Original Article

Cyclopropene Fatty Acid and Amide: Inhibitors of the Pheromone Biosynthesis in *Bombyx mori*

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11,12-Methylenehexadec-11-enoic acid and the amide were synthesized by the method of Baird *et al.* (1992) via a key intermediate, 1,2,2-tribromo-1-butylcyclopropane, which was debrominated and coupled with an alkylhalide resulting in a dialkylcyclopropene. Their activities as inhibitors of pheromone biosynthesis were examined with virgin females of the silkworm moth, *Bombyx mori*, *in vivo*. Bombykol titer diminished in the pheromone glands treated with the cyclopropenes, and experiments utilizing [1-¹⁴C] or [16,16,16-²H₃]-hexadecanoic acids as a biosynthetic precursor showed that the cyclopropenes completely inhibited incorporation of the isotopes into bombykol and (Z)-11-hexadecen-1-ol at the dose of 1 µg per pheromone gland but accelerated incorporation into hexadecan-1-ol. These results revealed the acid and amide with a propene ring at the 11-position are inhibitors of desaturation step(s), Δ11-desaturation and possibly Δ10,12-desaturation, in the biosynthetic pathway. The nitrile derivative with the propene ring did not inhibit the bombykol biosynthesis.

INTRODUCTION

Insect sex pheromones are useful tools for not only monitoring the emergence of pests but also preventing the damage directly by mass trapping and disruption of communication between males and females. The majority of the lepidopterous sex pheromones identified to date were composed of fatty alcohols with one or two C=C bonds and their derivatives (acetates and aldehydes),¹⁾ and it was estimated that their biosyntheses were accomplished by the following four steps: 1) construction of fatty acid(s), 2) desaturation, 3) chain shortening and/or elongation, and 4) modification of the terminal functional group.²⁾ As another approach applying pheromone

research, it is interesting to find an inhibitor that prevents the biosynthetic steps, particularly desaturation. Since not a few pheromones include the C=C bond at a characteristic position (*i.e.* 11-position), it is expected that the inhibitor specifically attacks this insect enzyme system.

Sterculic acid, 9,10-methyleneoctadec-9-enoic acid, was known to inhibit the Δ9-desaturation of octadecanoic acid in vertebrates.^{3,4)} Recently, Camps' group reported that 12,13-methylenehexadec-12-enoic acid and some ring-positional isomers acted as inhibitors of Δ11-desaturase in a noctuid moth, *Spodoptera littoralis*,^{5,6)} the pheromone of which is composed of the acetates of C₁₄ fatty alcohols. This paper deals with the synthesis of 11,12-methylenehexadec-11-enoic acid (**1**) and its derivatives, amide (**2**) and nitrile (**3**), and with the results of their inhibitory activities in the pheromone biosynthesis of the silkworm moth, *Bombyx mori*, the sex

Abbreviations: 16: Acid, hexadecanoic acid (palmitic acid); Z11-16: Acid, (Z)-11-hexadecenoic acid; 16: OH, hexadecan-1-ol; Z11-16: OH, (Z)-11-hexadecen-1-ol; bombykol, (10E,12Z)-10,12-hexadecadien-1-ol.

pheromone of which is a C₁₆ fatty alcohol with a conjugated diene system at the 10,12-position.

MATERIALS AND METHODS

1. Analytical Instruments

The NMR spectra of each compound in CDCl₃ were measured with a JEOL EX 270 Fourier transform spectrometer (270.2 MHz for ¹H, and 67.9 MHz for ¹³C) using TMS as an internal standard. Electron impact GC-MS was accomplished with a JEOL SX-102A mass spectrometer, using an ionization voltage of 70 eV and a TC-FFAP capillary column [0.25 mm i.d. × 30 m, GL Sciences (Tokyo)] for fatty alcohols in pheromone glands and DB-1 capillary column [0.25 mm i.d. × 30 m, J & W Scientific (Folsom, CA, U.S.A.)] for synthetic cyclopropenes. The ion source temperature was 240°C and the column temperature program was 40°C for 1 min, 50°C/min to 140°C, and finally 8°C/min to 220°C. Mass spectra (*m/z* 50–800) were measured twice per minute in a cyclic scan mode lasting from 5 to 20 min after the sample injection. IR spectra were recorded as thin films with a Hitachi 260-50 infrared spectrophotometer. HPLC analysis used a Shimadzu LC-6A system equipped with a UV spectrometric detector (SPD-6A), and a normal phase column [Nucleosil 5 NO₂ (8 mm i.d. × 15 cm)] or a reversed phase column [Nucleosil 5 ODS (8 mm i.d. × 15 cm)] packed by Senshu Kagaku Co. (Tokyo).

2. Synthesis

2.1 2-Bromo-1-hexene (4)

In accordance with the synthesis of 2-bromo-1-nonene,⁷⁾ the olefine (4) was prepared by addition of dry HBr to 1-hexyne in the presence of tetraethylammonium bromide in 46% yield; bp ~50°C/43 mmHg. ¹H NMR (ppm): H-1 5.38 (d, *J*=1.5 Hz) and 5.54 (d, *J*=1.5 Hz), H-3 2.42 (t, *J*=7.5 Hz), H-4 1.54 (tt, *J*=7.5, 7 Hz), H-5 1.34 (tq, *J*=7, 7 Hz), H-6 0.92 (t, *J*=7 Hz). ¹³C NMR (ppm): C-1 116.2, C-2 134.9, C-3 41.2, C-4 30.1, C-5 21.6, C-6 13.8. IR λ_{max} cm⁻¹: 2980, 2950, 2850, 1630, 1460, 1150, 890.

2.2 1,2,2-Tribromo-1-butylcyclopropane (5)

NaOH (20 N aqueous solution, 40 ml) was added to a stirred mixture of olefine (4) (20

mmol, 3.2 g), bromoform (40 mmol, 10.2 g) and cetyltrimethylammonium bromide (0.05 g).⁸⁾ After heating at 70°C for 12 hr, the reaction mixture was extracted with dichloromethane and dried over Na₂SO₄. After removing the unreacted olefine (4) with a vacuum pump, the residue was chromatographed on a silica gel column (50 g), that was developed with 10% benzene in *n*-hexane producing the tribromide (5) in 38% yield. ¹H NMR (ppm): H-3 2.02 (m), H-4 1.69 (m), H-5 1.39 (tq, *J*=7.5, 7 Hz), H-6 0.95 (t, *J*=7.5 Hz), CH₂ of cyclopropane 1.83 (d, *J*=9 Hz) and 1.96 (d, *J*=9 Hz). ¹³C NMR (ppm): C-1 45.8, C-2 33.2, C-3 41.4, C-4 29.8, C-5 22.1, C-6 14.0, CH₂ of cyclopropane 38.0. IR λ_{max} cm⁻¹: 2940, 2910, 2850, 1450, 1010, 690.

2.3 15-Bromo-5,6-methylenepentadec-5-ene (6)

To a dry THF (200 ml) solution of the tribromide (5) (10 mmol, 3.6 g) cooled in a dry ice/acetone bath, *n*-butyllithium (20 mmol, 14 ml of 1.8 M *n*-hexane solution) was added while stirring under nitrogen.⁹⁾ After the addition, the mixture was stirred in a cold bath for 30 min, and then the resulting lithio-cyclopropene was treated with a HMPA (50 ml) solution of 1-bromo-9-iodononane (10 mmol, 3.4 g), which was prepared from 9-bromononan-1-ol by a reaction with a mixture of iodine, triphenylphosphine and imidazole.¹⁰⁾ The reaction mixture was stirred for 1 hr in the same cold condition, and for 5 more hours at room temperature to complete the coupling reaction. The crude product was poured into cold water and extracted with *n*-hexane. After washing with water, 1 N HCl and NaHCO₃ solution, the extract was dried over Na₂SO₄ and concentrated *in vacuo*. The residue was chromatographed on a silica gel column (100 g) developed with *n*-hexane resulting in the bromide (6) in 25% yield. ¹H NMR (ppm): H-1 0.92 (t, *J*=7.5 Hz), H-2 and H-3 1.3–1.6 (m), H-4 and H-7 2.38 (t, *J*=6.5 Hz), H-8–H-13 1.3–1.6 (m), H-14 1.86 (tt, *J*=7, 7 Hz), H-15 3.41 (t, *J*=7 Hz), CH₂ of cyclopropene 0.77 (s). ¹³C NMR (ppm): C-1 13.9, C-2 22.5, C-3 29.5, C-4 and C-7 25.7 and 26.0, C-5 109.3, C-6 109.3, C-8 27.4, C-9–C-13 28.2–29.4, C-14 32.8, C-15 34.0, CH₂ of cyclopropene 7.4. IR λ_{max} cm⁻¹: 2930, 2850, 1670, 1460, 1005, 720, 640, 560.

2.4 11,12-Methylenehexadec-11-enenitrile (3)

A mixture of the bromide (6) (2 mmol, 512 mg), sodium cyanide (4 mmol, 192 mg), water (4 ml), and acetone (20 ml) was stirred at 60°C for 12 hr. Water (20 ml) was added to the mixture, and the product was extracted with *n*-hexane. After the usual workup, the preparative TLC yielded the nitrile (3) in 50% yield. GC-MS: *Rt* 9.14 min, *m/z* 247 (M^+ , 41%), 204 (15%), 81 (base ion). ^1H NMR (ppm): H-2 2.34 (t, $J=7$ Hz), H-3 1.64 (tt, $J=7,7$ Hz), H-4–H-9 1.3–1.6 (m), H-10 and H-13 2.38 (t, $J=6.5$ Hz), H-14 and H-15 1.3–1.6 (m), H-16 0.91 (t, $J=7.5$ Hz), CH_2 of cyclopropene 0.77 (s). ^{13}C NMR (ppm): C-1 119.8, C-2 17.1, C-3 25.4, C-4–C-8 28.7–29.3, C-9 27.3, C-10 26.0, C-11 and C-12 109.3 and 109.4, C-13 25.7, C-14 29.6, C-15 22.5, C-16 13.9, CH_2 of cyclopropene 7.4. IR λ_{max} cm^{-1} : 2930, 2850, 1700, 1660, 1460, 1250.

2.5 11,12-Methylenehexadec-11-enamide (2)

A mixture of the nitrile (3) (0.5 mmol, 128 mg), NaOH (5 *N* aqueous solution, 1.5 ml) and ethanol (15 ml) was stirred at 90°C for 12 hr. Water (15 ml) was added to the mixture, and the product was extracted with ethyl acetate. After the usual workup, the preparative TLC yielded the amide (2) in 26% yield. ^1H NMR (ppm): H-2 2.22 (t, $J=7.5$ Hz), H-3 1.63 (tt, $J=7.5, 7$ Hz), H-4–H-9 1.3–1.6 (m), H-10 and H-13 2.38 (t, $J=6.5$ Hz), H-14 and H-15 1.3–1.6 (m), H-16 0.92 (t, $J=7.5$ Hz), CH_2 of cyclopropene 0.77 (s), NH_2 5.58 (br s) and 5.97 (br s). ^{13}C NMR (ppm): C-1 176.1, C-2 36.0, C-3 25.5, C-4–C-8 29.2–29.4, C-9 27.4, C-10 26.0, C-11 109.3, C-12 109.3, C-13 25.7, C-14 29.5, C-15 22.5, C-16 13.9, CH_2 of cyclopropene 7.4. IR λ_{max} cm^{-1} : 3360, 3190, 2930, 2850, 1660, 1630, 1465, 1420, 1410, 1260, 1090, 1020, 800.

2.6 11,12-Methylenehexadec-11-enoic acid (1)

A mixture of 3 (0.5 mmol, 128 mg), NaOH (10 *N* aqueous solution, 1.5 ml) and ethanol (15 ml) was stirred at 90°C for 12 hr. 2 *N* HCl (15 ml) was added to the mixture, and the product was extracted with ethyl acetate. The preparative TLC yielded the acid (1) in 33% yield. GC-MS (methyl ester): *Rt* 9.57 min, *m/z* 280 (M^+ , 21%), 249 ($[\text{M}-\text{OCH}_3]^+$, 15%) 95 (base ion). ^1H NMR (ppm): H-2 2.35 (t, $J=7.5$ Hz), H-3 1.63 (tt, $J=7.5, 7$ Hz), H-4–

H-9 1.3–1.6 (m), H-10 and H-13 2.38 (t, $J=6.5$ Hz), H-14 and H-15 1.3–1.6 (m), H-16 0.91 (t, $J=7.5$ Hz), CH_2 of cyclopropene 0.77 (s). ^{13}C NMR (ppm): C-1 179.6, C-2 34.0, C-3 24.7, C-4–C-8 29.1–29.5, C-9 27.4, C-10 26.0, C-11 109.3, C-12 109.3, C-13 25.7, C-14 29.5, C-15 22.5, C-16 13.9, CH_2 of cyclopropene 7.4. IR λ_{max} cm^{-1} : 2930, 2850, 1710, 1465, 1455, 1410, 1290, 1005.

3. Labeled Chemicals

[1- ^{14}C]16: Acid (2.07 GBq/mmol), supplied by American Radiolabeled Chemical Inc. (St. Louis, MO, U.S.A.), was dissolved in dimethyl sulfoxide (DMSO) to a concentration of *ca.* 50,000 dpm/ μl . [16,16,16- ^3H]16: Acid (99.7 atom % ^3H), supplied by ISOTEC Inc. (Miamisburg, OH, U.S.A.), was dissolved in the same solvent to a concentration of *ca.* 10 $\mu\text{g}/\mu\text{l}$.

4. Insects

Larvae of *B. mori* [hybrides of Shunrei (female) $\times$ Shogetsu (male)] were reared on a semisynthetic diet (Nippon Nosan-Kogyo, Co., Yokohama) and maintained under 16:8 light-dark cycles at 25°C. Females were selected at the 5th larval instar.

5. Bioassay

5.1 Expt A

Five sex pheromone glands of *B. mori* were topically treated with one of the cyclopropene compounds, 1–3, at the rate of 1, 10 and 100 $\mu\text{g}/\text{gland}$ with 1 μl DMSO 21–23 hr after eclosion (6 hr after onset of the first scotophase). The glands were excised 7 hr after the treatment (5 hr after onset of the second photophase) and separately extracted with *n*-hexane for 30 min. Each extract was injected into a NO_2 column of HPLC in order to analyze bombykol (*Rt*: 11.72 min) quantitatively by monitoring UV 235 nm absorption. The HPLC solvent was 2% 2-propanol in *n*-hexane and the flow rate was 1.5 ml/min.

5.2 Expt B

The pheromone glands were topically treated with one of cyclopropenes 1–3 (1 μg of 1 and 2, and 10 μg of 3) in 1 μl DMSO 25–27 hr after eclosion (2 hr after onset of the second photophase). Three groups of three virgin

females were used per treatment. Two hours after the treatment another DMSO solution of ^{14}C -16: Acid ($1\ \mu\text{l}$, 50,000 dpm) was topically applied to the glands. After a further 3 hr incubation, the glands of each group were excised and extracted with ethyl acetate for 30 min. Fatty alcohols incorporating ^{14}C were purified by the same method established for the research of bombykol biosynthesis using two types of chromatography, normal phase TLC (silica gel 60F₂₅₄, Merck) and reversed phase HPLC with an ODS column.¹¹⁾ Solvent of the HPLC was 7% water in methanol (1.5 ml/min), and bombykol (9.0–10.5 min), Z11-16: OH (10.5–12.5 min) and 16: OH (15.0–17.0 min) were separately collected. Radioactivities incorporated into these compounds were measured by liquid scintillation counting on a Packard model TRI-CARB 300C.¹¹⁾

5.3 Expt C

The inhibitory activities of **1** and **2** were examined using ^3H -16: Acid ($10\ \mu\text{g}$ in DMSO) instead of ^{14}C -16: Acid as in Expt B. After treatment of three pheromone glands with an inhibitor and ^3H -16: Acid, fatty alcohols in the glands were extracted with *n*-hexane for 30 min, and an aliquot of this crude extract (0.06 female equivalent) was injected into a capillary column of GC-MS without any purification. From the residue of the extracts, bombykol was removed by HPLC with the NO_2 column used in Expt A, and the fraction eluted from 9–11 min, which included Z11-16: OH and 16: OH, was obtained. An aliquot

of this fraction (0.6 female equivalent) was also analyzed by GC-MS.

RESULTS

1. Synthesis of 11,12-Methylenehexadec-11-enoic Acid (**1**) and the Derivatives (**2** and **3**)

Compounds with a propene ring at the 11,12-position (**1–3**) were synthesized by modifying the method reported by Baird *et al.*⁹⁾ as shown in Fig. 1. The bromide (**4**) was prepared by the addition of HBr to 1-hexyne in the presence of tetraethylammonium bromide,⁷⁾ and converted into the tribromocyclopropane (**5**) by the addition of dibromocarbene.⁹⁾ The reaction of **5** with two mol equivalents of *n*-butyllithium and followed with one mol equivalent of 1-bromo-9-iodononane yielded the bromide with a propene ring (**6**). An alkyl chain of **6** was elongated by one carbon to yield the nitrile derivative (**3**), which was hydrated to yield the amide (**2**) and further hydrolyzed to yield the acid (**1**). These cyclopropenes, **1–3** and **6**, were characterized by NMR. The signals corresponding to the methylene protons of the ring appeared at δ 0.77 ppm (singlet) in the ^1H NMR spectra and the carbons at δ 7.4 ppm in the ^{13}C NMR spectra.^{9,12)} These uncommon chemical shifts are caused by the diamagnetic anisotropy of the C=C bond.

2. Effect of Cyclopropenes on Bombykol Titer

Figure 2 shows the bombykol titer of glands treated with cyclopropenes. The acid (**1**) and

Fig. 1 The synthetic route for cyclopropene fatty acid (**1**) and the derivatives (**2** and **3**).

Fig. 2 The pheromone titers of the glands treated with cyclopropenes (1–3).

The indicated amount of each cyclopropene in 1 μ l DMSO was topically applied to the sex pheromone gland of *Bombyx mori* and bombykol was quantitatively analyzed as described in Expt A (section 5.1 in MATERIALS AND METHODS). The bars represent the mean $\pm$ S.E. ($n=5$).

amide (2) decreased the titer, and exhibited similar activities at levels of 1–100 μ g/gland. The level at the highest dose was about half of the normal content. The nitrile (3) had no effect on the pheromone titer.

3. Effect of Cyclopropenes on the Incorporation of ^{14}C -16: Acid into Fatty Alcohols

When ^{14}C -16: Acid was topically applied to the pheromone gland in a normal virgin female of *B. mori*, ca. 1% of the radioactivity was observed in bombykol and also in two other fatty alcohols, Z11-16: OH and 16: OH.¹¹⁾ Table 1 shows the incorporation ratios (%) into the three alcohols in the glands pretreated with cyclopropenes (1–3) before the ^{14}C -16: Acid application. The acid (1) strongly inhibited the ^{14}C -incorporation into bombykol and Z11-16: OH at 1 μ g/gland dose, while increased the incorporation into 16: OH three times as much as blank or DMSO-pretreatment. The amide (2) completely inhibited the incorporation into the unsaturated alcohols and accelerated that into the saturated alcohol more than the acid (1) did at the same dose. The nitrile (3) did not inhibit the incorporation into bombykol even at a ten times higher dose.

Table 1 Incorporation ratios (%) of ^{14}C -16: Acid into fatty alcohols of a pheromone gland pretreated with cyclopropene compounds (1–3) in Expt B.

Treatment ^{a)}	Incorporation (%) of ^{14}C -16: Acid		
	Bombykol	Z11-16: OH	16: OH
Blank	0.98 $\pm$ 0.41	0.59 $\pm$ 0.21	1.13 $\pm$ 0.54
DMSO ^{b)}	0.75 $\pm$ 0.23	0.63 $\pm$ 0.17	0.87 $\pm$ 0.20
Acid (1) ^{c)}	0.02 $\pm$ 0.02	0.06 $\pm$ 0.03	2.78 $\pm$ 0.85
Amide (2) ^{c)}	0.00 $\pm$ 0.00	0.02 $\pm$ 0.01	4.52 $\pm$ 0.36
Nitrile (3) ^{d)}	0.58 $\pm$ 0.23	0.45 $\pm$ 0.11	0.87 $\pm$ 0.32

^{a)} Three groups of three insects each were used per treatment.

^{b)} Pretreated with 1 μ l/gland of DMSO only.

^{c)} Pretreated with 1 μ g/gland of the acid (1) or amide (2) dissolved in 1 μ l of DMSO.

^{d)} Pretreated with 10 μ g/gland of the nitrile (3) dissolved in 1 μ l of DMSO.

4. Effect of Cyclopropenes on the Incorporation of ^3H -16: Acid into Fatty Alcohols

Figure 3(A) shows the total ion chromatogram (TIC) of the pheromone gland extracts treated with ^3H -16: Acid and/or cyclopropenes (1 and 2). Although treatment with the

Fig. 3 The GC-MS analyses of the pheromone gland extracts treated with DMSO solution of cyclopropenes and/or $^2\text{H}_3$ -labeled precursor: (A) total ion chromatogram (TIC), (B) mass chromatogram monitoring bombykol at m/z 238 and 241, (C) mass chromatogram monitoring Z11-16: OH at m/z 222 and 225, (D) mass chromatogram monitoring 16: OH at m/z 224 and 227.

The chromatograms of (A) and (B) were obtained from the 0.06 female extract equivalent and those of (C) and (D) from the 0.6 female extract equivalent. Applied chemicals are as follows: a, DMSO only; b, $^2\text{H}_3$ -16 Acid (10 μg); c, acid (**1**) (1 μg); d, acid (**1**) (1 μg) and $^2\text{H}_3$ -16: Acid (10 μg); e, amide (**2**) (1 μg); f, amide (**2**) (1 μg) and $^2\text{H}_3$ -16: Acid (10 μg). $^2\text{H}_3$ -Labeled compounds flowed out from the capillary column a little bit faster than the corresponding unlabeled compounds. Details of the chemical application (Expt C) and the condition of GC-MS analyses are described in MATERIALS AND METHODS (sections 5.3 and 1, respectively).

biosynthetic precursor, 10 $\mu\text{g}/\text{gland}$ of $^2\text{H}_3$ -16: Acid, did not influence the content of each fatty alcohol in the gland, each cyclopropene (1 $\mu\text{g}/\text{gland}$) made the peak of bombykol a little bit smaller and the peak of 16: OH much larger than the blank. Bombykol and that labeled with $^2\text{H}_3$ were monitored by their molecular ions $[\text{M}]^+$ at m/z 238 and 241, respectively (Fig. 3(B)). While analyzing the extract of the glands treated with $^2\text{H}_3$ -16: Acid, the ion at m/z 241 was observed with *ca.* 5% relative intensity of the ion at m/z 238. The value indicates that the incorporation of $^2\text{H}_3$ -16: Acid into bombykol is *ca.* 0.3%. This $^2\text{H}_3$ -incorporation was not detected on the

GC-MS analyses of the extracts of glands pretreated with the cyclopropenes. Z11-16: OH and $^2\text{H}_3$ -Z11-16: OH were monitored by their $[\text{M}-\text{H}_2\text{O}]^+$ at m/z 222 and 225, respectively (Fig. 3(C)). The ion peak at m/z 225, which was observed with *ca.* 10% relative intensity in the glands treated only with $^2\text{H}_3$ -16: Acid, was diminished by pretreatment with acid (**1**) and disappeared by pretreatment with amide (**2**). Monitoring 16: OH and $^2\text{H}_3$ -16: OH by their $[\text{M}-\text{H}_2\text{O}]^+$ at m/z 224 and 227, respectively (Fig. 3(D)), the fragment ion at m/z 227 was observed with a remarkable relative ion intensity (*ca.* 40%) at m/z 224 in the extract of glands treated with the $^2\text{H}_3$ -16: Acid.

When the glands were pretreated with the cyclopropenes, the relative ion intensity at m/z 227 was decreased as shown in this figure. In spite of that the amount of $^3\text{H}_3\text{-16:OH}$, which was calculated with the relative ion intensity and the peak area of the saturated alcohol in TIC, was greatly increased by the pretreatment. The diminution of the ion peak at m/z 227 in Fig. 3(D) was caused by a much larger accumulation of the unlabeled alcohol rather than $^3\text{H}_3\text{-16:OH}$.

DISCUSSION

Based on the incorporation experiments using several precursors labeled with ^{14}C and ^3H , it has been estimated that the biosynthetic pathway of the sex pheromone of *B. mori*, bombykol, begins with the construction of the hexadecanoic moiety with acetyl CoA. This step is followed by $\Delta 11$ -desaturation to (*Z*)-11-hexadecenoic and further by $\Delta 10,12$ -desaturation to (10*E*,12*Z*)-10,12-hexadecadienoic intermediates. Finally, the dienoic acyl moiety is reduced to the primary alcohol.^{13,14} This study shows that the pheromone biosynthesis was strongly inhibited by cyclopropene fatty acid (**1**) in *B. mori* as reported in *S. littoralis*.⁹ Because the virgin females contain not a little amount of bombykol before a treatment of the inhibitor, bombykol was not completely diminished after the treatment of **1** even at a high dose (100 $\mu\text{g/gland}$). The titers were, however, clearly reduced by the inhibition of new supplements with the amount of inhibition depending on the dose level. The amide (**2**) was interestingly revealed to be another inhibitor of the biosynthesis, while the nitrile (**3**) did not show activities in our *in vivo* bioassay system. When a free fatty acid is applied to the pheromone gland, it will be easily metabolized by β -oxidation. The amide seems to be one of the derivatives promising to prevent this metabolism. Both of the incorporation experiments with ^{14}C - and $^3\text{H}_3\text{-16:Acid}$ suggested that **2** is a stronger inhibitor than **1**.

The saturated and monounsaturated alcohols, 16:OH and Z11-16:OH, are present in the pheromone gland of *B. mori*.^{15,16} Although their roles in the mating behavior are not known, these alcohols were produced in

addition to bombykol by reduction of the corresponding saturated and monounsaturated acyl intermediates. Cyclopropenes (**1** and **2**) also suppressed the biosynthesis of Z11-16:OH, indicating that these compounds inhibited the step of $\Delta 11$ -desaturation. Structure-activity relationships of cyclopropenes, which were examined by the desaturation enzyme system of hen liver,¹⁷ suggested that complete coincidence of the position of the propene ring and the double bond position of a desaturated product is not necessary to inhibit the enzyme. The propene ring positions of **1** and **2** indicate that the step of $\Delta 10,12$ -desaturation is also inhibited by the cyclopropenes. We are going to examine the effect of cyclopropenes on the incorporation of labeled Z11-16:Acid into bombykol in order to reveal their target step. The blocking of the unsaturation step(s) caused accumulation of 16:OH, and also high level incorporation of the labels into 16:OH. The cyclopropenes did not inhibit the reduction of the saturated acyl intermediate as expected. This result suggests that the reduction step in bombykol biosynthesis is also undisturbed by cyclopropenes.

We have investigated the bombykol biosynthesis using ^{14}C -labeled precursors. This time incorporation of $^3\text{H}_3\text{-16:Acid}$ into bombykol was successfully detected by the technique of capillary GC-MS, which also informed about the structure and quantity of unlabeled compounds. Results of Expt B tracing ^{14}C coincide well with those of Expt C tracing ^3H , although the pheromone glands were treated with a *ca.* one hundred times larger amount of the precursor in the latter experiment. Our preliminary assay indicated that 10 $\mu\text{g/gland}$ application is necessary for a reliable and optimal observation of the incorporation of $^3\text{H}_3\text{-16:Acid}$ into bombykol and Z11-16:OH. The biosynthetic system may not have been disturbed by this treatment, since the TIC pattern of gland extract treated with the acid was almost as same as that of the blank. If the damage of biosynthetic systems can be kept to a minimum, experiments with ^3H -labeled precursors are informative in the bioassay of inhibitors.

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要 約

シクロプロペン構造を有する脂肪酸とそのアミド体: カイコガ性フェロモン合成の阻害剤

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11, 12-メチレンヘキサデク-11-エノイック酸とそのアミド体を, トリプロモシクロプロパン化合物を脱プロム化後アルキル化する方法 (Baird ら, 1992) を用いて合成した. それら化合物のフェロモン合成に及ぼす影響をカイコガ雌を用いて検討したところ, フェロモン腺への処理はボンビコール含量の低下をもたらした. また, ^{14}C 標識あるいは ^3H 標識ヘキサデカン酸を利用した実験において, 上記のシクロプロペン化合物をフェロモン腺あたり $1\text{ }\mu\text{g}$ 前処理すると, 生合成前駆体のボンビコールおよび (Z)-11-ヘキサデセノールへの変換が完全に抑えられ, ヘキサデカノールへの変換は逆に促進された. 以上の結果より, これら 11-位にシクロプロペン構造をもつ化合物は, ボンビコール生合成の不飽和化の過程 (11-位とおそらく 10, 12-位不飽和化反応) を阻害することが明らかになった. 一方, ニトリル誘導体はフェロモン生合成阻害活性を示さなかった.