Sex Pheromone Biosynthetic Pathway in *Spodoptera littoralis* and Its Activation by a Neurohormone*

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Deuterium-labeled fatty acids have been used to elucidate the sex pheromone biosynthetic pathway in *Spodoptera littoralis*. Label from palmitic acid was incorporated during the scotophase into all the pheromone acetates and their corresponding fatty acyl intermediates. \( (Z,E)-9,11 \)-tetradecadienyl acetate, the major component of the pheromone blend, is synthesized from palmitic acid via tetradecanoic acid, which, by the action of a specific \( (E)-11 \) desaturase and subsequently a \( (Z)-9 \) desaturase, is converted into \( (Z,E)-9,11 \)-tetradecadienoate. By further reduction and acetylation, this compound leads to the diene acetate.

Deuterated precursors applied to the pheromone gland during the photophase were also incorporated into the pheromone. The percentage of labeled \( (Z,E)-9,11 \)-tetradecadienyl acetate relative to natural compound was significantly higher during the light period.

Label incorporation from different intermediates into the pheromone was stimulated by injection of brain-subesophageal ganglion extract during the photophase. The influence of the pheromone biosynthesis-activating neuropeptide on the biosynthetic pathway is discussed.

Chemical elucidation of a large variety of sex pheromones from Lepidoptera (Insecta) has been followed in recent years with increasing interest on the biosynthesis of these specific sexual semiochemicals. The analysis of pheromone gland composition in different species has revealed the occurrence of unusual fatty acids that have been proposed as precursors of pheromone components (1-4). Many lepidopteran sex pheromones are produced by limited \( \beta \) oxidation steps in conjunction with desaturation systems (5, 6). However, only in a few studies has a combination of different fatty acyl intermediates been used to demonstrate experimentally a pheromone biosynthetic pathway (e.g. 7-9).

The regulation of sex pheromone biosynthesis in *Lepidoptera* has also received special attention after Raina and Klun (10) reported that a neurohormone from the head

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*This work was supported by Comisión Asesora de Investigación Científica y Técnica Grant 84/0087, Consejo Superior de Investigaciones Científicas Grant 85/263, and a postdoctoral fellowship (to T. M.) from the Spanish Ministry of Education and Science. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: PBAN, pheromone biosynthesis-activating neuropeptide; Br-SOG, brain-subesophageal ganglion; FAMEs, fatty acyl methyl esters; GLC, gas liquid chromatography-mass spectrometry; MS, mass spectrometry; GLC-MS, coupled gas liquid chromatography-mass spectrometry. The compounds are abbre-viated: C14:Ac, tetradecyl acetate; \( (Z)-9 \) C14:Ac, \( (Z)-9 \) tetradecenyl acetate; \( (Z)-11 \) C14:Ac, \( (Z)-11 \)-tetradecenyl acetate; \( (E)-11 \) C14:Ac, \( (E)-11 \)-tetradecenyl acetate; \( (Z,E)-9,11 \)-C14:Ac, \( (Z,E)-9,11 \)-tetradecadienoate; \( (Z,E)-9,11 \)-tetradecadienyl acetate; \( (Z,E)-9,11 \)-tetradecadienoate; \( (Z,E)-9,11 \)-tetradecadienyl acetate. To abbreviate fatty acids and their corresponding methyl esters, Ac in the above acetates is replaced by acid and Me, respectively (e.g. C14:acid, tetradecanoic acid, C14:Me, methyl tetradecanoate). For the abbreviation of labeled compounds, the same nomenclature is followed preceded by either \( d_0 \) or \( d_2 \) according to the number of deuterium atoms in the \( \omega \) and \( \omega-1 \) positions (e.g. \( d_0 \) C14:acid, [14,14,14-\( ^2 \)H] tetradecanoic acid; \( d_0 \) (E)-11 C14:acid, [15,15,14,14,14-\( ^2 \)H] (E)-11 tetradecanoic acid).
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**Labeled Precursors**

[16,10,16-2H4]hexadecanoic and [14,14,14-2H4]tetradecanoic acids were supplied by IC Chemicalien (München, Federal Republic of Germany).

Deuterated unsaturated fatty acids were prepared by oxidation of the parent alcohols with CrO3 in acetic acid (19), except for [10,11,12-2H3][Z,E]-9,11-tetradecadienil-1-ol, which was oxidized with pyridinium dichromate in dimethylformamide (20).

(Z) and (E) [15,15,15,15-2H4]-11-tetradecenyl-1-ol were prepared by Grignard coupling of [7H]methyl in acetone (25).

Protective group. The above labeled aldehyde was synthesized by anesthetized with CO2 and fixed under netting in such a way that animal with the exception of d16:acid of which 1 pg was applied.

Application of Labeled Precursors—Females were shortly treated with different deuterated fatty acids, considered previously in S. littoralis (3), were found, including (Z)-11-C14:acid, (E)-11-C14:acid, and (Z)-9,11-C14:acid. Likewise, methyl hexadecanoate and tetradecanoate were also present in the methanolyzed extract, which has been found in very low proportions (0.5–1%) (31), was not detected in our strain.

The fatty acid content of sex pheromone glands was also analyzed, and some unusual fatty acyl moieties, identified previously in S. littoralis (3), were found, including (Z)-11-C16:acid, (Z)-9-C14:acid, (Z) and (E)-11-C14:acid, and (Z,E)-9,11-C14:acid (28–31). However, the proportions of these compounds in our strain differ from those reported by Dunkelblum et al. (31), the diene acetate being by far the most abundant compound (about 70%) of the pheromone blend; the other acetates were found in the following proportions: (Z)-11-C14:Ac, about 5%; (E)-11-C14:Ac, about 10%; (Z)-9,11-C14:Ac, about 13%; and C14:Ac, about 1% (14). A minor component of S. littoralis sex pheromone, (Z,E)-9,12-C14:Ac, which has been found in very low proportions (0.5–1%) (31), was not detected in our strain.

**RESULTS**

Elucidation of the Sex Pheromone Biosynthetic Pathway

Analysis of S. littoralis pheromone gland extracts revealed the presence of a previously identified mixture of C14 acetates, C14:Ac, (Z)-9-C14:Ac, (Z) and (E)-11-C14:Ac, and (Z,E)-9,11-C14:Ac (28–31). However, the proportions of these compounds in our strain differ from those reported by Dunkelblum et al. (31), the diene acetate being by far the most abundant compound (about 70%) of the pheromone blend; the other acetates were found in the following proportions: (Z)-11-C14:Ac, about 5%; (E)-11-C14:Ac, about 10%; (Z)-9,11-C14:Ac, about 13%; and C14:Ac, about 1% (14). A minor component of S. littoralis sex pheromone, (Z,E)-9,12-C14:Ac, which has been found in very low proportions (0.5–1%) (31), was not detected in our strain.

The fatty acid content of sex pheromone glands was also analyzed, and some unusual fatty acyl moieties, identified previously in S. littoralis (3), were found, including (Z)-11-C16:acid, (Z)-9-C14:acid, (Z) and (E)-11-C14:acid, and (Z,E)-9,11-C14:acid. Likewise, methyl hexadecanoate and tetradecanoate were also present in the methanolyzed extracts. These compounds were found in the proportions indicated on Table I.

The identification of the above acetates and FAME was accomplished by capillary GLC and GLC-MS, by comparison of their retention times and mass spectra with those of authentic samples.

Virgin females close to the onset of their second scotophase were treated with different deuterated fatty acids, considered...
to be putative intermediates in sex pheromone biosynthesis. The incorporation of deuterium was analyzed in fatty acyl moieties and acetates of pheromone gland extracts. The abundances of deuterated FAME relative to those of the natural compounds, as well as the amounts of labeled (Z,E)-9,11-C14:Ac, were determined for each precursor (Tables II and III).

\([16,16,16-^2H_3]_{\text{Hexadecanoic Acid}}\] - Analyses of sex pheromone extracts of females treated with \(d_3\)-C16:acid revealed that label was incorporated into all the pheromone components studied (Fig. 1). Ions \(m/z = 255\) and 199, corresponding to labeled (Z,E)-9,11-tetradecadienyl and tetradecyl acetates, respectively, appeared approximately 0.05 min before the natural compounds (ions \(m/z = 259\) and 196, respectively). Likewise, ion \(m/z = 197\) was detected at the retention times of deuterated (Z)-9-C14:Ac, (Z)-11-C14:Ac, and (E)-11-C14:Ac. These ions were not observed in controls.

When methanolyzed pheromone gland extracts were analyzed, label was found in all C14 and C16 FAME. Characteristic M+3 ions for deuterated (Z,E)-9,11-C14:Me (\(m/z = 241\)), (Z)-9-C14:Me, (Z)-11-C14:Me, and (E)-11-C14:Me (\(m/z = 243\)), C14:Me (\(m/z = 245\)), and (Z)-11-C16:Me (\(m/z = 271\)) were detected about 0.05 min before those of natural compounds only in experimental extracts (Fig. 2).

\([14,14,14-^2H_3]_{\text{Tetradecanoic Acid}}\] - Sex pheromone from insects treated with \(d_3\)-C14:acid contained labeled tetradecyl, (Z)- and (E)-11-tetradecenyl, and (Z,E)-9,11-tetradecadienyl acetates, as shown by the presence of their characteristic ions \(m/z = 199, 197,\) and 255, respectively, at the expected retention times. It is worth mentioning that deuterated (Z)-9-tetradecenyl acetate was not found in these experiments (Fig. 1). Methanolyzed pheromone gland extracts from experimental females contained the characteristic ions \(m/z = 245, 243,\) and 241 (see above). However, only a small amount of deuterated (Z)-9-C14:Me was detected (Fig. 2).

\([14,14,14-^2H_3]_{\text{(Z)-11-Tetradecanoic Acid}}}\] - The application of \(d_3\)-(Z)-11:C14:acid resulted in the formation of labeled (Z)-9-11-C14:Ac; its characteristic ion \(m/z = 197\) was observed about 0.04 min before natural (Z)-9-11-C14:Ac. No deuterated (Z,E)-9,11-C14:Ac was formed, as concluded from the absence of ion \(m/z = 253\) at the expected retention time (Fig. 1). Analysis of FAME revealed the presence of labeled (Z)-9-11-C14:Me (\(m/z = 243\)) but not that of deuterated (Z,E)-9,11-C14:Me (Fig. 2).

\([14,14,14-^2H_3]_{\text{(Z)-9-Tetradecanoic Acid}}}\] - After treatment with this acid, incorporation was detected only into (Z)-9-11-C14:Ac, as shown by the presence of ion \(m/z = 197\) at the expected retention time.

\([13,13,14,14,14-^2H_5]_{\text{(E)-11-Tetradecanoic Acid}}}\] - Sex pheromone components of insects treated with this acid included labeled (E)-11-tetradecenyl and (Z,E)-9,11-tetradecadienyl acetates, as shown by the appearance of their characteristic M+5 ions, \(m/z = 199\) and 257, respectively, which eluted about 0.08 min before natural (Z,E)-9,11-C14:Ac. No deuterated (Z,E)-9,11-C14:Me was detected (Fig. 2).

\([13,13,14,14,14-^2H_5]_{\text{(Z)-9-Tetradecanoic Acid}}}\] - After treatment with \(d_5\)-Z-9-C14:acid resulted in the formation of labeled (Z)-9-11-C14:Ac, which eluted about 0.08 min before natural compound (\(m/z = 194\)).

\([10,11,12,12-^2H_4]_{\text{(Z,E)-9,11-Tetradecadienoic Acid}}}\] - Sex pheromone analysis of females treated with this intermediate showed the presence of ion \(m/z = 255\), corresponding to \(d_5\) (Z,E)-9,11-C14:Ac, which eluted about 0.03 min before the natural acetate (\(m/z = 252\)). This ion was not observed in controls (Fig. 1).

From the results described above, the sex pheromone biosynthetic pathway depicted in Fig. 3 is proposed, which is analyzed in detail under "Discussion."

**Incorporation of Labeled Intermediates during the Photophase**

Application of deuterated fatty acids was also performed during the photophase, when pheromone biosynthesis is normally not activated and only low levels of pheromone are detected.

GLC-MS analyses of pheromone gland extracts revealed that label from different precursors was also incorporated into fatty acyl intermediates and pheromone acetates during the photophase. The amounts of labeled (Z,E)-9,11-C14:Ac were not statistically different from those detected during the scotophase. However, the relative abundance of labeled pheromone with respect to the natural compound was twice as high during the photophase (Table II).

The relative amounts of label incorporation into different intermediates (FAME) were also determined and compared between photophase and scotophase. Only for the last intermediate of pheromone biosynthesis, (Z,E)-9,11-C14:acid, were significant differences found with all the precursors used (Table III).

**Effect of Br-SOG Extract**

The injection of Br-SOG extracts shortly after treatment with \(d_5\)-C14:acid stimulated label incorporation into pheromone. However, the amounts of \(d_5\)-Z-9,11-C14:Me were significantly lower than in controls (saline-injected females) (Table IV).

Treatment with \(d_5\)-(Z,E)-9,11-C14:acid, prior to Br-SOG injection, also resulted in an increase in labeled pheromone and a decrease of this last precursor (Table IV).

The analysis of FAME from females treated with \(d_5\)-C14:acid and further injected with Br-SOG extracts showed that the amounts of the labeled diene acyl intermediate were significantly lower in those animals than in controls (Table IV).

**Table II**

Incorporation of labeled fatty acids into S. littoralis sex pheromone

Data represent means ± S.E. obtained from four replicates of 10-15 females. Differences between photophase and scotophase are statistically significant when indicated (**, p < 0.01; *, p < 0.02).

| Precursor       | Photophase Mean ± S.E. | %* | Scotophase Mean ± S.E. | %* |
|-----------------|------------------------|----|------------------------|----|
| \(d_5\)-C16:acid| 84 ± 23                | 1.4 ± 0.2 | 75 ± 6                | 0.7 ± 0.1** |
| \(d_5\)-C14:acid| 250 ± 28               | 6.5 ± 0.7 | 227 ± 35              | 3.6 ± 0.8** |
| \(d_5\)-(E)-11-C14:acid | 283 ± 20          | 17.2 ± 0.1 | 737 ± 155             | 8.9 ± 0.5** |
| \(d_5\)-(Z,E)-9,11-C14:acid | 307 ± 21    | 14.3 ± 1.8 | 530 ± 158             | 6.2 ± 1.0** |

* Labeled pheromone is expressed as percentage of the natural compound.
the minor component (Z,E)-9,12-C14:Ac, which could not be detected. Likewise, the fatty acid composition we found in the above acetates, except for the minor component (Z,E)-9,12-C14:Ac, and (Z,E)-9,11-C14:Ac; later, (Z)-9-C14:Ac, (E)-11-C14:Ac, and (Z,E)-9,11-C14:Ac were also identified (29-31). The pheromone produced by the strain used in the present study consists of a mixture of the above acetates, except for the minor component (Z,E)-9,12-C14:Ac, which could not be detected. Likewise, the fatty acid composition we found in pheromone glands is in agreement with that reported by Dunkelblum and Kehat (3).

These researchers proposed that (Z,E)-9,11-tetradecadienyl acetate, the main pheromone component, could be synthesized from palmitic acid by Δ-11 desaturation and further β-oxidation. Specific (E)-11 desaturation of the thus formed (Z)-9-tetradecanoic acid would give rise to (Z,E)-9,11-tetradecadienoic acid, the immediate precursor. Likewise, (Z) and (E)-11-tetradecanoic acids would be formed by the action of two specific (Z) and (E)-11 desaturases on tetradecanoic acid, derived from β-oxidation of palmitic acid.

The results presented in this paper disagree with some of the above assumptions. Label from palmitic acid was incorporated into all pheromone acetates and intermediates above precursors but not to labeled (Z)-9-C14:Ac. These results indicate that C14:acid, formed by β-oxidation of palmitic acid, is probably the substrate of two specific Δ-11 desaturase enzymes, one of them introducing the E double bond and the other one giving rise to the Z isomer. Reduction and acetylation of both (Z) and (E)-11-tetradecanoic acids would produce the corresponding (Z) and (E)-11-C14:Ac. The existence of Z/E isomerasers in the pheromone glands was ruled out, since females treated with d(Z)-11-C14:acid did not produce labeled (E)-11-tetradecenoyl moieties and vice versa. It is worth mentioning that only saturated C14:acid appears to be the substrate of these Δ-11 desaturases, since application of (Z)-9-tetradecanoic acid did not lead to the formation of the corresponding (Z,E)-9,11-C14 diene.

Our data show that palmitic acid is the precursor of (Z,E)-9,11-C14:Ac via tetradecanoic acid and not via (Z)-11-C14:acid. Biosynthetic pathways of pheromones with conjugated diene structures have been proposed in both the silkworm (8) and the codling moth (9); in these species, the second double bond was suggested to be formed by α-oxidation of one of the monoene intermediate allylic positions, followed by 1,4-elimination of water (29). In S. littoralis, however, (Z), (E)-11-tetradecadienyl acetate appears to be synthesized by a sequential introduction of the two double bonds. Accordingly, the action of a specific (Z)-9 desaturase on the previously formed (E)-11-tetradecanoic acid would produce (Z,E)-9,11-tetradecadienoic acid, which would be further reduced and acetylated. This assumption is supported by the fact that sex pheromone glands treated with d(Z)-11-tetradecanoic acid produce labeled (E)-11-tetradecenoyl and (Z,E)-9,11-tetradecadienylacetates. Both the substrate and the stereoselectivity of this (Z)-9 desaturase enzyme are evidenced by the finding that insects receiving d(Z)-11-tetradecanoic acid showed label incorporation into (Z)-11-tetradecenyl acetate, but (Z,Z)-9,11-tetradecadienyl acetate was not identified. Additionally, neither (E,Z)-9,11-C14:Ac nor the (E,E) isomer was found in extracts from insects treated with d(Z)-11-C14:acid and d(E)-11-C14:acid, respectively. The other hand, tetradecanoic acid does not seem to be a suitable substrate for this (Z)-9 desaturase, as concluded from the absence of detectable amounts of labeled (Z)-9-tetradecenyl acetate after treatment with dC14:acid.

Whereas a (E)-9 desaturase has been implicated in the biosynthesis of Cidia pomonella sex pheromone (9), the present evidence of the action of a (Z)-9 desaturase is, to our

### Table III

| Precursor | (Z)-1-C16 | (Z)-9-C14 | C14 | (E)-11-C14 | (Z,E)-9,11-C14 |
|-----------|----------|----------|-----|-----------|---------------|
| dC16:acid | 2.37 ± 0.36 | 1.92 ± 0.12 | 4.20 ± 0.43 | + | 0.32 ± 0.05 |
| dZ(1)-11-C14:acid | (+)* | 3.08 ± 0.11 | - | - | - |
| dC14:acid | - | 0.62 ± 0.12 | (36.88 ± 4.90) | 15.77 ± 2.97 | 1.45 ± 0.17 |
| dE(1)-11-C14:acid | - | - | (40.50 ± 4.40) | 6.67 ± 0.93 |
| d(E)-9,11-C14:acid | - | - | - | (4.72 ± 1.27) |

| Precursor | (Z)-1-C16 | (Z)-9-C14 | C14 | (E)-11-C14 | (Z,E)-9,11-C14 |
|-----------|----------|----------|-----|-----------|---------------|
| dC16:acid | 2.75 ± 0.38 | 1.32 ± 0.16** | 6.07 ± 1.39 | + | 0.19 ± 0.62*** |
| dZ(1)-11-C14:acid | (+) | 5.04 ± 1.27 | - | - | - |
| dC14:acid | - | 0.50 ± 0.05 | (46.90 ± 6.02) | 16.57 ± 2.82 | 0.90 ± 0.10* |
| dE(1)-11-C14:acid | - | - | (44.75 ± 5.15) | 3.90 ± 0.18* |
| d(E)-9,11-C14:acid | - | - | - | (2.77 ± 0.28) |

*Figures within parentheses refer to the applied precursors. +, these values could not be accurately calculated.
-*, no incorporation detected.
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In the light of the above results, a complex system of desaturases is proposed to be involved in the pheromone biosynthesis of S. littoralis: (a) a δ-11 palmitoyl-CoA desaturase, such as the one previously reported in Trichoplusia ni (5); (b) two specific (Z) and (E)-11 desaturases, similar to those implicated in Argyrotaenia velutinana (32), introducing (Z) and (E) double bonds in tetradecanoic acid; and (c) a specific (Z)-9 desaturase of (E)-11-Cl4:Acid.

In our studies on the pheromontropic activity of brain extracts in S. littoralis (14), we showed that the injection of brain-subesophageal ganglion homogenate at the onset of the scotophase restored pheromone production in decapitated females and stimulated pheromone biosynthesis during the photophase. These results suggested that the enzymatic sys-
in pheromone biosynthesis could be activated at any time of the photoperiod cycle.

The second objective of the present study was to gain a better understanding of the biochemical events involved in such activation. In this context, we expected that the analysis of labeled pheromone and intermediates produced from different deuterated precursors during photophase and scotophase could give us some clue to this question. In these experiments, we found that the availability of precursors seems to be important for pheromone production, since application of deuterated intermediates during the photophase resulted in label incorporation into the pheromone, and no significant difference was detected in the amounts of labeled pheromone at both periods of time (Table II). However, since calling behavior and pheromone release occur after lights-off, it cannot be excluded that the amounts of labeled (Z,E)-9,11-C14:Ac produced during the scotophase were higher than those found. Accordingly, some release of labeled pheromone during calling could account for the lower percentage of label found during the scotophase (Table II).

It is worth mentioning that titer of natural pheromone found in those females treated at the onset of scotophase (both controls and experimental animals) were considerably reduced as compared with those characteristic of second scotophase females. In order to see whether these low pheromone levels were due to insect handling, a group of females was treated as those in the experiments (C02-anesthetized and fixed under netting for 30 min), and another group was left undisturbed. The results showed that insect manipulation close to the scotophase may cause a 3-fold reduction in pheromone production.

In order to obtain a more reliable determination of the amounts of labeled pheromone produced, pheromone biosynthesis was stimulated during the photophase by injection of brain extract, which does not induce calling behavior in the recipient females (14). In these experiments, a significant increase of label incorporation into (Z,E)-9,11-C14:Ac was observed from different deuterated intermediates. Titer of natural pheromone were also increased in those animals. These results suggest that, although the supply of precursors may be important for pheromone production, a rate-limiting step may be activated (directly or indirectly) by the PBAN. However, Br-SOG extracts have been used in the present experiments, and brain factors other than PBAN might well be affecting pheromone biosynthesis.

Additionally, the study of FAME profiles in females treated with d5C16:acid, d5C14:acid, or (Z,E)-9,11-C14:acid and further injected with Br-SOG extract showed no significant differences when compared with control animals (injected with saline), except in the amounts of the diene acyl precursor, which were significantly lower in Br-SOG-injected females (Table IV). These results suggest that the transformation of this intermediate into the pheromone might be affected by the PBAN, but pure peptide will be necessary to clarify this point.

In this context, we have observed that as indicated for other moth species, *Hydraecia micacea* (33), the last enzymatic step in *S. littoralis* pheromone biosynthesis, by which alcohols are converted into acetates, is not under hormonal control, since (Z,E)-9,11-tetradecadien-1-01 applied to the pheromone gland was converted to the corresponding acetate regardless of the time of application (photophase or scotophase) (data not shown).

The mechanism(s) by which the PBAN activates pheromone biosynthesis in Lepidoptera is still unknown. It has been indicated that in the redheaded leafroller moth, *A. velatunana*, the brain hormone regulates pheromone production by activating the supply of octadecanoyl and/or hexadecanoyl intermediates (17). In *Heliothis armigera*, however, it has been suggested that the PBAN is involved in the regulation of fatty acid biosynthesis in the sex pheromone gland (15). Results from the present study show that label incorporation from several precursors into the pheromone can be stimulated by Br-SOG extracts. Our data do not enable us to determine which is the precise pathway affected by the PBAN, but we suggest that the reduction step may be affected by the brain factor in *S. littoralis*.

Acknowledgments—We thank M. Luz Gomez for her assistance in the synthesis of labeled precursors. Prof. Wendell L. Roelofs for sending us a manuscript prior to publication, and Dr. Jan A. Veenstra for critical reading of the manuscript.

### Table IV

Effect of Br-SOG injection on incorporation of labeled fatty acids into C14 acyl intermediates and pheromone

| Precursor       | C14:Me (Z)-9,14:Me | (Z,E)-9,11-C14:Me | (Z,E)-9,11-C14:Ac | n |
|-----------------|------------------|------------------|------------------|---|
| d5C16:acid C    | 6.63 ± 1.00      | 2.43 ± 0.33      | 0.74 ± 0.07      | 3 (10–13) |
| E               | 6.50 ± 0.53      | 1.10 ± 0.10      | 0.49 ± 0.04**    | 6 (10–13) |
| d5C14:acid C    | 57.73 ± 5.12     | 16.20 ± 1.53     | 5.30 ± 0.53*     | 7 (5)   |
| d5dienec acid   | 11.57 ± 0.78     | 0.168 ± 0.008    | 3 (6)            |
| E               | 8.07 ± 0.46**    | 0.781 ± 0.12*    | 4 (6)            |

* *No.* of females used/replicate.

* Only natural compound was quantified.

Data are means ± S.E. Percentages relative to the natural compound are indicated in parentheses. C, saline-injected females; E, Br-SOG-injected females. Significant differences between control and experimental animals were found when indicated (*, p < 0.05; **, p < 0.01; *** p < 0.001).
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Sex pheromone biosynthetic pathway in Spodoptera littoralis and its activation by a neurohormone.
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J. Biol. Chem. 1990, 265:1381-1387.

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