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IDENTIFICATION OF SEX ATTRACTANT PHEROMONE COMPONENTS OF THE TUSSOCK MOTH, *Euproctis taiwana* (SHIRAKI) (LEPIDOPTERA: LYMANTRIIDAE)

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Abstract—Two compounds were isolated and identified from abdominal tips of the female tussock moth, *Euproctis taiwana* (Shiraki), by a combination of gas chromatography–electroantennographic detection, coupled gas chromatography–mass spectrometric analysis, microreaction, and synthesis. These compounds were (*Z*)-16-methyl-9-heptadecenyl isobutyrate (74.8%) and 16-methylheptadecyl isobutyrate (25.2%). Their total amount was determined to be ca. 29.8 ng/female. Field bioassays showed that (*Z*)-16-methyl-9-heptadecenyl isobutyrate is a sex attractant for the male moths. These chemical compounds had not previously been found as the sex pheromone components in the genus *Euproctis*.

Key Words—Tussock moth, *Euproctis taiwana*, sex attractant pheromone, (*Z*)-16-methyl-9-heptadecenyl isobutyrate, 16-methylheptadecyl isobutyrate.

INTRODUCTION

The tussock moth, *Euproctis taiwana* (Shiraki), distributed in the Okinawan region of Japan and Taiwan, is polyphagous and a serious pest of a variety of crops. These include guava, mango (Azuma, 1968), rose (Wang, 1982a), gladiolus (Wang, 1982b), loquat (Miyatake et al., 1990), kidney beans, tea, cabbage, and corn. Furthermore, *Euproctis* larvae are covered with bristles that can lead to severe dermatitis when they contact human skin (Ogata, 1958; Su, 1981).

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In the genus *Euproctis*, sex pheromones have been identified for *E. chrysorrhoea* (Leonhardt et al., 1991), *E. psudoconspersa* (Wakamura et al., 1994), *E. similis similis* (Yasuda et al., 1994), and *E. similis xanthocampa* (Tan et al., 1984), but no common component has been found in the sex pheromones of these species of *Euproctis*.

This paper describes the isolation and identification of the female sex attractant pheromone of *E. taiwana*.

METHODS AND MATERIALS

Insects. In May 1993, *Euproctis taiwana* larvae were collected from fields of cabbage and kidney beans, Naha City, Okinawa Prefecture, Japan. The larvae were reared on an artificial diet at 25°C under a 16L:8D photoperiod.

Preparation of Pheromone Extracts. Abdominal tips were excised from 2-day-old virgin females 1 hr into photophase and extracted with hexane for 30 min at room temperature. The extracts were filtered through a piece of absorbent cotton, accumulated, and stored below −30°C until used.

Column Chromatography. For separation of the extracts, column chromatography on Florisil was carried out (Carroll, 1961). Components were successively eluted with hexane and 5%, 10%, and 50% ether in hexane.

For separation of geometric isomers, column chromatography on silica gel impregnated with silver nitrate ($AgNO_3$: 16.7%) ($AgNO_3$–SiO_2 chromatography) was used. Components were successively eluted with hexane and 1%, 2%, 3%, 4%, 5%, 7%, and 10% ether in hexane.

Gas Chromatograph–Electroantennographic Detector (GC-EAD). The fractions from Florisil chromatography were analyzed by a GC-EAD system (Struble and Arn, 1984). Gas chromatography was performed with a Hewlett-Packard HP 5890 Series II gas chromatograph with a DB-WAX column (polyethylene glycol 20M, 30 m × 0.25 mm ID × 0.25-μm film thickness) and a flame ionization detector (FID) and electroantennographic detector (EAD). FID and EAD responses were simultaneously recorded. The column temperature was set at 50°C for 1 min, programmed at 20°C/min to 150°C, and then programmed at 5°C/min to 220°C and held for 10 min. Helium was the carrier gas.

Gas Chromatograph–Mass Spectrometer (GC-MS). GC-MS analyses were conducted with a JEOL JMS-SX102A mass spectrometer combined with a Hewlett-Packard HP 5890 Series II gas chromatograph using the following columns: DB-1 (100% dimethyl polysiloxane, 30 m × 0.25 mm ID × 0.25-μm film thickness), HP-5 (5% diphenyl–95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 0.33-μm film thickness), DB-WAX and DB-23 (50% cyanopropyl polysiloxane, 30 m × 0.25 mm ID × 0.25-μm film thickness). The column temperature conditions were the same as those in the GC-EAD system. Helium was

the carrier gas. High-resolution mass spectra (HRMS) were obtained by the same system.

Chemical Reactions. The monounsaturated components were hydrogenated with diimide to determine the acid moieties (Yamaoka et al., 1976). The diimide reduction products were analyzed by the GC-MS system. Esters of 1-octadecanol were used as authentic standards.

Microozonolysis was used to determine the double-bond positions of alcohol moieties (Beroza and Bierl, 1967). Oxygen with ozone was blown into a hexane solution of the sample below −50°C for 1 min, and it was then immediately injected into the GC-MS system.

Naturally occurring esters were hydrolyzed in 1% KOH in ethanol at 50°C for 22 hr. After evaporation of the solvent, the residue was extracted with hexane and poured on a silica gel column. Compounds were successively eluted with hexane and 5%, 10%, 25%, and 50% ether in hexane. A part of the 25%-ether-in-hexane fraction, which contained alcohols, was concentrated and chlorinated with methanesulfonyl chloride and dimethylformamide at 65°C for 16 hr (Evans et al., 1968). The reaction mixture was then placed directly onto a silica gel column, and the chloride derivatives were eluted with hexane and then injected into the GC-MS system with the DB-WAX column.

Synthesis. 8-Methyl-1-nonyne was synthesized from 1-bromo-3-methylbutane and 2-propyn-1-ol following the methodology of Naoshima et al. (1990). (*Z*)-16-Methyl-9-heptadecen-1-ol (16Me-Z9-17:OH) was synthesized from 8-methyl-1-nonyne and 8-bromo-1-octanol following the methodology of Hendry et al. (1975). 16-Methyl-1-heptadecanol (16Me-17:OH) was prepared from methyl 16-methylheptadecanoate by reduction with lithium aluminum hydride. Corresponding esters were obtained from 16Me-Z9-17:OH and 16Me-17:OH reacted with acid anhydride. (*Z*)-16-Methyl-9-heptadecenyl isobutyrate (16Me-Z9-17:iBu) was purified by $AgNO_3$–SiO_2 chromatography.

Field Bioassay. Field tests were conducted at the Okinawa Prefectural Agricultural Experiment Station, Naha City, using sticky traps (SE trap, Sankei Chem. Co.). Traps were placed 0.5–1.5 m above ground at intervals greater than 5 m. Hexane solutions of test samples were impregnated into a piece of filter paper (2 × 5 cm, Advantec No. 2) and placed into the trap. The reference trap was baited with three 3-day-old virgin females. Trap data ($x + 0.5$) were subjected to square-root transformation before analysis of variance, and Tukey's test was used for comparing means where the variance analysis (general linear models procedure) indicated a significant F value (SAS Institute, 1988).

RESULTS

The accumulated extract of 262 virgin females was fractionated with the Florisil column. Each fraction was checked by the GC-EAD analysis at 1 female equivalent. The 5%-ether-in-hexane fraction showed two EAD-active peaks

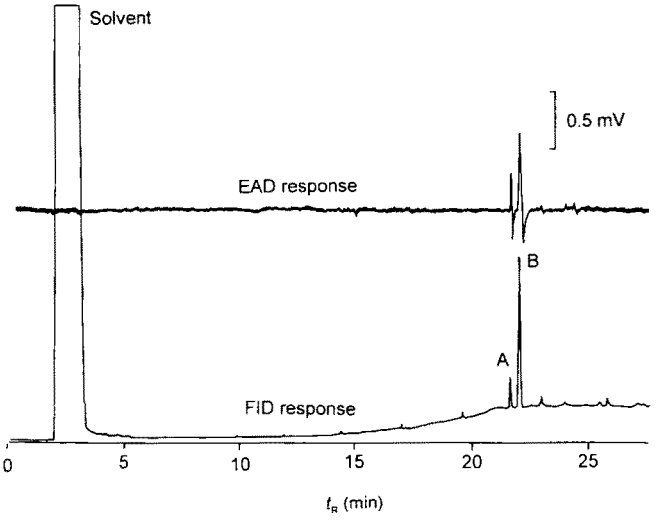

FIG. 1. GC-EAD profile of female gland extract of *Euproctis taiwana*. One female equivalent of 5% ether-in-hexane fraction from Florisil column was injected. GC: DB-WAX, operated at 50°C for 2 min, programmed at 20°C/min to 150°C, and then programmed at 5°C/min to 220°C, where it was kept for 10 min.

(Figure 1, peaks A and B). The ratio of the areas of these two compounds was 1:3, and the total amount was estimated to be ca. 29.8 ng/female by GC analysis. GC-MS analysis with the DB-WAX column of the 5% fraction led to an estimate that compound A was an isomer of an 18-carbon saturated alcohol ester of a 4-carbon carboxylic acid, which showed the following characteristic fragment ions: *m/z* 89 ($C_3H_7CO_2$ + 2H, base peak), 252 (M−88, 29%) and 340(M^+, 32%) (HRMS found: 340.3338, calcd. for $C_{22}H_{44}O_2$: 340.3341). Compound B was also estimated to be an isomer of an 18-carbon monounsaturated alcohol ester of a 4-carbon carboxylic acid, which showed the following characteristic fragment ions: *m/z* 82 (base peak), 89 ($C_3H_7CO_2$ + 2H, 44%), 250 (M−88, 58%) and 338 (M^+, 2%) (HRMS found: 338.3126, calcd. for $C_{22}H_{42}O_2$: 338.3185).

The 5% fraction was further chromatographed by $AgNO_3$–SiO_2 chromatography to confirm the degree of unsaturation of the components. Compound A was eluted in 2%-ether-in-hexane fraction and compound B was eluted in the 4%-, 5%-, and 7%-ether-in-hexane fractions.

One peak was detected in the products of diimide reduction of compound B, the 5% fraction of $AgNO_3$–SiO_2 chromatography, by GC-MS analysis with the DB-WAX column. This compound was shown to be identical to compound

A by cochromatography with the 2% fraction from $AgNO_3$-SiO_2 chromatography and by GC-MS analysis. Furthermore, the product of diimide reduction of compound B showed a clear EAD response.

The retention time of compound A on DB-1 column was, however, lower than on those of authentic esters possessing a straight chain of the corresponding number of carbon atoms in the alcohol moiety. This indicated that the compound had alkyl branch(es) in the alcohol moiety. The alkyl chloride derived from the ester in the 2% fraction from $AgNO_3$-SiO_2 chromatography after hydrolysis and subsequent chlorination was analyzed with the HP-5 column. One peak was observed at 16.83 min. The mass spectrum of this compound showed a molecular ion at *m/z* 288 (15%) and an M+2 ion at *m/z* 290 (5%), which indicated that this compound contained 18 carbon atoms and one chlorine atom, that is, it was derived from the alcohol moiety of compound A. Diagnostic fragment ions were observed at *m/z* 245 (M−43, 74%) and 273 (M−15, 34%), which were accompanied by the isotopic ions at *m/z* 247 (23%) and 275 (11%), respectively. This suggested the presence of one methyl branch at the position 16 of the 17-carbon chain. This was confirmed by GC-MS analysis of authentic 1-chloro-16-methylheptadecane. The mass spectrum and retention time of compound A were identical to those of synthetic 16-methylheptadecyl isobutyrate (16Me-17:iBu) in subsequent GC-MS analysis, but did not fit with those of synthetic 16-methylheptadecyl *n*-butyrate. Compound A was, therefore, identified as 16Me-17:iBu.

GC-MS analysis with the DB-WAX column of reductive ozonolysis products of the 5% fraction from $AgNO_3$-SiO_2 chromatography showed two major compounds: a 9-carbon aldehyde and 8-formyloctyl isobutyrate. These indicated the double bond at carbon 9 in the 18-carbon alcohol moiety of compound B. Furthermore, the alkyl chlorides derived from the products of diimide reduction of compound B showed properties identical to those of compound A by GC-MS analysis. Compound B was estimated to be 16-methyl-9-heptadecenyl isobutyrate on the basis of these microreactions.

The synthetic compounds were analyzed by GC-MS with the DB-23 column. The retention time of compound B fit well with synthetic (8)-16-methyl-9-heptadecenyl isobutyrate (16Me-Z9-17:iBu), and (*E*)-16-methyl-9-heptadecenyl isobutyrate did not fit with compound B. The mass spectrum of synthetic 16Me-Z9-17:iBu was identical to that of compound B. Therefore, compound B was identified as 16Me-Z9-17:iBu.

The mixture of the two synthetic esters at the natural ratio was analyzed by the GC-EAD. This showed two clear EAD responses to the synthetic mixture, which were similar to those occurring when the 5% fraction from Florisil chromatography was injected.

Field tests were conducted to determine the activity of synthetic 16Me-Z9-17:iBu (Table 1). More *E. taiwana* males were caught with 16Me-Z9-17:iBu

TABLE 1. TRAP CATCHES OF MALE *Euproctis taiwana* WITH (Z)-16-METHYL-9-HEPTADECENYL ISOBUTYRATE (16ME-Z9-17:IBU) (OCTOBER 18–20, 1994, NAHA)[a]

16Me-Z9-17:iBu (μg/filter paper)	No. of males caught/trap/night (mean ± SE)[b]
0	0.0 ± 0.0 a
30	0.2 ± 0.2 a
100	0.8 ± 0.6 a
300	0.7 ± 0.7 a
1000	0.3 ± 0.3 a
3VF[c]	0.0 ± 0.0 a

[a] SE sticky traps were used.
[b] Values indicated are means and standard errors with three traps for two nights. Data were transformed to $\sqrt{(x + 0.5)}$ and submitted to analysis of variance. The means followed by the same letter in the same column are not significantly different at 5% level by Tukey's test.
[c] Three 3-day-old virgin females.

alone than with three 3-day-old females, but the difference was not statistically significant. The male catch increased when the dose was increased from 30 μg to 100 μg. These results suggest that this is the optimum dose level for male attraction.

Field tests were also conducted to determine the activity of synthetic 16Me-17:iBu, 16Me-Z9-17:iBu, or their blends (Table 2). From June 22 to July 4, males were caught with three 3-day-old females and also with 300 μg of 16Me-Z9-17:iBu alone. No significant increase or decrease was observed when 10, 100, or 200 μg of 16Me-17:iBu was added to 300 μg of 16Me-Z9-17:iBu. From October 20 to 22, no males were caught with 100 μg of 16Me-17:iBu alone, although many males were caught with 300 μg of 16Me-Z9-17:iBu alone or a blend of 300 μg of 16Me-Z9-17:iBu and 100 μg of 16Me-17:iBu.

DISCUSSION

Two compounds were identified as possible sex pheromone components in *Euproctis taiwana*. The major component was characterized as 16Me-Z9-17:iBu and the minor one as 16Me-17:iBu. These two components have not previously been reported as natural products. Field bioassays showed that 16Me-Z9-17:iBu is essential to attract male *E. taiwana* moths. Synergistic effects on male attraction by 16Me-17:iBu were not apparent in the field tests (Table 2), although 16Me-17:iBu was EAG-active. Further studies are necessary to check the behavioral activity of the minor components and to develop an effective synthetic

TABLE 2. TRAP CATCHES OF MALE *Euproctis taiwana* WITH (Z)-16-METHYL-9-HEPTADECENYL ISOBUTYRATE (16ME-Z9-17 : iBU), 16-METHYLHEPTADECYL ISOBUTYRATE (16ME-17 : iBU) OR THEIR BLENDS (1994, NAHA)[a]

Chemicals (μg/filter paper)		No. of males caught/trap (mean ± SE)[b]		
16Me-Z9-17 : iBu	16Me-17 : iBu	June 20–22	June 22–July 4	October 20–22
0	0			0.0 ± 0.0 a
10	3	0.0 ± 0.0 a		
30	10	0.2 ± 0.2 a		
100	30	0.2 ± 0.2 a		
300	0		3.7 ± 1.5 a	1.5 ± 0.8 a
300	10		3.3 ± 1.5 a	
300	100	3.7 ± 1.5 b	1.0 ± 0.0 a	1.0 ± 0.5 a
300	200		3.3 ± 3.3 a	
0	100			0.0 ± 0.0 a
3VF[c]		0.3 ± 0.2 ab	2.5 ± 0.0 a	0.0 ± 0.0 a

[a] SE sticky traps were used.
[b] Values indicated are means and standard errors with three traps. Data were transformed to $\sqrt{(x + 0.5)}$ and submitted to analysis of variance. The means followed by the same letter in the same column are not significantly different at 5% level by Tukey's test.
[c] Three 3-day-old virgin females.

TABLE 3. SEX PHEROMONE COMPONENTS IN GENUS *Euproctis*

Species	Components	Ratio (%)	Reference
Euproctis chrysorrhoea	(Z,Z,Z,Z)-7,13,16,19-docosatetraenyl isobutyrate	100	Leonhardt et al. (1991)
Euproctis psudoconspersa	10,14-dimethylpentadecyl isobutyrate	94.3	Wakamura et al. (1994)
	14-methylpentadecyl isobutyrate	5.7	
Euproctis similis similis	(Z)-7-octadecenyl 2′-methylbutyrate	53.5	Yasuda et al. (1994)
	(Z)-7-octadecenyl isovalerate	32.1	
	(Z)-7-octadecenyl isobutyrate	2.7	
	(Z)-7-octadecenyl *n*-butyrate	2.7	
	(Z)-9-octadecenyl 2′-methylbutyrate	4.3	
	(Z)-9-octadecenyl isovalerate	4.8	
Euproctis similis xanthocampa	(Z)-7-octadecenyl isovalerate	92.0	Tan et al. (1984)
	6-octadecenyl isovalerate	1.4	
	6-octadecenyl *n*-butyrate	1.6	
	9-octadecenyl isovalerate	5.0	
Euproctis taiwana	(Z)-16-methyl-9-heptadecenyl isobutyrate	74.8	This paper
	16-methylheptadecyl isobutyrate	25.2	

lure for monitoring field populations and/or use as a tool in integrated pest management strategy.

In the genus *Euproctis*, sex pheromones have already been identified for three species and one subspecies (Table 3). As volatile sex pheromones, these chemical compounds have relatively high molecular weights. The alcohol moieties of the esters identified from *Euproctis* include 16-, 17-, 18- or 22-carbon alcohols that includes saturated or unsaturated and straight or methyl-branched chains. The acid moieties of the esters are derived from 4- or 5-carbon organic acids.

In many closely related species of Lepidoptera, the sex pheromones include one or more common components (Arn et al., 1992). For example, 2-methylheptadecane is one of the sex pheromone components in the genus *Holomelina* (Arctiidae) and (*Z,E*)-9,12-tetradecadienyl acetate has been identified as a common component of sex pheromones in many species of the genus *Spodoptera* (Noctuidae). However, no such common compound has been found in the genus *Euproctis*.

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