Short Communication

Incorporation of ^{14}C -Fatty Acids into Bombykol, a Female Sex Pheromone of the Silkworm MothTetsu ANDO, Rika ARIMA,
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To date, more than 80 sex pheromonal compounds have been identified from female moths.¹⁾ Many of them are composed of $\text{C}_{10} \sim \text{C}_{18}$ straight chains with a terminal functional group and one or two $\text{C}=\text{C}$ double bonds. From this structural feature, they seem to be derived from fatty acids by modification of the acid function, dehydration, and change of the chain length. The biosynthetic pathways of some lepidopterous pheromones have been investigated,²⁾ but proof of the pathway seems insufficient. Pheromones are biosynthesized only at the pheromone glands in very limited amounts, and a trace of ^{14}C -labelled precursors is incorporated. The female of the silkworm moth (*Bombyx mori* L.) may reliably incorporate ^{14}C into its pheromone bombykol, (10*E*, 12*Z*)-10,12-hexadecadien-1-ol, since it secretes in micrograms. In this paper we report on the experimental conditions established for good ^{14}C incorporation and for selective incorporation of hexadecanoic (palmitic) acid among the ^{14}C -fatty acid series.

Silkworm moths*¹ were reared in a 16:8 light-dark cycle throughout their lives. To

each pheromone gland of the calling females in the light period [$^{16}\text{-}^{14}\text{C}$]hexadecanoic acid (see *²) was applied topically. A few minutes after the application, females took on the calling pose again. After several hours, the abdominal tips were excised and extracted with ethyl acetate for 30 min. The extract showed only three radioactive spots on a silica gel TLC plate (benzene:ethyl acetate=4:1, *R_f* values; 0.80, 0.34 and 0.12). Co-chromatography showed that the top and bottom spots were caused by the triacylglycerol having incorporated radiolabel from ^{14}C -hexadecanoic acid and the unconverted ^{14}C -acid, respectively. The radioactive material recovered from the middle spot was re-chromatographed on a silica gel TLC plate impregnated with AgNO_3 , and gave two separate spots (benzene:ethyl acetate=3:1, *R_f* values; 0.40 and 0.33). The *R_f* values of the upper and lower spots coincided with those of hexadecan-1-ol (cetyl alcohol) and bombykol, respectively. For easy measurement of incorporation of radiolabel into bombykol and the saturated alcohol, an extract of the pheromone gland was analyzed on HPLC with the functionalized polar Nucleosil NO_2 column, as shown in Fig. 1. Radioactivities recovered from the HPLC column could be counted better without loss than on a GC column, although the separation by GC was better than by HPLC. Bombykol could be analyzed quantitatively using the UV detector at 235 nm. Hexadecan-1-ol was not detected by UV, but GC analysis on the HPLC elution of synthetic bombykol mixed with authentic hexadecan-1-ol revealed that the bombykol fraction was contaminated with only 2% of the saturated alcohol.

The incorporation ratio of ^{14}C -hexadecanoic acid into bombykol was measured at different times (1, 3, 6, 12, 24, and 48 hr) after treatment. A long time was unnecessary; incorporation was highest 3 hr after treatment. This result was desirable, because the real

*¹ Fl hybrids of C 140 (female) and J 140 (male) strains.

*² All ^{14}C -acids were obtained from CEA (France). Their specific activities were 59, 54.5, 57, 54, 51, or 55 mCi/mmol. Each acid was dissolved in dimethyl sulfoxide at the concentration of about 50000 dpm/ μl , and 1 μl of the solution was applied to a female pheromone gland.

FIG. 1. HPLC of Pheromone Gland Extract and Incorporation of Radiolabel from Topically Applied [16- ^{14}C]Hexadecanoic Acid.

An extract obtained from 3 abdominal tips topically treated with [16- ^{14}C]hexadecanoic acid was injected into a HPLC column, Nucleosil 5 NO_2 (8 mm i.d. $\times$ 15 cm), with 2% 2-propanol in *n*-hexane at a flow rate of 1.5 ml/min. Recovered material was collected every 0.5 min and mixed with a solution of PPO and POPOP in toluene for counting its radioactivity by liquid scintillation. Triacylglycerol, hexadecan-1-ol, and bombykol were almost completely (>97%) collected in the fractions 9 to 10, 21 to 23, and 24 to 26, respectively. Incorporation of the ^{14}C -acid into 1,3- and 1,2-diacylglycerol (*R_t* values; 13.7 and 21.4 min) was not observed.

biosynthetic pathway of the pheromone might be shrouded by other metabolism, such as β -oxidation of a fatty acid, in long-term experiments. Next, incorporation was examined every three hours after eclosion. The moths usually emerged from the pupae 1~3 hr after lighting in our rearing conditions, and the pheromone of the gland was more abundant in light periods than in dark periods. The incorporation of radiolabel (about 2%) was highest in 2-day-old virgin female moths 4 hr after lighting (25~27 hr after eclosion). Values of the incorporation ratio increased in light periods and decreased in dark periods, synchronizing with changes in the amount of pheromone. This observation indicated that bombykol was biosynthesized *via* hexadecanoic acid rapidly and released immediately from the pheromone gland.

A series of ^{14}C -labelled fatty acids,*² [1-

^{14}C]dodecanoic (lauric) acid, [1- ^{14}C]tetradecanoic (myristic) acid, [1- ^{14}C] and [16- ^{14}C]hexadecanoic acids, and [1- ^{14}C] and [18- ^{14}C]octadecanoic (stearic) acids, was applied to 2-day-old female moths 4 hr after lighting. As with hexadecanoic acid, extracts of the glands treated with the other fatty acids showed ^{14}C incorporation into triacylglycerol, saturated alcohol, bombykol, and the rest of the fatty acid. Their incorporation ratios at 3 hr of incubation are listed in Table I. Incorporation of both [1- ^{14}C] and [16- ^{14}C]hexadecanoic acids into bombykol was much larger than that of other fatty acids the carbon number of which was different from bombykol. The selective incorporation of hexadecanoic acid indicates that the carbon chain of hexadecanoic acid was directly converted into bombykol. Incorporation *via* ^{14}C -acetyl CoA derived from ^{14}C -fatty acids by β -oxidation seems to be minor.

TABLE I. INCORPORATION RATIOS (%) OF ^{14}C -FATTY ACIDS^a

	Triacylglycerol ^b	Saturated alcohol ^b	Bombykol ^b	Fatty acid ^c (Unconversion)
[1- ^{14}C]Dodecanoic acid	0.64 ± 0.18	0.33 ± 0.13	0.23 ± 0.06	0.77 ± 0.52
[1- ^{14}C]Tetradecanoic acid	2.53 ± 0.87	0.20 ± 0.12	0.13 ± 0.06	2.79 ± 1.59
[1- ^{14}C]Hexadecanoic acid	8.85 ± 2.62	2.45 ± 0.88	1.73 ± 0.48	14.13 ± 5.60
[16- ^{14}C]Hexadecanoic acid	7.98 ± 0.21	2.20 ± 0.45	1.50 ± 0.29	10.44 ± 0.41
[1- ^{14}C]Octadecanoic acid	5.44 ± 0.84	1.22 ± 0.45	0.12 ± 0.16	20.88 ± 10.38
[18- ^{14}C]Octadecanoic acid	4.50 ± 0.95	0.84 ± 0.15	0.09 ± 0.09	23.04 ± 8.00

^a At least 3 groups of 3 insects each were used per treatment.

^b Data were obtained by recovery from a HPLC column.

^c Data were obtained by recovery from a TLC plate.

This result confirms that hexadecanoic acid is a precursor of bombykol biosynthesis, as suggested by Inoue and Hamamura in 1972.³⁾

For the biosynthesis of bombykol, two contradictory pathways have been proposed. One is the pathway *via* hexadecan-1-ol³⁾ and the other, *via* (Z)-11-hexadecenoic acid and (10E,12Z)-10,12-hexadecadienoic acid.^{4,5)} Our experiments showed that hexadecanoic acid was smoothly converted to hexadecan-1-ol rather than to bombykol in the pheromone gland, supporting the suggestion of the former pathway. The other fatty acids, however, were also changed to the corresponding saturated alcohols to the extents shown in Table I. If hexadecadienoic acid is a precursor, perhaps the reductase of hexadecadienoic acid can universally reduce the fatty acids.

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