Structure–Activity Relationships of Cyclopropene Compounds, Inhibitors of Pheromone Biosynthesis in *Bombyx mori*

Tetsu Ando,* Ryuta Ohno, Kazuhisa Ikemoto, and Masanobu Yamamoto

Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

According to the synthetic route for 11,12-methylenehexadec-11-enoic acid [10-(2-butyl-1-cyclopropenyl)decanoic acid] and the amide, their related cyclopropene compounds, which possessed a propene ring at the 7,8-, 9,10-, or 13,14-position in a C_{16} chain and the 11,12-position in a C_{14} or C_{18} chain, were synthesized via the corresponding 1-alkyl-1,2,2-tribromocyclopropane. Their activities as biosynthetic inhibitors of bombykol [(10*E*,12*Z*)-10,12-hexadecadien-1-ol; sex pheromone of the silkworm moth *Bombyx mori* L.] were measured with virgin female silkworm moths in vivo. The 7,8-methylene compounds were inactive even at the dose of 10 μg/gland, but other compounds at 1 μg/gland inhibited the conversion of [16,16,16-$^2H_3$]hexadecanoic acid to bombykol to some extent. Each amide showed stronger inhibitory activity than the corresponding acid, and the 11,12-methylene amide with a C_{16} chain was the strongest (I_{50} = 0.016 μg/gland) among the tested compounds. Furthermore, experiments comparing the incorporation of [1-^{14}C]hexadecanoic acid into bombykol and another alcohol component in the pheromone gland, (*Z*)-11-hexadecen-1-ol, suggested that the Δ11-desaturation was blocked by 9,10- and 11,12-methylene compounds and the subsequent Δ10,-12-desaturation by 11,12- and 13,14-methylene compounds.

Keywords: *Cyclopropene; desaturase pheromone biosynthesis; cyclopropenes structure–activity relationships; bombykol biosynthesis; Δ11-desaturation*

INTRODUCTION

The female moth of the silkworm *Bombyx mori* L. secretes sex pheromone bombykol from its pheromone gland in the abdominal tip to communicate with the male moth. Biosynthesis of bombykol starts from acetyl CoA with the construction of a saturated acyl compound with a C_{16} straight chain (maybe CoA of 16:Acid), into which a C=C bond with (*Z*)-configuration is introduced at the 11-position by a Δ11-desaturase. The (*Z*)-11-hexadecenoic intermediate is converted into a conjugated dienic acyl compound by a Δ10,12-desaturase, and the acyl moiety is finally reduced to a primary hydroxyl group to produce the fatty alcohol pheromone (Ando et al.1988b).

If pheromone biosynthesis can be blocked by an inhibitor, chemical communication between female and male moths will be interrupted and their chance of mating is expected to be very low. It appears promising that a chemical that prevents the desaturation step can be found because many lepidopterous pheromones include the C=C bond at a characteristic position and an inhibitor might specifically attack the insect enzyme system. Sterculic acid, 9,10-methyleneoctadec-9-enoic acid [9-(2-octyl-1-cyclopropenyl)octadecanoic acid], is known to inhibit the Δ9-desaturation of octadecanoic acid in vertebrates (Raju and Reiser, 1967; Fogerty et al., 1972). Recently, we reported that 11,12-methylenehexadec-11-enoic acid [10-(2-butyl-1-cyclopropenyl)decanoic acid, **1c**] and the amide derivative (**2c**) acted as inhibitors of bombykol biosynthesis (Ando et al., 1995). In this study we investigated the syntheses and inhibitory activities of several cyclopropanes to define their structure–activity relationships.

* Author to whom correspondence should be addressed [telephone (0423) 67-5698; fax (0423) 60-8830; e-mail antetsu@cc.tuat.ac.jp].

MATERIALS AND METHODS

Analytical Instruments. The NMR spectra of each compound in $CDCl_3$ were measured with JEOL EX 270 Fourier transform spectrometer (270.2 MHz for ^{1}H, and 67.9 MHz for ^{13}C) using tetramethylsilane (TMS) as an internal standard. Electron-impact GC-MS was accomplished with a JEOL SX-102A mass spectrometer, with ionization voltage of 70 eV and a TC-FFAP capillary column (0.25 mm i.d. × 30 m; GL Sciences, Tokyo, Japan). 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. The Shimadzu LC-6A system used for HPLC analyses was equipped with a UV spectrometric detector (SPD-6A) and either a normal-phase column (Nucleosil 5 NO_2; 8 mm i.d. × 15 cm) or a reversed-phase column (Nucleosil 5 ODS; 8 mm i.d. × 15 cm) packed by Senshu Kagaku Company (Tokyo, Japan). The solvent for the NO_2 column was 2% 2-propanol in *n*-hexane (flow rate, 1.5 mL/min). The retention time (R_t) of bombykol detected at UV 235 nm was 11.72 min. For the ODS column, the solvent was 7% water in methanol (flow rate, 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 collected separately.

Cyclopropene Compounds. In our previous paper (Ando et al., 1995), syntheses of 11,12-methylenehexadec-11-enoic acid (**1c**) and 11,12-methylenehexadec-11-enoamide (**2c**) were described in detail. According to the method for these compounds, their ring positional isomers were synthesized as shown in Figure 1. Namely, 2-bromo-1-decyne (**4a**) was prepared by the addition of dry HBr to 1-decyne (**3a**) in the presence of tetraethylammonium bromide (Cousseau, 1980) and converted into 1,2,2-tribromo-1-octylcyclopropane (**5a**) by the addition of dibromocarbene (Baird et al., 1985). The reaction of **5a** with 2 mol equivalents of *n*-butyllithium followed by 1 mol equivalent of 1-bromo-5-iodopentane yielded 1-bromo-6,7-methylenepentadec-6-ene (**6a**). An alkyl chain of the bromide (**6a**) was elongated by one carbon to yield 7,8-methylenehexadec-7-enenitrile (**7a**), which was hydrated to yield 7,8-methylenehexadec-7-enoamide (**2a**) and further hydrolyzed to yield 7,8-methylenehexadec-7-enoic acid (**1a**). 9,-10-Methylenes with a C_{16} chain (**1b** and **2b**) and 11,12-

S0021-8561(95)00757-6 CCC: $12.00

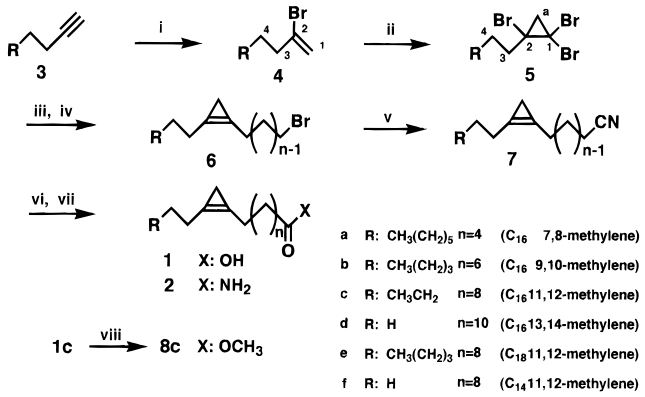

Figure 1. Synthetic route for cyclopropene fatty acids (**1a**−**f**), the amide derivatives (**2a**−**f**), and methyl ester (**8c**): (i) HBr/$Et_4N^+ \cdot Br^-/CH_2Cl_2$; (ii) $CHBr_3/C_{16}H_{33}N^+(CH_3)_3 \cdot Br^-$/aq. NaOH; (iii) 2eq. BuLi/HMPA/THF; (iv) $ICH_2(CH_2)_{n-1}CH_2Br$; (v) NaCN/acetone-H_2O; (vi) NaOH/EtOH; (vii) HCl; (viii) CH_2N_2/EtOEt.

methylenes with a C_{18} chain (**1e** and **2e**) were synthesized by the same manner starting from 1-octyne (**3b**), and 13,14-methylenes with a C_{16} chain (**1d** and **2d**) and 11,12-methylenes with a C_{14} chain (**1f** and **2f**) were synthesized from 1-butyne (**3d**). Treatment of **1c** with diazomethane yielded methyl 11,-12-methylenehexadec-11-enoate (**8c**). After confirming the chemical structures by ^1H and ^{13}C NMR (see Table 2), these synthetic cyclopropenes were dissolved in dimethyl sulfoxide (DMSO) to bioassay their inhibitory activities against bombykol biosynthesis.

Labeled Chemicals. [16,16,16-$^2H_3$]16:Acid (99.7 atom% ^2H), supplied by ISOTEC Inc. (Miamisburg, OH), was dissolved in DMSO at a concentration of ~10 μg/μL. [1-^{14}C]16:Acid (2.07 GBq/mmol), supplied by American Radiolabeled Chemical Inc. (St. Louis, MO), was dissolved in the same solvent at a concentration of ~50 000 dpm/μL.

Insects. Larvae of *B. mori* [hybrids of Shunrei (female) × Shogetsu (male)] were reared on a semisynthetic diet (Nippon Nosan-Kogyo Company, Yokohama, Japan) under 16 h light−8 h dark cycles at 25 °C. Female pupae and moths were incubated under the same conditions.

Bioassay with $^2H_3$-16:Acid (Experiment A). The pheromone gland was topically treated with one of the cyclopropenes [**1a**−**f**, **2a**−**f**, and **8c** (10−0.005 μg)] in 1 μL of DMSO, 25−27 h after eclosion (2 h after onset of the second photophase). Three groups of three virgin females were used per treatment. Two hours after the treatment, another DMSO solution (1 μL) of $^2H_3$-16:Acid (10 μg) was topically applied to the glands. After a further 3 h of incubation, the glands of each group were excised and extracted with ethyl acetate for 30 min. The extract was injected into a normal-phase column of HPLC, and pentadecan-1-ol (10 ng) was added to the recovered bombykol fraction as the internal standard. An aliquot of this fraction was analyzed by GC-MS in a selected-ion-monitoring (SIM) mode. Peak areas of the $[M - H_2O]^+$ ion of pentadecan-1-ol at m/z 210 (R_t, 12.5 min) and M^+ ion of $^2H_3$-bombykol at m/z 241 (R_t, 16.2 min) were measured to quantitatively analyze the bombykol incorporating $^2H_3$-16:Acid. The inhibitory activity (I_{50} value) of each cyclopropene was calculated by comparing its incorporation in the females treated with the inhibitor to that in females treated only with DMSO before the application of $^2H_3$-16:Acid. The average amount of $^2H_3$-bombykol extracted from the female glands untreated with the cyclopropene compounds was 26 ± 5 ng/gland.

Bioassay with ^{14}C-16:Acid (Experiment B). The inhibitory activities of **1a**−**f**, **2a**−**f**, and **8c** were also examined with ^{14}C-16:Acid (50 000 dpm in 1 μL of DMSO) instead of $^2H_3$-16:Acid, as in Experiment A. After treatment of three pheromone glands with an inhibitor and ^{14}C-16:Acid, fatty alcohols incorporating ^{14}C were extracted with ethyl acetate for 30 min and purified by the same method established for the research of bombykol biosynthesis by two types of chromatography, normal-phase TLC (silica gel 60F_{254}, Merck) and reversed-phase HPLC with an ODS column (Ando et al., 1988a, 1995). Radioactivities incorporated into bombykol, Z11-16:OH, and 16:OH were measured by liquid scintillation counting on a Packard model TRI-CARB 300C.

Table 1. NMR Assignments of Synthetic Compounds 4a−d and 5a−d

	NMR chemical shifts (ppm) for indicated position										
compd	1	2	3	4	5	6	7	8	9	10	CH_2[a]
					[A] ^1H NMR						
4a[b]	5.38 (d) 5.55 (d)		2.41 (t)	1.55 (tt)	~1.3	~1.3	~1.3	~1.3	~1.3	0.88 (t)	
4b[c]	5.38 (d) 5.55 (d)		2.41 (t)	1.53 (tt)	~1.3	~1.3	~1.3	0.89 (t)			
4c[d]	5.38 (d) 5.54 (d)		2.42 (t)	1.54 (tt)	1.34 (tq)	0.92 (t)					
4d[e]	5.37 (d) 5.56 (d)		2.46 (q)	1.13 (t)							
5a[f]			2.0 (m)	1.7 (m)	~1.3	~1.3	~1.3	~1.3	~1.3	0.89 (t)	1.84 (d) 1.94 (d)
5b[g]			2.0 (m)	1.7 (m)	~1.3	~1.3	~1.3	0.90 (t)			1.82 (d) 1.95 (d)
5c[h]			2.0 (m)	1.7 (m)	1.39 (tq)	0.95 (t)					1.83 (d) 1.96 (d)
5d[i]			2.02 (dq) 2.16 (dq)	1.25 (t)							1.83 (d) 1.96 (d)
					[B] ^{13}C NMR						
4a	116.2	135.0	41.5	28.4	27.9	29.2[j]	29.3[j]	31.9	22.7	14.1	
4b	116.2	135.0	41.5	27.9[j]	28.1[j]	31.5	22.6	14.1			
4c	116.2	134.9	41.2	30.1	21.6	13.8					
4d	115.2	136.3	35.0	13.2							
5a	45.9	33.2	41.7	29.0[j]	29.2[j]	29.4[j]	27.7	31.8	22.6	14.1	38.1
5b	45.9	33.2	41.7	28.6	27.7	31.7	22.6	14.1			38.1
5c	45.8	33.2	41.4	29.8	22.1	14.0					38.0
5d	47.0	33.0	35.4	12.0							37.9

[a] CH_2 of propane ring. [b] $J_{1,1} = 1.5$ Hz, $J_{3,4} = 7.5$ Hz, $J_{4,5} = 7$ Hz, $J_{9,10} = 6.5$ Hz. [c] $J_{1,1} = 1.5$ Hz, $J_{3,4} = 7.5$ Hz, $J_{4,5} = 7$ Hz, $J_{7,8} = 6.5$ Hz. [d] $J_{1,1} = 1.5$ Hz, $J_{3,4} = 7.5$ Hz, $J_{4,5} = 7$ Hz, $J_{5,6} = 7$ Hz. [e] $J_{1,1} = 1.5$ Hz, $J_{3,4} = 7.5$ Hz. [f] $J_{9,10} = 6.5$ Hz, $J_{a,a} = 9$ Hz. [g] $J_{7,8} = 6.5$ Hz, $J_{a,a} = 9$ Hz. [h] $J_{4,5} = 7$ Hz, $J_{5,6} = 7.5$ Hz, $J_{a,a} = 9$ Hz. [i] $J_{3,3} = 14.5$ Hz, $J_{3,4} = 7$ Hz, $J_{a,a} = 9$ Hz. [j] Assignments for these carbons are not unambiguous and may be reversed in the same row.

Table 2. NMR Assignments of Synthetic Cyclopropene Compounds 1a−d and 2a−d

	NMR chemical shifts (ppm) for indicated position									
compd	1	2	3	=C	———CH_2———	CH_2	14	15	16	CH_2[a]
					[A] ^{1}H NMR					
1a		2.36 (t)	1.64 (tt)		2.37 (t)	∼1.5	∼1.3	∼1.3	0.88 (t)	0.78
1b		2.36 (t)	1.64 (tt)		2.37 (t)	∼1.5	∼1.3	∼1.3	0.89 (t)	0.77
1c		2.35 (t)	1.63 (tt)		2.38 (t)	∼1.5	∼1.3	∼1.3	0.91 (t)	0.77
1d		2.35 (t)	1.63 (tt)		2.38 (t)	∼1.5		2.39 (q)	1.13 (t)	0.78
2a		2.23 (t)	1.64 (tt)		2.37 (t)	∼1.5	∼1.3	∼1.3	0.88 (t)	0.78
2b		2.23 (t)	1.64 (tt)		2.37 (t)	∼1.5	∼1.3	∼1.3	0.89 (t)	0.77
2c		2.22 (t)	1.63 (tt)		2.38 (t)	∼1.5	∼1.3	∼1.3	0.92 (t)	0.77
2d		2.22 (t)	1.64 (tt)		2.38 (t)	∼1.5		2.39 (q)	1.13 (t)	0.78
					[B] ^{13}C NMR					
1a	179.2	33.9	24.5	108.9	25.8	27.0	31.9	22.7	14.1	7.4
				109.7	26.0	27.4				
1b	179.9	34.0	24.6	109.2	26.0	27.3	31.7	22.6	14.1	7.4
				109.5	26.1	27.3				
1c	179.6	34.0	24.7	109.3	25.7	27.4	∼29.5	22.5	13.9	7.4
				109.3	26.0					
1d	179.2	33.9	24.7	109.1	26.0	27.5		19.7	12.0	7.4
				110.6						
2a	175.4	35.8	25.3	109.0	25.8	27.1	31.9	22.7	14.1	7.4
				109.7	26.0	27.4				
2b	175.8	35.9	25.5	109.2	26.0	27.3	31.7	22.6	14.1	7.4
				109.5	26.1	27.3				
2c	176.1	36.0	25.5	109.3	25.7	27.4	∼29.5	22.5	13.9	7.4
				109.3	26.0					
2d	175.6	35.9	25.5	109.1	26.0	27.5		19.7	12.0	7.4
				110.6						

[a] CH_2 of propene ring.

RESULTS

Synthesis of Cyclopropene Compounds. Cyclopropene acids (**1a**−**f**) and amides (**2a**−**f**) were synthesized by a modification of the method reported by Baird et al. (1992), as shown in Figure 1. Chemical structures of all the synthetic compounds were confirmed by ^{1}H and ^{13}C NMR. The NMR assignments of synthetic intermediates **4a**−**d** and **5a**−**d** are shown in Table 1, and those of the characteristic signals of the final products **1a**−**d** and **2a**−**d**, which included the unique methylene signals of the propene ring (δ 0.77−0.78 ppm in ^{1}H NMR and δ 7.4 ppm in ^{13}C NMR) caused by the diamagnetic anisotropy of the C=C bond, are shown in Table 2. The NMR spectra of 11,12-methylenes with a C_{18} chain (**1e** and **2e**) and 9,10-methylenes with a C_{16} chain (**1b** and **2b**) are quite similar because these compounds include the same terminal alkyl structure. Furthermore, 11,12-methylenes with a C_{14} chain (**1f** and **2f**) showed almost the same NMR data as those of 13,14-methylenes with a C_{16} chain (**1d** and **2d**), exceptfor CH_2 signals of the long chains in a side of the acyl group. Yields of the acids (**1a**−**f**) and amides (**2a**−**f**) were ∼0.7 and 0.5%, respectively, from the corresponding 1-alkynes (**3a**−**d**).

Experiment A. $^2H_3$-Labeled bombykol in the pheromone gland treated with $^2H_3$-16:Acid was quantitatively measured by GC-SIM analyses with pentadecan-1-ol as an internal standard. When the $^2H_3$-16:Acid was applied at 10 μg/gland in the most fertile period of bombykol biosynthesis (Ando et al., 1988a), 0.26% of it was incorporated into bombykol. Inhibitory activities of the synthetic cyclopropene compounds were evaluated based on this rate, and I_{50} (μg/gland) values are shown in Table 3 (doses at these levels block half of the incorporation observed for untreated females). Among the cyclopropene compounds with a C_{16} chain tested in this study, 11,12-methylenes [**1c** (I_{50} = 0.040 μg/gland) and **2c** (I_{50} = 0.016 μg/gland)] were the strongest inhibitors. 9,10-Methylenes (**1b** and **2b**) moderately inhibited the incorporation, whereas activities of 13,14-methylenes (**1d** and **2d**) were very weak. Although 11,12-methylenes with a C_{18} chain (**1e** and **2e**) and a C_{14} chain (**1f** and **2f**) showed rather strong activities, I_{50} values of **1c** and **2c** with a C_{16} chain were much smaller than those of the compounds. Each amide more potently blocked bombykol biosynthesis than the corresponding acid. The activity of methyl ester (**8c**) was weaker than the parent acid **1c**. 7,8-Methylenes (**1a** and **2a**) were inactive at the dose of 10 μg/gland.

Table 3. I_{50} (μg/Gland) Values of Cyclopropene Compounds[a] (1a−f and 2a−f) Blocking the Incorporation of $^2H_3$-16:Acid into Bombykol

inhibitor; carbon length and position of cyclopropene		I_{50} (μg/gland)	
		acid (**1**)	amide (**2**)
C_{16}	7,8-methylene (**a**)	> 10	> 10
C_{16}	9,10-methylene (**b**)	0.73	0.23
C_{16}	11,12-methylene (**c**)	0.040	0.016
C_{16}	13,14-methylene (**d**)	2.2	0.50
C_{18}	11,12-methylene (**e**)	0.32	0.17
C_{14}	11,12-methylene (**f**)	0.21	0.10

[a] The I_{50} value of methyl 11,12-methylenehexadec-11-enoate (**8c**) is 0.15 μg/gland.

Experiment B. The inhibitory activities of the cyclopropene compounds were also compared in the experiments using ^{14}C-16:Acid. In the pheromone gland of *B. mori*, the ^{14}C-labeled acid was converted into bombykol and two other fatty alcohols, Z11-16:OH and 16:OH, occurring specifically in the gland. Their ^{14}C-incorporation, which can be easily counted because of their good separation by HPLC, is ∼1% in the normal virgin female (Ando et al., 1988a). The incorporation ratios (%) into the three alcohols in glands pretreated with 1 μg of each cyclopropene compound (**1a**−**f**, **2a**−**f**, and **8c**) before the ^{14}C-16:Acid application are shown in Figure 2. The effect of these cyclopropenes on the ^{14}C-incorporation into bombykol is similar to that on the $^2H_3$-incorporation examined in Experiment A. Their inhibitory activities decreased in the following order: 11,12-methylenes (**1c** and **2c**), 9,10-methylenes (**1b** and

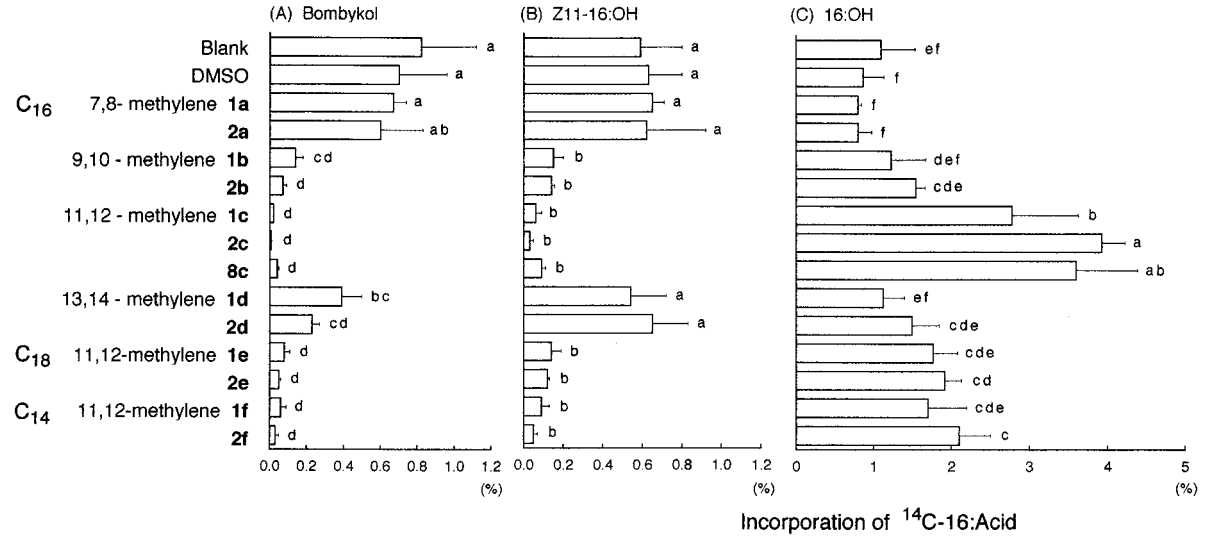

Figure 2. Incorporation (%) of ^{14}C-16:Acid into fatty alcohols [bombykol (A), Z11-16:OH (B), and 16:OH (C)] in the pheromone glands of *B. mori* that were pretreated with cyclopropene compounds (**1a**−**f**, **2a**−**f**, and **8c**; 1 μg).

2b) and 13,14-methylenes (**1d** and **2d**) among C_{16} chain compounds, and C_{16} (**1c** and **2c**), C_{14} (**1f** and **2f**), and C_{18} (**1e** and **2e**) chain compounds among 11,12-methylenes. Inhibitions by the amides were stronger than those by the corresponding acids. The 7,8-methylene compounds (**1a** and **2a**) were inactive toward inhibition. The ^{14}C-incorporation of Z11-16:OH was also blocked by the 11,12- and 9,10-methylenes, whereas the 7,8- and 13,14-methylenes showed no effect. On the contrary, the ^{14}C-incorporation of 16:OH increased by more than three times because of the pretreatment of the 11,12-methylenes. This incorporation was moderately increased by the 9,10- and 13,14-methylenes, whereas 7,8-methylenes had no effect.

DISCUSSION

It has been reported that the desaturation of octadecanoic acid is inhibited by naturally occurring cyclopropene fatty acid, sterculic acid, in the livers of vertebrates (Raju and Reiser, 1967; Fogerty et al., 1972), and desaturation of 16:Acid is inhibited by some synthetic analogs in the pheromone glands of a noctuid moth, *Spodoptera littoralis* (Arsequell et al., 1989; Gosalbo et al., 1992). Our study revealed that the desaturation in pheromone biosynthesis of *B. mori* was also inhibited by some cyclopropene acids, which prevented both the incorporation of $^{2}H_3$- and ^{14}C-labeled 16:Acid into the pheromone bombykol. Furthermore, the incorporation was blocked by the amide derivatives; their inhibitory activities being stronger than those of the parent acids. Among the tested cyclopropene compounds, C_{16} 11,12-methylene amide (**2c**) showed the strongest inhibitory activities against bombykol biosynthesis. This amide (**2c**) blocked half of the conversion of ^{14}C-16:Acid into bombykol at a dose of <0.1 nmol/gland, and the I_{50} values in Table 3 show that **2c** is 2.5 times more active than the corresponding acid **1c**. The I_{50} values of C_{16} 9,10-methylene amide (**2b**) and C_{18} and C_{14} 11,12-methylene amides (**2e** and **2f**), with moderate inhibitory activity, are from one-half to one-third of the values of the corresponding acids **1b**, **1e**, and **1f**, respectively. The amides seem to prevent the metabolism by β-oxidation, which easily metabolizes the acids applied to the pheromone gland. Also some other reasons for the stronger activity of amides can be postulated; for example the aminocarbonyl group might enhance its penetration into the target site and affinity for it. Our previous paper showed that **7c**, the cyano analog of **1c**, was inactive (Ando et al., 1995). This experiment revealed that esterification of **1c** decreased the activity. The functional group, which optimizes the activity, should be dynamically studied in the future.

Based on the incorporation experiments with several precursors, it has been estimated that there are two desaturation steps, Δ11- and Δ10,12-desaturation, in the bombykol biosynthesis (Ando et al., 1988b). The C_{16} saturated acyl compound, which is constructed with acetyl CoA, is converted into a monounsaturated acyl compound (derivative of Z11-16:Acid) by Δ11-desaturase, and successively into a diunsaturated acyl compound (derivative of E10,Z12-16:Acid) by Δ10,12-desaturase. Finally, the acyl moiety was transformed into alcohol by reductase to accomplish the bombykol production. The pheromone gland of *B. mori* possesses not only bombykol but also two other alcohols, Z11-16:OH, which are produced from the acyl intermediates by reductase. Although their roles in the mating communication are unknown, progress in the desaturation steps can be estimated by counting their ^{14}C-incorporation from ^{14}C-16:Acid. 11,12-Methylene compounds with a C_{16} chain (**1c** and **2c**) significantly diminished the ^{14}C-incorporation of Z11-16:OH and that of bombykol much more, as shown in Figure 2. This result indicates that the 11,12-methylenes blocked both of the two desaturation steps. This blocking of the unsaturation steps caused high level ^{14}C-incorporation into 16:OH. It is interesting that C_{18} and C_{14} 11,12-methylenes (**1e**, **2e**, **1f** and **2f**) also showed rather strong inhibitory activities against the enzymes acting in the biosynthesis of the pheromone with a C_{16} chain. The steps inhibited by other cyclopropene compounds were also suggested from the results of Experiment B. The 9,10-methylenes (**1b** and **2b**) might selectively inhibit Δ11-desaturation, because the ^{14}C-incorporation of bombykol and Z11-16:OH decreased to a similar level by the treatment of these cyclopropenes. On the contrary, the 13,14-methylenes (**1d** and **2d**) selectively inhibited Δ10,12-desaturation because only the ^{14}C-incorporation of bombykol decreased. The biosynthetic pathway of bombykol and the steps that are expected to be inhibited by cyclopropene compounds are summarized in Figure 3. We plan to examine the effect of cyclopropenes on the incorpora-

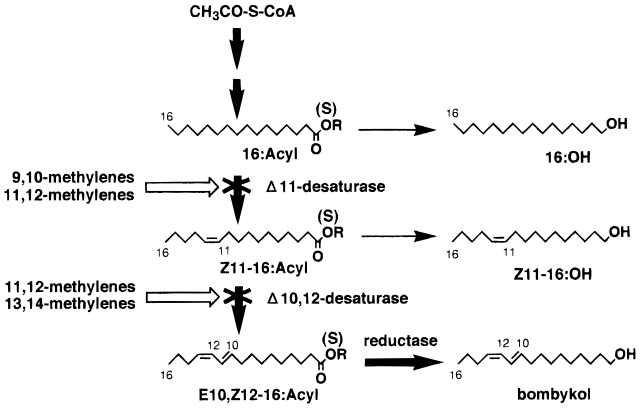

Figure 3. Biosynthetic pathway of bombykol and the prospective steps that were inhibited by cyclopropene compounds. The biosynthetic pathway for bombykol is shown by the thick arrows, and some of the related metabolic reactions by the thin arrows. The desaturation steps in this pathway proceed on fatty acyl groups in a lipid or an activated derivative. The acyl carrier, which has not been identified, is represented by −OR or −SR.

tion of labeled Z11-16:Acid into bombykol to reveal their critical target steps. The Δ10,12-desaturation is the novel step that is recognized as a retro-1,4-addition, and the mechanism has not been estimated by any experiments. We hope to obtain some information about the mechanism by the additional investigations with these inhibitors.

The structure−activity relationships of cyclopropene fatty acids were studied by Fogerty et al. (1972) by first using the desaturase enzyme system of hen liver. In this system, Δ9-desaturation of octadecanoic acid was strongly inhibited by 9,10-methyleneoctadec-9-enoic acid (sterculic acid) and 10,11-methylenenonadec-10-enoic acid, and moderately by 8,9-methyleneheptadec-8-enoic acid (malvalic acid). Inhibition was not observed by the treatment with the 11,12-methylene derivative. Recently, Gosalbo et al. (1992) reported the effect of three synthetic cyclopropene acids on the Δ11-desaturation of 16:Acid in the pheromone gland of a noctuid moth *Spodoptera littoralis*. This Δ11-desaturation was inhibited by all of the tested cyclopropenes, **1c**, and the 10,11- and 12,13-methylene analogs. The latter two compounds possess the propene ring at the adjoining position of the 11,12-bond. These results indicate that the position of a propene ring in the active compounds is not strictly coincident with the position of the C=C bond introduced by desaturase. It is noteworthy that Δ11-desaturase in the pheromone gland of *B. mori* was inhibited by even the 9,10-methylene compounds (**1b** and **2b**), which had the ring at the position removed by one bond from the C=C bond. The Δ11-desaturation is one of the key steps in pheromone biosynthesis of lepidopterous insects. Pheromonal components with a C_{14} chain unsaturated at the 9-position and a C_{12} chain unsaturated at the 7-position are produced via a chain shortening reaction after the Δ11-desaturation (Bjostad et al., 1987). Further comparative studies of inhibitors against many lepidopterous species will help to define the Δ11-desaturase.

ABBREVIATIONS USED

16:Acid, hexadecanoic acid (palmitic acid); Z11-16:Acid, (*Z*)-11-hexadecenoic acid; 10E,Z12-16:Acid, (10*E*,-12*Z*)-10,12-hexadecadienoic acid; 16:OH, hexadecan-1-ol; Z11-16:OH, (*Z*)-11-hexadecen-1-ol; bombykol (10*E*,12*Z*)-10,12-hexadecadien-1-ol.

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Received for review November 20, 1995. Revised manuscript received July 17, 1996. Accepted July 31, 1996.[⊗]

JF950757N

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1996.