Enzymatic Conversion and Degradation of Sex Pheromones in Antennae of the Male Silkworm Moth *Antheraea polyphemus*

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Dedicated to Professor Adolf Butenandt on the occasion of his 85th birthday

Moth, Antennae, Pheromones, Metabolites, Enzymes

In living antennae of the male silkworm moth *Antheraea polyphemus* the pheromone compounds [6,7-^{3}H]-6,11-hexadecadienyl acetate and [12,13-^{3}H]-10,12-hexadecadienol are enzymatically converted to their corresponding fatty alcohols, aldehydes, acids and long-chained fatty acid esters. In antennae of freshly hatched moths the ^{3}H-labeled pheromones are degraded at high rates to volatile polar metabolites. The half-life of the pheromone acetate is about 3 min. In dried antennae the pheromone acetate is merely hydrolyzed to the fatty alcohol.

Introduction

Sex pheromones are essential for the orientation and attraction of the sex partners of many butterflies and moths and are becoming increasingly important in biological pest control. Large efforts have been made for a quarter of a century to isolate sex pheromones from abdominal sex glands of female moths and to identify the active components with chromatographic and spectroscopic methods.

Studies on the metabolism of moth pheromones were started after the identification of Bombykol, the primary pheromone component produced by the female silkworm moth *Bombyx mori*. Bombykol was described thirty years ago as (*E,Z*)-10,12-hexadecadien-1-ol by Butenandt *et al.* [1]. The Bombykol isomer (*E,E*)-10,12-hexadecadien-1-ol and (*E,Z*)-10,12-hexadecadien-1-al (Bombykal) were found in gland extracts of the female moths [2–4]. For an historical retrospect on Bombykol and related compounds see Hecker and Butenandt 1984 [5]. (*E,Z*)-6,11-hexadecadienyl acetate and (*E,Z*)-6,11-hexadecadien-1-al have been identified as the main pheromone components of the female moth of *Antheraea polyphemus* by Kochansky *et al.* [6]. Recently, a third pheromone component (*E,Z*)-4,9-tetradecadienyl acetate has been found in *Antheraea pernyi* by Bestmann *et al.* [7].

The first stage of pheromone uptake and enzymatic conversion begins with the adsorption of the airborne molecules on the long sensory hairs [8]. Possibly, the molecules diffuse on the outer surface, reaching pores and pore tubuli which lead them through the hair wall into the hair lumen. The pheromone molecules migrate along the hair surface and/or within the hair lumen towards the branches of the antennae [9]. Pheromone inactivation is important after the interaction of pheromone molecules with the proposed receptor molecules of the dendritic membranes [10]. The time course of electrophysiological responses (EAGs and single cell recordings) indicate that pheromone molecules must be inactivated within seconds after interaction with the receptors, whereas the half-life of pheromone conversion in intact moth antennae was found in the order of some minutes. Therefore, the enzymatic transformation is not sufficient and a rapid inactivation mechanism was postulated [11, 12]. Pheromone conversion as described and found in antennae, wings, legs and other organs of the insect body prob-

Abbreviations: LC, liquid chromatography; TLC, thin-layer chromatography; LSC, liquid scintillation counting; ITS, internal standard; EAG, electroantennogram; BHT, 2,6-ditertiary-butyl-4-hydroxy-toluene;
E10,Z12-16:Ol, (*E,Z*)-10,12-hexadecadienol, Bombykol;
E10,Z12-16:Al, (*E,Z*)-10,12-hexadecadienal, Bombykal;
E6,Z11-16:Ac, (*E,Z*)-6,11-hexadecadienyl acetate;
Z6,Z11-16:Ac, (*Z,Z*)-6,11-hexadecadienyl acetate;
Z6,E11-16:Ac, (*Z,E*)-6,11-hexadecadienyl acetate;
E6,Z11-16:Al, (*E,Z*)-6,11-hexadecadienal;
[^{3}H]Bombykol, [12,13-^{3}H]*E*10,*Z*12-16:Ol, [12,13-^{3}H](*E,Z*)-10,12-hexadecadienol;
[^{3}H]HDAc, [6,7-^{3}H]-6,11-16:Ac, [6,7-^{3}H]-6,11-hexadecadienyl acetate (mixture of three *E*/*Z*-isomers, see Tables I and II);
[^{3}H]HDOl, [6,7-^{3}H]-6,11-hexadecadienol;
[^{3}H]HDAl, [6,7-^{3}H]-6,11-hexadecadienal;
[^{3}H]HDAcid, [6,7-^{3}H]-6,11-hexadecadienoic acid;
[^{3}H]HDEster, [6,7-^{3}H]-6,11-hexadecadienoic acid ester.

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ably serves as a cleaning mechanism which prevents pheromone accumulation in the insect integument and adaptation of the highly sensitive receptor cells [13].

Experiments on the enzymatic conversion of Bombykol in the silkworm moth *Bombyx mori* were performed with [12,13-^{3}H](*E,Z*)-10,12-hexadecadienol ([^{3}H]Bombykol) [14]. [^{3}H]Bombykol and related fatty alcohols applied onto antennae of male and female moths of *Bombyx mori* were oxidized to the corresponding fatty acids and partly esterified to long-chained fatty acid esters [9, 15].

Similar investigations showed the enzymatic conversion of [7,8-^{3}H](*Z*)-7,8-epoxy-2-methyl-octadecane ([^{3}H]Disparlure) in the gypsy moth, *Lymantria dispar* [16] and the enzymatic hydrolysis of [^{3}H](*Z*)-7-dodecenyl acetate and analogues in the cabbage looper moth, *Trichoplusia ni* [17–22].

It should be noted that esterases, alcohol oxidases and aldehyde dehydrogenases, which play a role in pheromone conversion and degradation in insect antennae, are also involved in the biosynthesis of sex pheromones as found in the pheromone glands of female moths of *Choristoneura fumiferana* [23–26] and *Heliothis virescens* [27]. Studies with [6,7-^{3}H]- and [11,12-^{3}H](*E,Z*)-6,11-hexadecadienyl acetate have provided information on pheromone uptake and transport, enzymatic hydrolysis of the pheromone and its interaction with a binding protein in the sensillum lymph of *Antheraea polyphemus* and *Antheraea pernyi* [28–39].

This paper reports on "pheromone conversion and degradation" using [6,7-^{3}H]-6,11-hexadecadienyl acetate ([^{3}H]HDAC) as the pheromone source with male moths of *Antheraea polyphemus* and includes the study of the volatile polar metabolites which has been previously neglected.

The term "conversion" describes the enzymatic transformation of long-chained pheromones at their functional groups and the term "degradation" denotes the enzymatic decomposition of pheromones to short-chained metabolites.

Materials and Methods

Insects

Pupae of *Antheraea polyphemus* (Cramer) were supplied by E. Taschenberg, Fredonia, N.Y. (U.S.A.) and M. Furr, Germantown, T.N. (U.S.A.). The cocoons were kept at 4 °C with a light-dark cycle of 11:13 h. For emergence the cocoons were warmed up to 22 °C at 65% rel. humidity in a light-dark cycle of 13:11 h for 3 weeks.

Experiments were performed using freshly excised antennae. In control experiments air dried antennae were used which had been stored either in a desiccator for 14 days or at −20 °C for several (up to 7) years.

Radiochemicals

Pheromones and stereoisomers were generally produced by multistep synthesis *via* the Wittig reaction. The three isomers (*E,Z*)-, (*Z,Z*)-, and (*Z,E*)-6,11-hexadecadienyl acetate were labeled at the 6,7-position and obtained by tritiating the acetylenic bond of (*Z*)-11- and (*E*)-11-hexadecen-6-ynyl acetate (68.3:31.7) with tritium gas and the Lindlar catalyst (Amersham and Buchler). The precursors were obtained from H. J. Bestmann and O. Vostrowsky (Erlangen). In the following biological experiments a mixture of the 3 isomers was used (Table I). [^{3}H]Bombykol was produced by tritiating (*E*)-10-hexadecen-12-yn-1-ol provided by P. E. Schulze (Schering, Berlin) [14].

The ^{3}H-labeled compounds were regularly analyzed and purified twice by thin-layer chromatography to a purity of over 98%. The ^{3}H-labeled pheromones were diluted and stored in pentane and heptane (Conc.: 10 ppm) in darkness under nitrogen at −20 °C. The antioxidant BHT (Merck) was added to the autoxidisible compounds. The specific activities and molecular concentrations per dpm and nanogram are shown (Table II). ^{3}H-labeled water and toluene were used as internal standards (ITS) for liquid scintillation counting and ^{3}H-labeled methanol (Packard Instruments) as a marker in column chromatography. The specific radioactivities were determined by using radiogas chromatography and LSC. In some cases the purity of the radioactive compounds was checked with liquid chromatography and UV-, IR-, NMR-, and mass-spectrometry.

Solvents and reagents

The solvents for the radiochemicals were generally hydrocarbons, chloroform and methanol. Standard solvent (I) for TLC: hexane, diisopropyl ether, methanol, and NH_3 (25%) (100:20:10:0.15, by vol.). Standard solvent (II) for LC: hexane, diisopropyl ether, ethyl acetate, and methanol

(25:25:25:200, by vol.). Cellosolve (glycolmonoethylether) was found to be the best solvent for aqueous and nonaqueous solutions. Scintillator: PPO and POPOP in Rotiszint 22 (Roth), PPO without POPOP in cellosolve for wet oxidation. Oxidizers for the antennae: 30% hydrogen peroxide and 60% perchloric acid. The chemicals were all of analytical grade and obtained from Merck (Darmstadt), Fluka (Buchs, Switzerland) and Roth (Karlsruhe).

Chromatography

Thin-layer chromatography was generally used to separate the radioactive pheromone compounds from their metabolites. The ^{3}H-labeled compounds were identified by comparison with reference compounds as described earlier [15]. After TLC the radio-chromatograms (30 × 150 mm) were completely scraped in the form of 30 zones into 20 ml scintillation vials with semi-automatic scrapers [40, 41] obtained from Desaga (Heidelberg) and Hölzel (Dorfen). LSC was carried out at 5 °C in Tricarb 3390 counters or at 25 °C in a Minaxi 4430 counter (Packard Instrument, U.S.A.). The results in cpm or dpm were either plotted immediately or recorded on paper tapes or diskettes which were analyzed by electronic data processing (PDP 11/40, Digital Equipment or IBM Personal Computer) and plotted in the form of radiochromatograms with peak area integration and percent calculation (Fig. 1).

Liquid chromatography was used to separate the volatile from the nonvolatile metabolites without loss of ^{3}H-activity (Fig. 2). The separated compounds were automatically collected in common scintillation vials with a Labocol Vario-10 (Labomatic, Sinsheim). After elution the radioactivity of the fractions was measured using LSC in Rotiszint 22. The data were handled as described above.

Incubation and elution

The pheromones were applied as airborne compounds by using a stream of compressed air (10 seconds at 100 ml/s ≡ 2.6 m/s) blown onto the antennae. The pheromone molecules were loaded in solution onto cotton wool plugs inserted into connecting capillary tubes up-stream of the antennae [42]. The amount of the absorbed [^{3}H]HDAc per antenna was between $10^2 - 5 \times 10^3$ picograms ($2 \times 10^{11} - 10^{13}$ molecules). To determine the pheromone half-life in the antennae, the molecules were allowed to incubate for periods of 1, 5, 10 or 30 min at 20 °C unless stated otherwise.

In other experiments the antennae were treated with the pheromone dissolved in pentane. The solvent was evaporated from the antennae within 30 sec in an air current.

After pheromone application and incubation the antennae were extracted with 5 ml pentane and afterwards with 5 ml chloroform-methanol (2:1, by vol.) or only with 2 ml chloroform-methanol (2:1, by vol.). The ^{3}H-activity remaining in the antennae (^{3}H-residue) was determined after wet oxidation with 200 µl $HClO_4$ and 400 µl H_2O_2 for 8 h at 70 °C. Thereafter the residue was dissolved in 10 ml Cellosolve and treated with 10 ml PPO scintillation cocktail and the ^{3}H-activity measured by LSC.

Evaporation and partition

The pheromone isomers (Table I) are strongly adsorbed on activated silica gel. The evaporation of the pheromone compounds was investigated after it was pipetted onto silica gel but not subjected to chromatographic separation. Samples of [^{3}H]HDAc in 5 µl pentane were topically applied onto marked squares of the TLC plates, dried and incubated for periods of 0.25–100 min before scraping and LSC. The ^{3}H-activity of each sample was measured before and after scraping, the difference (^{3}H-loss) is considered as the amount of pheromone evaporation. Similarly, the evaporation rates of the volatile ^{3}H-metabolites from TLC plates were determined at 0 °C and 20 °C (Table IV).

The hydrophilic character of the ^{3}H-metabolites obtained from living and dried antennae was determined by partitioning between water and diethyl ether (Table V).

Errors

The total error of the cited operations was estimated at ± 3–6%. Additional errors of ± 1–3% from pheromone evaporation after TLC and scraping were found using solvent I.

Results

Time dependency

[^{3}H]HDAc adsorbed from an air stream onto freshly cut antennae was partly converted into less volatile but mainly degraded into highly volatile ^{3}H-

Table I. Pheromone isomers as prepared by hydrogenation [^{1}H] and tritiation [^{3}H] of the precursor mixture (*Z*)-11- and (*E*)-11-hexadecen-6-ynyl acetate (68.3:31.7). ± SD: standard deviation.

Stereoisomer	^{3}H [%]	^{1}H [%]
*Z*6,*Z*11-16:Ac	63.2 ± 3.0	64.3 ± 3.0
*Z*6,*E*11-16:Ac	27.1 ± 5.0	28.0 ± 5.0
*E*6,*Z*11-16:Ac	9.7 ± 2.0	7.7 ± 2.0

metabolites. With increasing incubation time the amounts of [^{3}H]HDAc in freshly cut antennae decreased and those of the ^{3}H-metabolites increased.

After a short incubation (1 min) of freshly isolated antennae (3 week old insects) with [^{3}H]HDAc only small amounts of ^{3}H-metabolites were detected (Fig. 1). About 16% of the pheromone was converted to nonvolatile ^{3}H-metabolites (incl. ^{3}H-residue) and about 11% to volatile ^{3}H-metabolites (Table III, A–C). While most of the ^{3}H-activity in the pentane fraction was nonvolatile, more than 50% from the chloroform-methanol fraction was calculated as volatile (Table III, A–B).

After a long incubation (30 min) only 9.3% nonvolatile ^{3}H-metabolites but 79.2% volatile ^{3}H-metabolites were determined in the pentane and chloroform-methanol fractions (Table III, D–E). A residual ^{3}H-activity of 9.2% was not extractable with the solvents used (Table III, F).

The overall half-life of the pheromone was reached at an incubation time of 3.0 ± 0.3 min (Fig. 3). During this time the amounts of newly formed alcohols increased to 14% (at 2 min) but later decreased to 4.5% (at 30 min) (Table VI).

[^{3}H]Bombykol was metabolized more slowly than [^{3}H]HDAc in freshly cut antennae of male moths of *Antheraea polyphemus* (Table VI).

Volatile metabolites

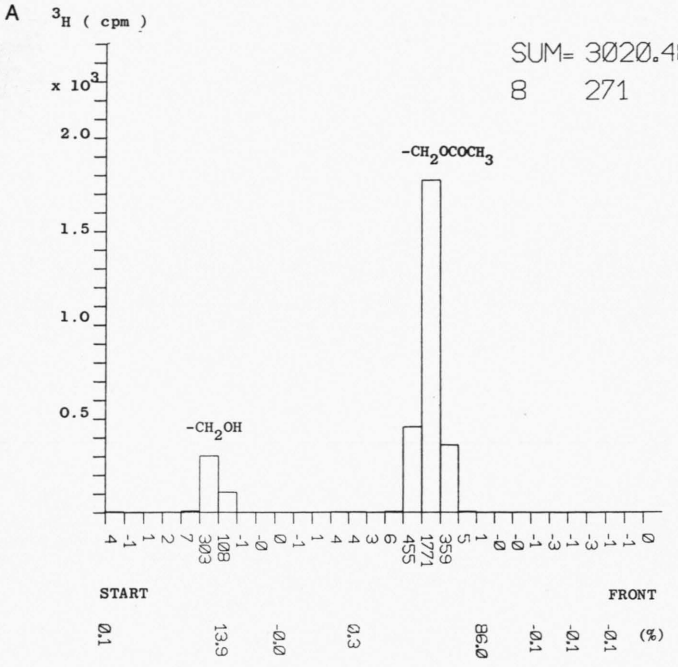
Control experiments with [^{3}H]HDAc (141 pg) on TLC plates showed that after short incubations the ^{3}H-evaporation remained between 4–8% of the initial ^{3}H-activity. The maximum loss was 10% after 100 min incubation. After TLC ^{3}H-evaporation was only 5%. This low evaporation of the ^{3}H-labeled pheromone contrasts with the high evaporation of

Table II. Specific activities of ^{3}H-labeled pheromones and numbers of pheromone molecules per dpm and nanogram. [6,7-^{3}H]*E*6/*Z*11-16:Ac is a mixture of 3 *E*/*Z*-isomers (see Table I). ± SD: standard deviation.

Pheromone compound	Specific activity		Number of molecules	
	mCi/mg	Ci/mmol	1 dpm =	1 ng =
[6,7-^{3}H]*E*6/*Z*11-16:Ac	110.0 ± 10.0	31.0 ± 3.0	8.78×10^6	2.13×10^{12}
[12,13-^{3}H]*E*10,*Z*12-16:Ol	242.0 ± 12.0	58.1 ± 3.0	4.67×10^6	2.49×10^{12}

Table III. Fractional elution of [^{3}H]HDAc and ^{3}H-metabolites from freshly cut antennae of *Antheraea polyphemus* with pentane and chloroform–methanol (2:1, by vol.). Incubation, elution, TLC and LSC as described (Fig. 1). [^{3}H]HDAc per antenna: 2.31 ng; ^{3}H-activities of fractions: A–C: 1.12×10^6 dpm (100%), D–F: 9.0×10^5 dpm (100%).

Fraction	Solvent	Incubation time [min]	^{3}H-activity [%]			Nonvolatile ^{3}H-activity [%]			
			total	volatile	nonvolatile	acetate	alcohol	aldehyde	acid	ester
A	pentane	1	92.1	7.6	84.5	72.5	11.8	0.2	–	–
B	chloroform methanol	1	7.0	3.6	3.4	0.4	1.7	–	1.0	0.3
C	residue	1	0.9	–	0.9	–	–	–	–	–
A–C		1	100.0	11.2	88.8	72.9	13.5	0.2	1.0	0.3
D	pentane	30	5.5	2.7	2.8	1.6	0.9	–	0.3	–
E	chloroform methanol	30	85.3	76.5	8.8	0.7	2.5	0.4	3.9	1.3
F	residue	30	9.2	–	9.2	–	–	–	–	–
D–F		30	100.0	79.2	20.8	2.3	3.4	0.4	4.2	1.3



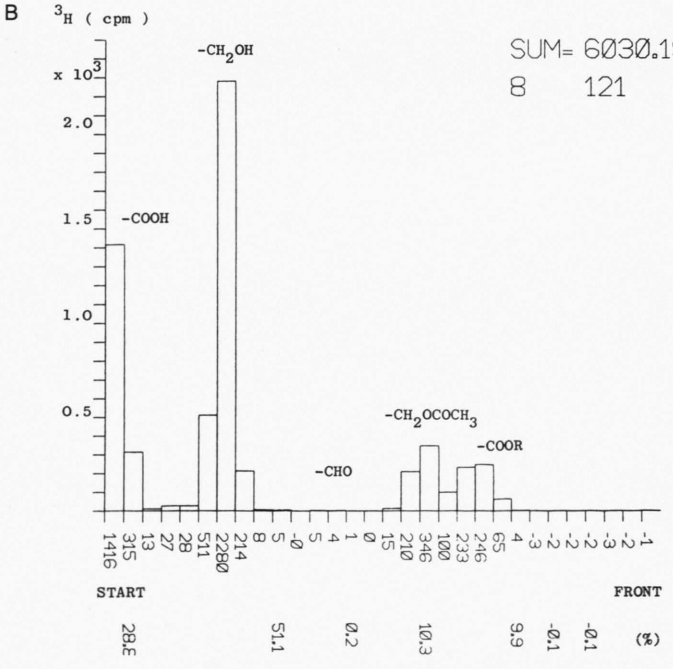


Fig. 1. Separation of ^{3}H-metabolites by TLC. The metabolites were obtained by applying [^{3}H]HDAc in an air current to freshly cut antennae of male moths of *Antheraea polyphemus*. [^{3}H]HDAc per antenna: 2.31 ng. The antennae were incubated (1 min) at 20 °C and eluted (10 min) with pentane (A) and chloroform–methanol (2:1, by vol.) (B). Aliquots of the extracts (Table III, A and B) were analyzed by TLC on precoated plates 200 × 200 × 0.25 mm with concentration zones (Merck). The chromatograms were developed with solvent I and the scraped zones measured by LSC. The negative data (cpm or percentage) result from background subtraction. The [^{3}H]SUM does not contain ^{3}H-background.

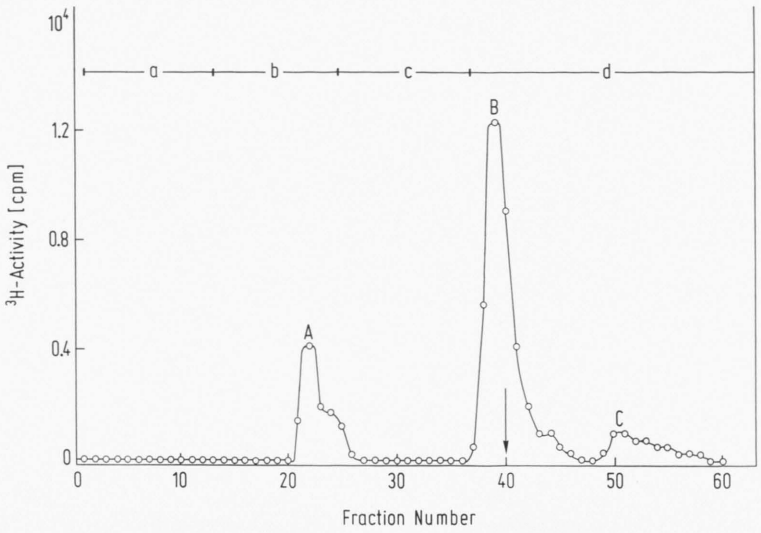


Fig. 2. Separation of neutral and acidic pheromone metabolites by LC. The ^{3}H-metabolites were obtained from freshly cut antennae which were incubated (30 min) with [^{3}H]HDAc and eluted (20 min) with chloroform–methanol (2:1, by vol.). [^{3}H]HDAc per antenna: 2.34 ng. Aliquots of the extract (1.6×10^5 dpm) were chromatographed with a column (10×300 mm) of Florisil (Merck) and a gradient of solvent II with a flow rate of 1 ml/min. Eluents: (a) 25 ml hexane, (b) 25 ml diisopropyl ether, (c) 25 ml ethyl acetate, (d) 200 ml methanol. The solvents (a)–(c) were treated with 40 µl ammonia and solvent (d) with 80 µl acetic acid. ^{3}H-activities of the peaks: A: 19.3% ([^{3}H]HDAc, [^{3}H]HDOl, [^{3}H]HDEster); B: 64.8% (volatile ^{3}H-metabolites); C: 15.9% ([^{3}H]HDAcid). The arrow indicates the fraction in which the ITS (^{3}H-labeled methanol) appeared.

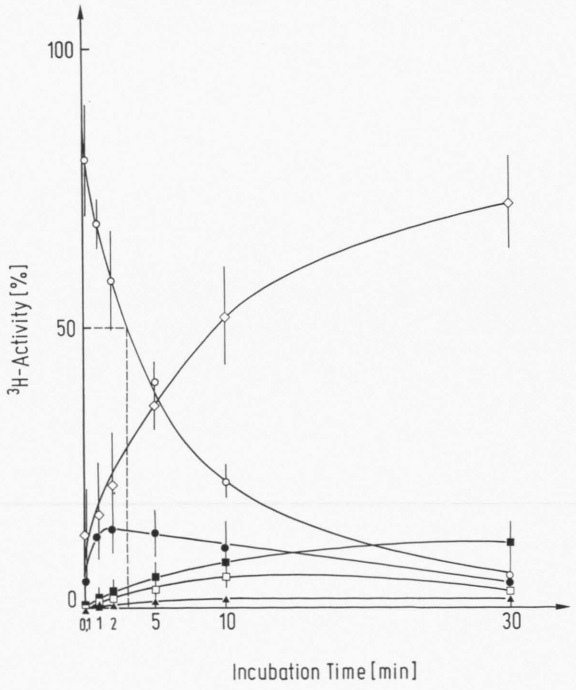


Fig. 3. Time course of pheromone conversion and degradation in freshly cut antennae. The ^{3}H-metabolites were recovered from antennae which were applied for 10 s with [^{3}H]HDAc and incubated (0.1–30 min) in air. [^{3}H]HDAc per antenna: 2.88–4.12 ng. Incubation, elution, TLC and LSC as described (Fig. 1). Pheromone half-life: 3.0 ± 0.3 min; n = 4 (number of experiments), error bars: ± SD (standard deviation). Symbols: [^{3}H]HDAc (○), [^{3}H]HDOl (●), [^{3}H]HDAl (△), [^{3}H]HDEster (▲), [^{3}H]HDAcid (□), ^{3}H-residue (■), volatile ^{3}H-metabolites (◇), nonvolatile ^{3}H-metabolites (◆).

Table IV. Evaporation of [^{3}H]HDAc and ^{3}H-metabolites from TLC plates before (a, b) and after (c) chromatography. [^{3}H]HDAc standards and chloroform–methanol extracts (Fig. 3, 30 min incubation) were used. Aliquots were applied onto the plates and scraped after 1 or 100 min incubation. Thin-layer material: (a) silica gel, (b) silanized silica gel (RP-2/8, Merck), (c) chromatography with solvent I; n = number of experiments, ± SD: standard deviation.

^{3}H-Compound	Incubation time [min]	Conc. per layer [pg/cm^2]	Temperature [°C]	^{3}H-Evaporation [%]	n
[^{3}H]HDAc	100	80[a,c]	20	5.1 ± 3.0	26
	1	60[a]	20	6.7 ± 1.7	12
	100	60[a]	20	10.6 ± 2.1	16
^{3}H-Activity from antennae	1	20[a]	0	46.7 ± 7.1	6
	1	20[a]	20	58.5 ± 6.8	6
	1	20[b]	0	52.4 ± 3.0	6
	1	20[b]	20	64.3 ± 0.9	6

the ^{3}H-metabolites from the freshly cut antennae. The experiments with untreated and silanized silica gel clearly show the difference between pheromone and metabolite evaporation (Table IV).

The volatile ^{3}H-metabolites from the freshly cut antennae were shown by LC on Florisil to be hydrophilic (Fig. 2). The highest amount of ^{3}H-activity was eluted with methanol (Fig. 2, B). This peak was identical with the control peak obtained with tritiated methanol as the internal standard (ITS).

Partition experiments between water and diethyl ether were performed (Table V). The volatile ^{3}H-metabolites from freshly cut antennae (Fig. 3) were more soluble in water than the less volatile ^{3}H-metabolites from the dried antennae (Fig. 4).

Pretreatment effects

Dried antennae of male moths of *Antheraea polyphemus* were incubated with [^{3}H]HDAc. About 75% was hydrolyzed to the corresponding fatty alcohol after 30 min incubation (Fig. 4, A; Table VI). The overall half-life of the [^{3}H]HDAc was reached at an incubation time of 2.8 ± 0.3 min. The amounts of the volatile ^{3}H-metabolites were all below 10% and within the error limits imposed by [^{3}H]HDAc evaporation from the TLC plates. About 21% of [^{3}H]Bombykol was oxidized in dried antennae of *Antheraea polyphemus* to the corresponding fatty aldehyde after 30 min incubation (Table VI).

Antennae predried in air (14 days in a desiccator) were incubated with [^{3}H]HDAc. After 30 min incubation 83% was hydrolyzed to the corresponding fatty alcohol (Fig. 4, B).

Pentane pretreated antennae yielded, after 30 min incubation with [^{3}H]HDAc in pentane, 37% fatty alcohols, 20% fatty acids, and 7% fatty acid esters (Fig. 5). The overall half-life of the pheromone was reached at an incubation time of 2.0 ± 0.3 min.

Table V. Partition of ^{3}H-metabolites between diethyl ether and water. Freshly cut and dried antennae were applied and incubated (30 min) with [^{3}H]HDAc (Fig. 1) and extracted as described (Fig. 3 and 4). Aliquots (9×10^4 dpm) of the eluted ^{3}H-compounds were distributed in 2 ml of both solvents in each experiment; n = number of experiments, ± SD: standard deviation.

Phase (20 °C)	^{3}H-Activity [%] from fresh antennae	^{3}H-Activity [%] from dried antennae	n
Diethyl ether	14.7 ± 4.6	97.7 ± 1.7	8
Water	85.3 ± 4.8	2.3 ± 1.5	8

Table VI. Conversion and degradation of [^{3}H]HDAc and [^{3}H]Bombykol in freshly cut and stored antennae of *Antheraea polyphemus*. [^{3}H]HDAc per antenna: 1.48–4.12 ng. Incubation (30 min), elution, TLC, and LSC as described (Fig. 3 and 4). [^{3}H]Bombykol per antenna: 188–469 pg. Incubation (30 min), TLC and LSC as described (Fig. 1). [^{3}H]Bombykol and ^{3}H-metabolites were eluted within 20 min with 2 ml chloroform–methanol (2:1, by vol.); n = number of experiments, ± SD: standard deviation.

^{3}H-Compound	^{3}H-Activity [%] from fresh antennae		^{3}H-Activity [%] from dried antennae	
	[^{3}H]HDAc	[^{3}H]Bombykol	[^{3}H]HDAc	[^{3}H]Bombykol
Acetate	5.9 ± 3.3	–	5.1 ± 4.5	–
Alcohol	4.5 ± 1.2	11.3 ± 1.6	75.3 ± 7.0	51.0 ± 4.9
Aldehyde	0.2 ± 0.1	2.8 ± 2.7	1.7 ± 1.5	21.4 ± 2.9
Acid	3.7 ± 0.6	11.8 ± 1.3	2.2 ± 2.1	4.8 ± 0.6
Ester	2.3 ± 1.1	3.8 ± 1.0	4.3 ± 3.5	3.5 ± 1.0
Residue	11.4 ± 3.7	6.7 ± 0.8	1.6 ± 1.2	4.4 ± 0.9
Volatile	72.0 ± 8.2	63.6 ± 0.6	9.8 ± 4.8	14.9 ± 0.3
	n = 4	n = 3	n = 5	n = 3

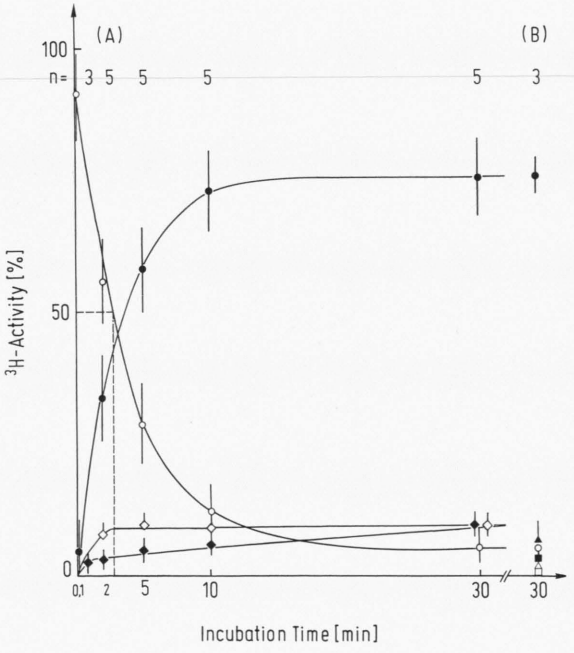


Fig. 4. Time course of pheromone conversion in dried antennae. The ^{3}H-metabolites were recovered from stored (A) and predried (B) antennae. [^{3}H]HDAc per antenna: 1.48–2.14 ng. Incubation, elution, TLC and LSC as described (Fig. 1). [^{3}H]HDAc and the ^{3}H-metabolites were eluted from the antennae within 2 h with chloroform–methanol (2:1, by vol.). Pheromone half-life: 2.8 ± 0.3 min; n = number of experiments, error bars: ± SD. Symbols: see Fig. 3.

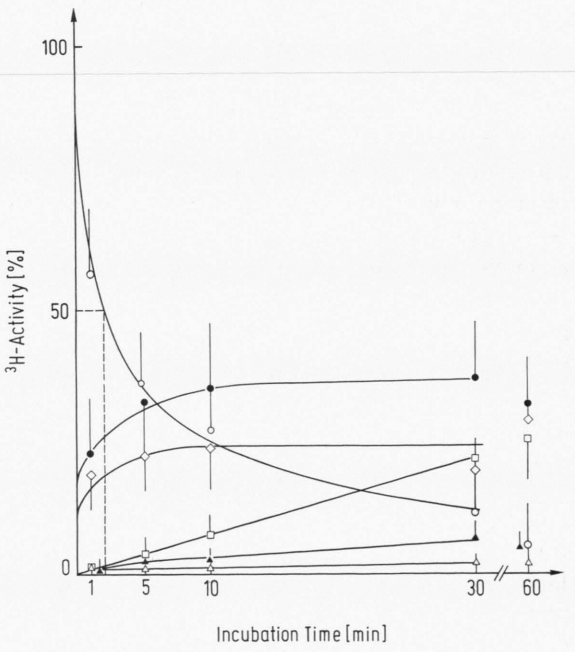


Fig. 5. Time course of pheromone conversion and degradation after pretreatment with pentane. The ^{3}H-metabolites were obtained from freshly cut antennae after pretreatment (10 s) with 50 µl pure pentane and thereafter applied with [^{3}H]HDAc in 10 µl pentane. [^{3}H]HDAc per antenna: 247–311 pg. Elution, TLC and LSC as described (Fig. 1). Pheromone half-life: 2.0 ± 0.3 min; n = 3, error bars: ± SD. Symbols: see Fig. 3.

Discussion

The time course of enzymatic transformation of pheromone acetates and alcohols within intact antennae of *Antheraea polyphemus* can be interpreted in the sense that two stages of pheromone inactivation take place. The first, pheromone conversion, includes the hydrolysis of the pheromone acetates by esterases and the oxidation of the pheromone alcohols by oxidases and/or dehydrogenases to the corresponding fatty acids according to the following scheme:

$$RCH_2OCOCH_3 \rightarrow RCH_2OH \rightarrow [RCHO] \rightarrow RCOOH. \quad (1)$$

The second stage, pheromone degradation, involves the enzymatic oxidation at the carbon-carbon double bonds and/or the total decomposition of the pheromone.

Only a small proportion of the fatty alcohols and/or fatty acids is converted into long-chained fatty acid esters. The fact that fatty aldehydes were found suggests that they are produced as intermediate metabolites. Bombykol is metabolized in a similar but not identical manner in male moths of *Antheraea polyphemus*. This could indicate different substrate specificities of the enzymes involved. Kasang and Kaissling demonstrated that E10,Z12-16:Ol is also converted enzymatically in antennae and other parts of the silkmoth *Bombyx mori* [9, 11, Kasang, in preparation]. Metabolic pathways were discussed in a number of plant and animal tissues where fatty alcohols were irreversibly converted to fatty acids [43]. In insects it is the primary and secondary hydroxyl groups of long-chained fatty alcohols that are preferably oxidized [44–46].

The results of this paper show that ^{3}H-labeled pheromones and isomeric compounds of E6,Z11-16:Ac and E10,Z12-16:Ol are metabolized in living antennae of *Antheraea polyphemus* with a substantial formation of volatile ^{3}H-metabolites. Thin-layer and liquid chromatography have demonstrated that the pheromone acetates were converted to fatty alcohols or aldehydes but not further degraded in dried antennae of *Antheraea polyphemus*. Parallel studies with Bombykol and the isomers of E6,Z11-16:Ac in antennae of *Bombyx mori* have also revealed substantial production of volatile ^{3}H-metabolites (Kasang *et al.*, in preparation).

It has been shown that in antennae of both species of moths the pheromones were mainly degraded to polar volatile compounds. Further experiments (in preparation) will give more information on the nature of these metabolites and the mechanism of pheromone oxidation.

The enzymatic conversion and degradation with overall half-lives of 3 min for [^{3}H]HDAc in male *Antheraea polyphemus* and 2.8 min for [^{3}H]Bombykol in male *Bombyx mori* is strikingly similar. Furthermore, because the metabolic curves of *Antheraea polyphemus* and *Bombyx mori* have different slopes during different incubation periods in half-logarithmic plots [34], each may be interpreted as being the sum of at least two different inactivation processes. The half-lives, estimated at 0.1 min of incubation, are more than 10 times shorter than those found after this period. Presumably, after short incubations of up to 1 min, predominantly those pheromone molecules which have penetrated hydrophilic compartments of the hairs or antennal branches were converted or degraded [37, 39, 42]. It appears that in *Antheraea polyphemus* most of the pheromone molecules were metabolized in the sensory hairs whereas in *Bombyx mori* the main conversion took place in the branches [9, 11, 12, 39].

In dried antennae of *Antheraea polyphemus* the pheromone acetates were mainly hydrolyzed to the corresponding fatty alcohols. In these cases the oxidizing enzymes were probably deactivated by drying. However, the cuticle bound esterases seem to be extremely stable against drying, freezing and hydrocarbons [37, 36]. About 20% of the applied Bombykol was enzymatically oxidized within 30 min to Bombykal. Presumably, this conversion is a result of the specificity of the involved enzyme for fatty alcohols with conjugated and isolated double bonds. However, as yet it is not possible to interpret the production of this extraordinary high amount of aldehyde.

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