moth: Characterization with electroantennogram technique. *Science* 174:297–299.

TABER, D. 1982. TLC mesh column chromatography. *J. Org. Chem.* 47:1351–1352.

WARTHEN, J.D., Jr., and JACOBSON, M. 1973. Insect sex attractants; XIV. All-*trans*-alkenol acetates via sodium–liquid ammonia reduction. *Synthesis* 10:616–617.

YAMAOKA, R., TANIGUCHI, Y., and HAYASHIYA, K.. 1984. Bombykol biosynthesis from deuterium-labeled (Z)-11-hexadecenoic acid. *Experientia* 40:80–81.