Inhibition of sex pheromone production in female lepidopteran moths by 2-halofatty acids

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Abstract Inhibition of sex pheromone production has been observed after topical treatment of pheromonal glands with DMSO solutions of 2-bromohexadecanoic acid, in three lepidopteran insects: *Spodoptera littoralis, Thaumotopoea pityocampa,* and *Bombyx mori.* It has been shown that this effect was brought about by action on the reductases and acetyltransferases of the final steps of the pheromones biosynthesis. Other halofatty acids, such as 2-fluoro- and 2-chlorohexadecanoic acids, were less active than the above bromoderivative whereas 2-bromotetradecanoic acid and 2-bromooctanoic acid exhibited activities quite comparable to the C-16 bromoacid. These results indicate that bromosubstitution is very important for this inhibitory action and chain length is of secondary importance.—**Hernanz, D., G. Fabrias, and F. Camps.** Inhibition of sex pheromone production in female lepidopteran moths by 2-halofatty acids. *J. Lipid Res.* 1997. **38:** 1988–1994.

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Many lepidopteran female sex pheromones are biosynthesized from fatty acids by peroxisomal β-oxidation in conjunction with specific desaturation, reduction, and acetylation reactions (1). It has been shown that in many moths sex pheromone production is controlled by the pheromone biosynthesis activating neuropeptide (PBAN), a peptide hormone produced in the subesophageal ganglion. This hormone, which has been isolated and sequenced from brains of different insect species, is a 33 amino acid peptide with an amidated carboxyl terminal portion and exhibits a high primary sequence homology in all the species so far investigated (2).

The studies carried out in different insects revealed differences in the mode of action of PBAN as far as transport, target site, and the particular enzymes activated in the biosynthetic pathways. Thus, it has been reported that in *Argyrotaenia velutinana* (3), *Helicoverpa zea* (4), and *Mamestra brassicae* (5) the peptide acts at a step in or prior to fatty acid synthesis. However, in *Chrysodeixis chalcites* (6) it has been proposed that PBAN affects a desaturation step, whereas in *Spodoptera littoralis* (7), *Thaumetopoea pityocampa* (8), and *Bombyx mori* (9) this neuropeptide appears to act at the last steps of the biosynthesis, such as reduction of fatty acyl moieites.

We anticipated that the development of specific inhibitors of the enzymes affected by PBAN would be an interesting putative procedure for insect biorational control by blocking the sex pheromone production. Our interest in this topic led us to search for candidate inhibitors from the corresponding literature in the field of mammals. Thus, in previous articles (10) we reported on the activity of several cyclopropene fatty acids, patterned after sterculic acid, as highly efficient insect desaturase inhibitors. However, although these derivatives clearly inhibited the desaturation of selected labeled precursors in the sex pheromone biosynthesis of *S. littoralis* (10) and *T. pityocampa* (8), the production of the natural pheromone was not perturbed (8). In fact, these negative results were in agreement with previous results of our laboratory suggesting that fatty acyl reduc-

Abbreviations: DMSO, dimethyl sulfoxide; FAME, fatty acid methyl ester; GC, gas–liquid chromatography; GC–MS, gas–liquid chromatography coupled to mass spectrometry; PBAN, pheromone biosynthesis activating neuropeptide; SIM, selected ion monitoring. Compounds are abbreviated: 2Br-16:Acid, 2-bromohexadecanoic acid; 2Br-8:Acid, 2-bromooctanoic acid; 2Br-14:Acid, 2-bromotetradecanoic acid; 2Cl-16:Acid, 2-chlorohexadecanoic acid; 2F-16:Acid, 2-fluorohexadecanoic acid; d_5Z9,E11-14:Me, methyl (13,13,14,14,14-$^2H_5$) (*Z,E*)-9,11-tetradecadienoate; d_3E11-14:Me, methyl (14,14,14-$^2H_3$) (*E*)-11-tetradecenoate; d_3Z11-14:Me, methyl (14,14,14-$^2H_3$) (*Z*)-11- tetradecenoate; 13:Me, methyl tridecanoate; d_{31}16:Acid, perdeuterated hexadecanoic acid; d_{25}Z11-16:Me, perdeuterated methyl (*Z*)-11-hexadecenoate; Z9,E11-14:OH, (*Z,E*)-9,11-tetradecadien-1-ol; Z9,E11-14:OAc, (*Z,E*)-9,11-tetradecadienyl acetate; $d_3$14:Acid, (14,14,14-$^2H_3$) tetradecanoic acid; d_5E11-14:Acid, (13,13,14,14,14-$^2H_5$) (*E*)-11 tetradecenoic acid; 13:OAc, tridecyl acetate.

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tases but not desaturases were activated by PBAN in the sex pheromone biosynthesis of these insects (7, 8).

A literature search of possible inhibitors of reductase enzymes revealed a recent report on the activity of 2Br-16:Acid (2-bromohexadecanoic acid) and its derivatives as nonspecific inhibitors of membrane enzymes involved in lipid metabolism in mammals (11). Because fatty acyl reductases are membrane-bound enzymes (12), we envisaged that 2Br-16:Acid might impair the production of natural pheromone in those species in which reduction of acyl moieties is the hormone controlled step in the sex pheromone biosynthetic pathway. Therefore, we undertook the investigation of the putative activity of 2Br-16:Acid on sex pheromone production by the female *Spodoptera littoralis* (Lepidoptera: Noctuidae), one of our model insects, to extend our previous preliminary studies on β-oxidation inhibition by this compound and other α- or β-halofatty acids (13). Additionally, the inhibitory effect of 2Br-16:Acid on sex pheromone production has also been investigated in two other species: *Thaumetopoea pityocampa* and *Bombyx mori*.

MATERIALS AND METHODS

Insects

S. littoralis specimens were reared in our laboratory as reported elsewhere (7). *B. mori* pupae were kindly provided by Dr. Bernard Mauchamp (INRA, Lyon, France) and *T. pityocampa* pupae were supplied by the Spanish Instituto para la Conservación de la Naturaleza (ICONA). All species were maintained in a 16 h:8 h light:dark cycle. Only virgin females were used in the experiments.

Chemicals

Dimethyl sulfoxide (DMSO) was obtained from Sigma (St. Louis, MO), perdeuterated hexadecanoic acid (d_{31}16:Acid) and (14,14,14-$^2H_3$) tetradecanoic acid ($d_3$14:Acid) from IC Chemikalien (Munich, Germany), and 2-bromooctanoic (2Br-8:Acid), 2-bromotetradecanoic (2Br-14:Acid), and 2Br-16:Acid from Aldrich Chemie (Milwaukee, WI). 2-Chlorohexadecanoic acid (2Cl-16:Acid), 2-fluorohexadecanoic acid (2F-16:Acid), 2-bromohexadecan-1-ol, (*Z,E*)-9,11-tetradecadien-1-ol (Z9,E11-14:OH) and (13,13,14,14,14-$^2H_5$) (*E*)-11-tetradecenoic acid (d_5E11-14:Acid) (7) were synthesized in our laboratories.

Treatments

Inhibition of desaturation reactions. These bioassays were performed as described in previous articles (10). Briefly, 60 min before the onset of their second scotophase, *S. littoralis* females were immobilized under netting and their pheromone glands were topically treated with 0.1 μl of DMSO (controls) or 0.1 μl of DMSO containing 1 μg of 2Br-16:Acid. After a 30-min incubation, the suitable tracer in 0.1 μl of DMSO was topically applied to the glands and the insects, still immobilized, were placed back in the rearing chamber. Glands were excised 4 h later and processed for fatty acid methyl ester (FAME) analysis as indicated below. Tracers used were: (*Z*)-11 desaturation of hexadecanoic acid, d_{31}16:Acid (4 μg); (*Z*)- and (*E*)-11 desaturation of tetradecanoic acid, $d_3$14:Acid (1 μg) and (*Z*)-9 desaturation of (*E*)-11-tetradecenoic acid, d_5E11-14:Acid (1 μg).

Inhibition of acetylation of Z9,E11-14:OH. S. littoralis females were decapitated 1 h before the onset of their third scotophase and their pheromone glands were topically treated with a solution of 2Br-16:Acid (0.1 μL, 10 μg/μL in DMSO). Controls received 0.1 μL of DMSO. After 30 min of incubation, treated and control glands were dosed with a solution of Z9,E11-14:OH (0.1 μL, 10 μg/μL in DMSO) and tissues were dissected 30 min later and processed for pheromone analysis. The experiment was carried out in the light.

Inhibition of sex pheromone production. Thirty min before lights-off, intact 2-day-old *S. littoralis* females were immobilized and their pheromone glands were treated with serial dilutions of 2Br-16:Acid (0.1 μL, 1 to 100 μg/μL in DMSO). Controls received 0.1 μL of DMSO. Insects were released at the beginning of the scotophase, placed back in the rearing chamber, and their pheromone glands were dissected after 2 h and processed for pheromone analysis as indicated below. Experiments with other 2-halofatty acids were similarly carried out.

All the experiments that involved PBAN stimulation of pheromone production were performed in the light with decapitated insects as follows: *S. littoralis* 2-day-old insects that had been decapitated 1 h before performing the experiment; *B. mori*, 1-day-old insects decapitated 24 h before the experiment, and *T. pityocampa*, 1-day-old insects decapitated 16 h before the experiment. In all cases, 30 min before the beginning of the dark period the immobilized females were treated with 0.1 μL of a solution of 2Br-16:Acid in DMSO (*S. littoralis*, 20 μg/μL; *B. mori*, 100 μg/μL, and *T. pityocampa*, 100 μg/μL). After incubation with the inhibitor (*S. littoralis* and *B. mori*, 30 min and *T. pityocampa*, 3 h), insects were released, anesthetized by brief cooling and injected with 10 μL of a solution of PBAN in Meyer and Miller's saline (14) (*S. littoralis*, 1 pmol/μL; *B. mori*, 0.4 pmol/μL, and *T. pityocampa*, 5 pmol/μL. Pheromone glands were excised either 1 h (*B. mori*)

or 2 h (*S. littoralis* and *T. pityocampa*) after the injection and processed for pheromone analysis.

Preparation of extracts

For pheromone titer determinations, individual glands were extracted with 100 μL of hexane containing 10 ng of tridecyl acetate (13:OAc) as internal standard (1 h, room temperature) and the samples were analyzed by GC–MS as indicated below.

For FAME analysis, glands were directly submitted to base methanolysis as described elsewhere (15). Methyl tridecanoate (13:Me) (10 ng/gland) was included in the hexane for extraction to allow quantification.

Analytical methods

Analyses were carried out by electron impact GC–MS, using a Fisons gas chromatograph (8000 series) coupled to a Fisons MD800 mass selective detector equipped with the following fused silica capillary columns: nonpolar Hewlett-Packard HP-1 (30 m × 0.20 mm I.D.) or polar SGE BP-20 (30 m × 0.20 mm I.D.). Helium was used as carrier gas at a pressure of 15 Psi. Source temperature was 200°C and injector temperature was 250°C. Unless otherwise indicated, analyses were performed under the selected ion monitoring (SIM) mode. Specific analytical conditions were as follows.

Pheromone analyses. Individual gland extracts were analyzed on the HP-1 column that was programmed from 80°C to 280°C at 10°C/min. The ratios between ions corresponding to the pheromone component ((*Z,E*)-9,11-tetradecadienyl acetate (Z9,E11-14:OAc), 252; (*E,Z*)-10,12-hexadecadien-1-ol, 238) and the internal standard (13:OAc, 182) were calculated. Analyses of *T. pityocampa* pheromone extracts were performed under SCAN mode and the ratios between the areas of the peaks corresponding to (*Z*)-13-hexadecen-11-ynyl acetate and 13:OAc were determined.

(Z)-11 Desaturation of $d_3$11:Acid. Groups of two pheromone glands were extracted and methanolyzed and the ratios between ions 297 and 228 (molecular ions for perdeuterated methyl (Z)-11-hexadecenoate (d_{29}Z11-16:Me) and 13:Me, respectively) were determined. The HP-1 column was used with the following temperature program: 80°C to 170°C at 5°C/min, 15 min at 170°C, and then to 280°C at 10°C/min.

(Z)- and (E)-11 Desaturation of $d_3$14:Acid. Individual glands were extracted and methanolyzed under usual conditions and the ratios between ions 243 and 228 (molecular ions for methyl (14,14,14-$^2H_3$) (*Z*)-11-tetradecenoate (d_3Z11-14:Me) and methyl (14,14,14-$^2H_3$) (*E*)-11-tetradecenoate (d_3E11-14:Me) and 13:Me, respectively) were determined for each isomer. The polar BP-20 column was used with the following program: 80°C to 170°C at 2°C/min, 10 min at 170°C, and then to 260°C at 5°C/min.

(Z)-9 Desaturation of d_5E11-14:Acid. Individual glands were extracted and methanolyzed under standard conditions and the ratios between ions 243 and 228 (molecular ions for methyl (13,13,14,14,14-$^2H_5$) (*Z,E*)-9,11-tetradecadienoate (d_5Z9,E11-14:Me) and 13:Me, respectively) were determined. The HP-1 column was used with the following temperature program: 80°C to 170°C at 5°C/min, 15 min at 170°C, and then to 280°C at 10°C/min.

RESULTS

Inhibition of sex pheromone production

As shown in **Table 1,** 2Br-16:Acid inhibited sex pheromone production in *S. littoralis* in a dose-dependent manner, as concluded from the ratios between ions 252 and 182, corresponding to Z9,E11-14:OAc and 13:OAc, respectively, in the GC–MS analyses.

Other 2-halopalmitic acids, such as 2Cl-16:Acid and 2F-16:Acid were assayed. As shown in Table 1, whereas 2Cl-16:Acid caused a 74% inhibition of pheromone production at 10 μg/gland, it was not active at 1 μg/gland, as occurred with 2F-16:Acid at both doses. Likewise, 2Br-14:Acid and 2Br-8:Acid exhibited activities comparable to 2Br-16:Acid at doses of 1 μg/gland.

In another group of experiments, the effect of 2Br-16:Acid on PBAN stimulation of sex pheromone pro-

TABLE 1. Inhibition of sex pheromone production by 2-halofatty acids in *S. littoralis*

2-Haloacid	Dose	Amounts of Pheromone	n	% Inhibition
	μg			
2Br-16:Acid	0	8.6 ± 0.04[a]	20	
	0.33	9.10 ± 1.13[a]	16	n.i.
	1	2.5 ± 0.3[b]	9	71
	10	1.8 ± 0.2[b]	9	79
2Cl-16:Acid	0	7.1 ± 0.8[a]	13	
	1	8.8 ± 2.2[a]	11	n.i.
	10	1.8 ± 0.8[b]	4	74
2F-16:Acid	1	14.8 ± 3.8[a]	11	n.i.
	10	6.7 ± 1.5[a]	4	n.i.
2Br-14:Acid	0	11.8 ± 1.1[a]	11	
	1	2.5 ± 0.3[b]	9	79
2Br-8:Acid	1	4.0 ± 0.6[c]	9	66

Relative amounts of pheromone were calculated as the ratios (252/182) × 10, where 252 and 182 are the abundance of ions corresponding to Z9, E11-14:OAc and 13:OAc, respectively, in the GC–MS analyses. Results are given as means ± SE; n.i., no inhibition observed; n, number of replicates.

[a], [b], [c], For each experiment, significant differences between means are indicated by different superscript letters; unpaired two-tail *t*-test, $P \leq 0.05$.

TABLE 2. Inhibition of PBAN stimulation of sex pheromone production by 2Br-16:Acid in *S. littoralis*, *B. mori*, and *T. pityocampa*

Species	Amounts of Pheromone: DMSO	n	2Br-15:Acid	n	% Inhibition
S. littoralis	22.0 ± 3.8	4	8.9 ± 2.9[a]	4	60
B. mori	12.4 ± 2.3	8	0.5 ± 0.0[b]	7	96
T. pityocampa	214 ± 35	10	48 ± 14[c]	14	78

Amounts of pheromone were determined from the GC–MS analyses as 10 times the ratios between the areas of the peaks corresponding to the pheromone component and 13:OAc. Results are given as means ± SE. Treatments were performed as indicated in Materials and Methods. Doses of 2Br-16:Acid were: *S. littoralis*, 2 μg; *B. mori* and *T. pityocampa*, 10 μg. Incubations with the inhibitor were carried out for 30 min (*S. littoralis* and *B. mori*) or 3 h (*T. pityocampa*). Amounts of PBAN injected were: *S. littoralis*, 10 pmol; *B. mori*, 4 pmol; *T. pityocampa*, 50 pmol. Pheromone glands were dissected 1 h (*B. mori*) or 2 h (*S. littoralis* and *T. pityocampa*) after PBAN injection and extracted for pheromone titer determination.

[a] $P = 0.033$; [b] $P = 0.0004$; [c] $P = 0.0001$, differences between means were statistically significant; unpaired two-tail *t*-test.

duction was also tested. As shown in **Table 2,** in *S. littoralis* females 2Br-16:Acid caused a 60% inhibition of PBAN ability to induce pheromone production. This effect was also observed in two other moth species, *B. mori* and *T. pityocampa,* which produced significantly less amounts of pheromone when injected with PBAN after treatment with 2Br-16:Acid as compared to controls.

Inhibition of desaturases

The effect of 2Br-16:Acid on the (*Z*)-11 desaturation of hexadecanoic acid was investigated using d_{31}16:Acid as substrate. As shown in **Table 3,** the ratios between ions 297 and 228, corresponding to d_{29}Z11-16:Me and 13:Me, respectively, were similar in extracts from glands that had been treated with 2Br-16:Acid and in controls.

Likewise, 2Br-16:Acid had no effect on the (*Z*)-9 desaturase of (*E*)-11-tetradecenoic acid which was investigated using d_5E11-14:Acid as tracer. Thus, in the GC–MS analyses of FAME extracts, the ratios between the ions 243 and 228, corresponding to d_5Z9,E11-14:Me and 13:Me, respectively, had comparable values in glands treated with 2Br-16:Acid and in controls (Table 3).

Finally, *S. littoralis* pheromone glands treated with 2Br-16:Acid and further incubated with $d_3$14:Acid produced amounts of both d_3Z11-14:Me and d_3E11-14:Me not significantly different from controls, as concluded from the similar ratios between the ions 243 and 228, corresponding to d_3Z11- and d_3E11-14:Me and 13:Me, respectively, obtained for each isomer in the GC-MS analyses (Table 3).

Inhibition of acetyltransferase

The amounts of Z9,E11-14:OAc extracted from *S. littoralis* females that had been treated with Z9,E11-14:OH (1 μg) after a 30-min incubation with 2Br-16:Acid (1 μg) (mean ± S.E.: 3.82 ± 0.84 ng/gland, n = 10) were significantly lower than amounts produced by controls (8.57 ± 2.05 ng/gland, n = 10). In a similar experiment, the amounts of Z9E11-14:OAc obtained from insects that had received 2-bromohexadecan-1-ol and then Z9,E11-14:OH at the same doses were significantly lower (3.89 ± 0.65 ng/gland, n = 12) than amounts found in controls (9.90 ± 2.32 ng/gland, n = 13).

DISCUSSION

In *S. littoralis*, Z9,E11-14:OAc, the main component of the sex pheromone, is biosynthesized from hexadecanoic acid by β-oxidation followed by sequential (*E*)-11 and (*Z*)-9-desaturations and final reduction and acetylation (**Fig. 1**) (7).

In a previous article we reported on the inhibitory effect of 2Br-16:Acid on chain-shortening of 16:Acid in the biosynthesis of *S. littoralis* sex pheromone (13). One of the aims of this work was to test the effect of

TABLE 3. Inhibition of desaturation reactions by 2Br-16:Acid in the biosynthesis of *S. littoralis* sex pheromone

Desaturation Reaction	$M^+/228$: DMSO	n	2Br-16:Acid	n
(*Z*)-11 desaturation of d_{31}16:Acid	2.2 ± 0.3	7	3.2 ± 0.7	9
(*Z*)-11 desaturation of $d_3$14:Acid	5.0 ± 1.6	13	3.9 ± 0.9	11
(*E*)-11 desaturation of $d_3$14:Acid	3.0 ± 0.7	13	3.3 ± 0.6	11
(*Z*)-9 desaturation of d_5E11-14:Acid	35.7 ± 8.2	10	28.3 ± 6.0	15

M^+ corresponds to the molecular ion of the desaturation reaction product (297 for d_{29}Z11-16:Me, (*Z*)-11 desaturation of d_{31}16:Acid; 243 for d_3Z11-14:Me and d_3E11-14:Me, (*Z*)-11 and (*E*)-11 desaturation of $d_3$14:Acid and 243 for d_5Z9,E11-14:Me, (*Z*)-9 desaturation of d_5E11-14:Acid); 228 corresponds to the molecular ion 13:Me, which was used as internal standard. The area of each ion was calculated in the GC–MS chromatograms.

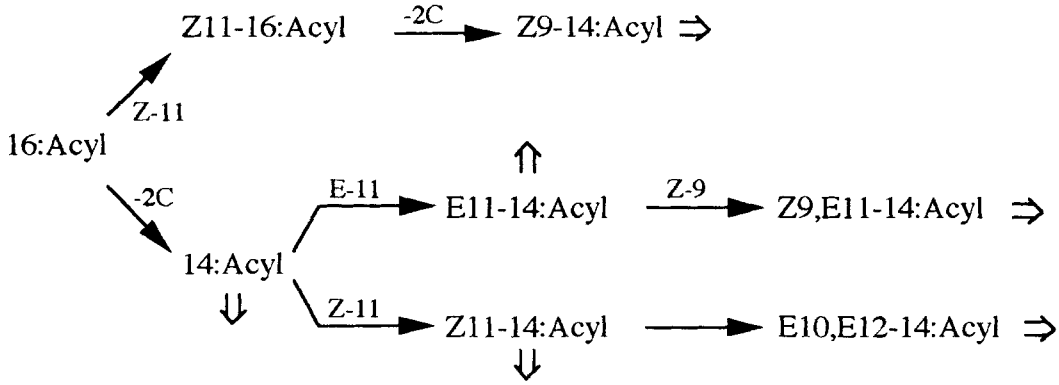

Fig. 1. Biosynthetic pathway of *S. littoralis* sex pheromone blend. Compounds are the acyl derivatives of: 16:Acyl, hexadecanoic acid; Z11-16:Acyl, (Z)-11-hexadecanoic acid; Z9-14:Acyl, (Z)-9-tetradecenoic acid and so on. The reactions involved are: (Z)-11 desaturation (Z11); (E)-11 desaturation (E11); (Z)-9 desaturation (Z9) and β-oxidation (-2C). Open arrows indicate reduction and acetylation.

2Br-16:Acid on other steps of the sex pheromone biosynthetic pathway of this species.

2-Bromofatty acids or their corresponding CoA esters have been shown to exert both specific (16, 17) and nonspecific effects (11, 17, 18) in biological systems. In the last case, authors claim that the inhibitory properties of 2-bromofatty acyl compounds are caused by the ability of the acyl chains to associate with membranes and, thus, to interact closely with membrane proteins. We therefore tested the effect of 2Br-16:Acid on desaturases, other intrinsic membrane proteins involved in the biosynthesis of moth sex pheromones (1). A complex system of desaturases is involved in the biosynthetic pathway of *S. littoralis* sex pheromone (7): a (*Z*)-11 desaturase of hexadecanoic acid, a (*Z*)-9 desaturase of (*E*)-11-tetradecenoic acid and two specific (*Z*)- and (*E*)-11 desaturases of tetradecanoic acid. One of the above-mentioned enzyme activities was affected by 2Br-16:Acid. Therefore, it appears that the effect of 2Br-16:Acid on the insect biosynthetic enzymes is rather specific depending on its affinity for an acyl-chain binding region of the enzyme.

Because, as mentioned in Results, 2Br-16:Acid inhibited sex pheromone production in *S. littoralis* whereas desaturation reactions were not affected, other enzyme(s) acting after the formation of the dienoate intermediate should be inhibited by the bromoacid. Candidate enzymes were the reductase and the acetyltransferase (19).

Reductases are also membrane-bound enzymes that transform acyl-CoA thioesters into the corresponding alcohols, which are immediately transformed into the corresponding acetates by nonspecific acetyltransferases (20, 21). To check whether the acetyltransferase was inhibited by 2Br-16:Acid in *S. littoralis*, pheromone glands were incubated with the bromoacid and then with Z9,E11-14:OH and the amounts of the resulting Z9,E11-14:OAc were then measured. In order to avoid intrinsic production of natural pheromone, these assays were performed with decapitated females (22). These experiments revealed that acetylation of Z9,E11-14:OH was inhibited by 2Br-16:Acid. At this point, we cannot conclude whether 2Br-16:Acid or 2-bromohexadecan-1-ol, resulting from its reduction, is the actual inhibitor, as inhibition of the acetyltransferase by the bromoalcohol does occur (D. Hernanz, unpublished experiments). However, analyses of methanolyzed extracts of pheromone glands treated with 2Br-16:Acid failed to reveal the presence of any 2-bromohexadecan-1-ol. Because, as will be discussed below, the reductase enzyme is also inhibited by 2Br-16:Acid, it seems unreasonable to think that the bromoacid will be reduced into the corresponding alcohol and, therefore, it appears that in vivo inhibition of acetyltransferase by 2Br-16:Acid is caused by the acid itself.

Likewise, also in the processionary moth, *T. pityocampa*, 2Br-16:Acid impaired PBAN-stimulated sex pheromone production. In this species, the sex pheromone biosynthetic pathway from palmitic acid involves desaturation, reduction, and acetylation reactions to give (Z)-13-hexadecen-11-ynyl acetate and it has been shown that the regulatory neuropeptide acts also at the level of reduction of acyl intermediates (8).

Fatty alcohols resulting from reduction of acyl derivatives are never found free in acetyltransferase-containing pheromone glands as they are immediately acetylated to the corresponding acetates. Therefore, *S. littoralis* and *T. pityocampa* were not good models to investigate the effect of 2Br-16:Acid on the reductase, as the resulting alcohols cannot be monitored. Therefore, these experiments were conducted using *B. mori*, whose pheromone gland lacks acetyltransferase enzyme and

reduction of (*E,Z*)-10,12-hexadecadienoic acid to (*E,Z*)-10,12-hexadecadien-1-ol, the main pheromone component, is the last reaction of the biosynthetic pathway (23). Furthermore, such reduction is the step activated by the neurohormone PBAN (24). Therefore, should 2Br-16:Acid have an inhibitory effect on the reductase, it would interfere with PBAN ability to stimulate sex pheromone production. Again, in order to avoid production of intrinsic pheromone, these experiments were carried out with decapitated females (22). The results obtained clearly showed that pheromone production was almost abolished in *B. mori* females that had been treated with 2Br-16:Acid before PBAN injection, thereby indicating that reduction of the (*E,Z*)-10,12-hexadecadienoyl moiety into the corresponding alcohol was inhibited by the bromoacid.

Our next step was to investigate the influence of a halogen atom and chain length on inhibition of sex pheromone production in *S. littoralis*. First, the activities of 2Cl-16:Acid and 2F-16:Acid were determined. As shown in Table 1, these activities were considerably lower than those of the bromoderivative. On the other hand, 2Br-14:Acid and 2Br-8:Acid exhibited activities quite comparable to those of the C-16 analog. These results indicate that bromosubstitution is very important for this inhibitory action and chain length is of secondary importance.

In conclusion, we have demonstrated that topical application on the sex pheromone gland of different amounts of 2-bromohexadecanoic acid and related derivatives thereof reduces pheromone production in the three lepidopteran insects so far investigated, *S. littoralis*, *T. pityocampa*, and *B. mori*. This reduction appears to be mainly originated by inhibition of enzymes implicated in the final steps of the biosynthetic pathway, such as acyl reductases in *B. mori* and, probably also in *S. littoralis* and *T. pityocampa*, although in these last two insects inhibition of acyl transferases cannot be rigorously excluded. The present results might be useful for application as biorational methods of insect-control by preventing the sexual communication of the insect. In preliminary behavioral studies carried out in a windtunnel, *S. littoralis* virgin females treated with 2-bromopalmitic acid were clearly less sexually attractive than untreated females to males of this species, in agreement with the above reported decrease in sex pheromone production. The results of these studies will be reported elsewhere. Further studies along this line are being pursued in our laboratories.

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