Bull. Inst. Zool., Academia Sinica **31**(4): 246-250 (1992)

CHEMICAL COMPOSITION OF SEX PHEROMONE GLAND EXTRACT IN FEMALE ORIENTAL ARMYWORM *PSEUDALETIA SEPARATA* WALKER (LEPIDOPTERA: NOCTUIDAE) IN TAIWAN[1]

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(Accepted January 14, 1992)

Rong Kou, Yien-Shing Chow and Hsiao-Yung Ho (1991) Chemical composition of sex pheromone gland extract in female oriental armyworm *Pseudaletia separata* Walker (Lepidoptera: Noctuidae) in Taiwan. (Z)-11-hexadecenyl acetate (Z11-16:OAc), (Z)-11-hexadecenol (Z11-16:OH), hexadecanol (16:OH), and hexadecanyl acetate (16:OAc) were isolated and identified as major chemical components from the female sex pheromone gland of the oriental armyworm, *Pseudaletia separata*, in Taiwan. The average amount of each component in one female gland was 60.0, 29.3, 9.3, and 9.0 ng/♀, respectively, in a ratio of 56:27:9:8.

Key words: Sex pheromone, *Pseudaletia separata.*

In Japan, the sex pheromone of the female oriental armyworm (*Leucania* (=*Pseudaletia*) *separata* Walker) was identified as a blend of (Z)-11-hexadecenyl acetate (Z11-16:OAc) and (Z)-11-hexadecenol (Z11-16:OH) at a ratio of 8:1 by Takahashi *et al.* (1979). However, in mainland China the male oriental armyworm was not attracted to the sex pheromone identified by Takahashi *et al.* in 1979; instead, (Z)-11-hexadecenal (Z11-16:Ald), hexadecanal (16:Ald), and (Z)-11-hexadecenol (Z-11-16:OH) were identified as the female sex pheromone components in the mainland China research (Zhu *et al.*, 1987). These different results encouraged our investigation of the chemical composition in the sex pheromone glands of the female *P. separata* in Taiwan in order to further determine the existence of pheromone polymorphism or of different species living in different parts of Asia.

MATERIALS AND METHODS

Insects

Mature larvae were collected from corn fields and reared with corn to pupation; after adults mated, eggs were laid. Hatched larvae were reared on an artificial diet modified from Shory and Hale (1965), and sexes were separated at pupal stage. All tested insects were maintained under a 16L:8D light regime at 24-26°C.

1. Paper No. 367 of the Journal Series of the Institute of Zoology, Academia Sinica
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Preparation of Sex Pheromone Gland Extract

Ovipositors from one hundred calling 4- to 5-day-old virgin females were excised and then immersed in hexane for 10 min. Calling females are easily recognized by their extruding ovipositors, similar to those found in other noctuid moths (Kou and Chow, 1987). Extracts were stored at 0°C for structure identification.

GC and GC-MS Analysis

Gas chromatography (GC) analysis of the extract was performed on three capillary columns. A Shimadzu 14-A gas chromatograph equipped with a flame ionization detector was used with a 30-m × 0.25-mm (ID) fused silica capillary column of Carbowax 20 M phase, a 30-m × 0.25-mm fused silica column of DB-17 phase, and a 30-m × 0.25-mm fused silica column of DB-225 phase. In the Carbowax 20 M and DB-225 columns, column temperatures were isothermal at 190°C and 200°C, respectively. In the DB-17 column, the column temperature was increased from 200 to 260°C at an increment of 5°C/min. Chromatographic conditions were: nitrogen carrier 0.5 Kg/cm^2. Gas chromatographic-mass spectrometric (GC-MS) analysis was conducted using a Finnigan Mat Incos 50 spectrometer. The GC-MS data were obtained using a 30 m × 0.25 mm column of DB-1701 phase. The column temperature was increased from 120 to 260°C at 3°C/min, then held at 260°C for 5 min using helium as the carrier gas. Electron impact (EI) mass spectra were collected at 70 eV with separator and source at 180°C.

The chemical composition of the sex pheromone gland extract was identified by comparing their mass spectra with those of authentic standards. Subsequently, the identification was verified by comparing the natural product GC retention time to an authentic standard under the same GC conditions described above. Standards of hexadecanyl acetate (16:OAc), hexadecanol (16:OH), 16:Ald, Z11-16: Ald, Z11-16:OAc, and Z11-16:OH were purchased from Sigma Chemical Co.

Titer Determination

Three hundred 4- to 5-day-old virgin female ovipositors were excised and pooled in hexane; extraction was performed during the animals's calling period (7-8 hr into the scotophase). Each microliter of pooled extract contained one ovipositor. The extract was subsequently analyzed for Z11-16:OAc, Z11-16:OH, 16:OH, and 16:OAc using the external standard method of quantitative analysis.

RESULTS AND DISCUSSION

Chemical Analysis

The gas chromatograph of the female sex pheromone gland extract is shown in Fig. 1. A total of four sharp peaks were found showing the extract is composed of 16:OAc, Z11-16:OAc, 16:OH, and Z11-16:OH. The mass spectral data for these compounds are listed in Table 1.

Titer determination

The amounts of those compounds identified as Z11-16:OAc, Z11-16:OH, 16:OH, and 16:OAc were determined to be 60.0, 29.3, 9.3, and 9.0 ng/♀, respectively, with a ratio of 56:27:9:8 (Table 2). If the 16:OH and 16:OAc are precursors, then the ratio between Z11-16:OAc and Z11-16:OH should be 67.2:32.8, a ratio close to 2:1.

In this study, the chemical extraction method and conditioning of test insects were identical to those reported by Takahashi *et al.* (1979) and Zhu *et al.* (1987); although the chemical composition of the sex pheromone gland extract of *P. separata* in Taiwan

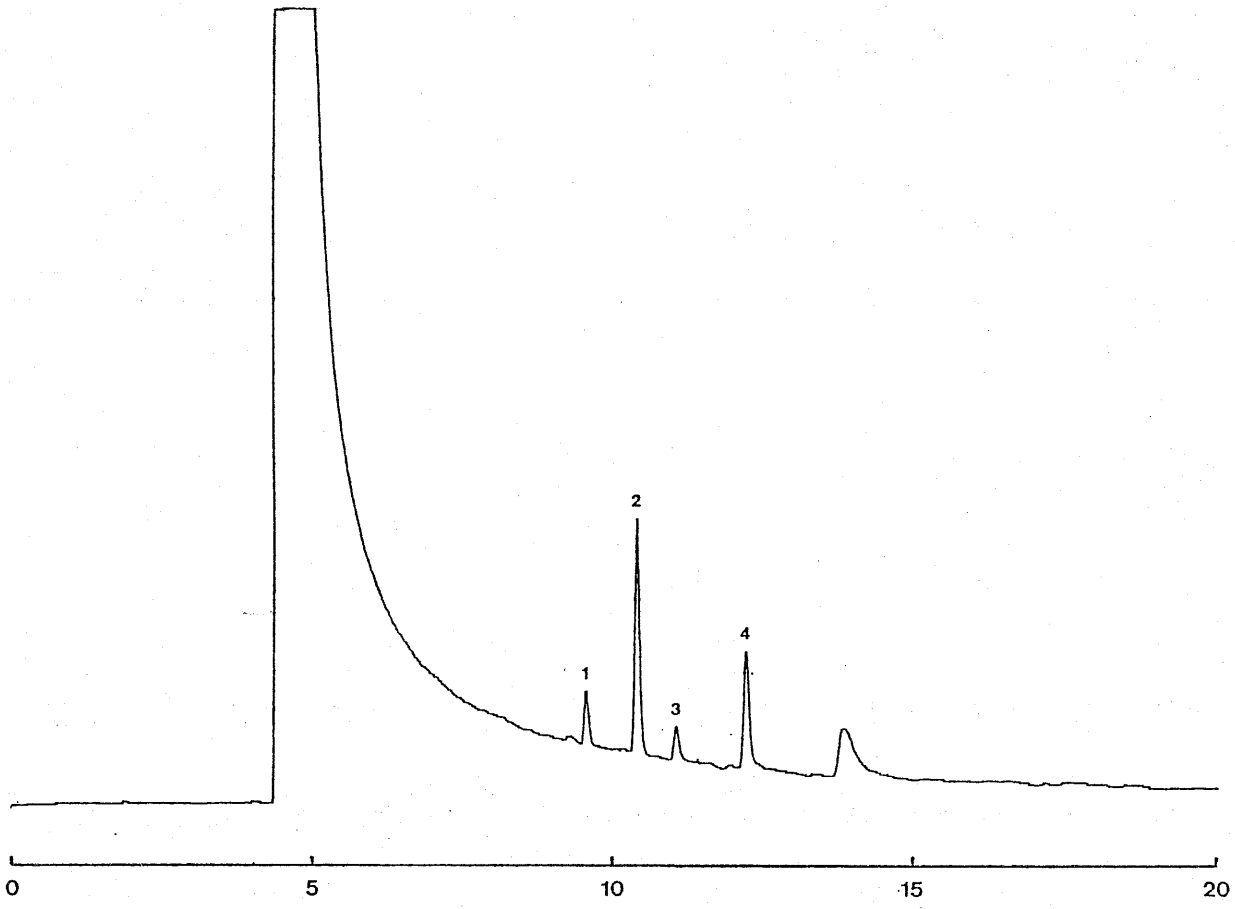

Fig. 1. Gas chromatogram of female sex pheromone gland crude extract. The gas chromatography was performed on a 30-m × 0.25-mm Carbowax 20M column; temperature was isothermal at 190 C. 1. 16:OAc; 2. Z11-16:OAc; 3. 16:OH; 4. Z11-16:OH.

Table 1

Mass spectra of four compounds identified from sex pheromone gland of female *Pseudaletia separata*

Compound	Mass spectral data M/Z (intensity, %)
Z11-16:OAc	43(100), 55(99), 82(85), 67(67), 96(65), 110(20), 61(12), 124(10), 138(5), 222(3), 152(2), 166(0.1)
Z11-16:OH	55(100), 41(71), 82(63), 67(53), 96(40), 109(13), 123(5), 222(0.3), 152(0.1)
16:OH	69(100), 43(91), 55(88), 57(85), 83(75), 97(48), 67(20), 111(13)
16:OAc	43(100), 69(50), 55(44), 83(42), 41(39), 57(37), 71(32), 97(29), 61(28), 85(27), 67(12), 111(10), 125(0.5)

Table 2

Average quantity (ng/♀) and percentage of Z11-16:OAc, Z11-16:OH, 16:OH, and 16:OAc produced by the sex pheromone gland of 4- to 5-day-old *Pseudaletia separata* virgin females

Component	Quantity (ng)	Percentage(%)
Z11-16:OAc	60.0	56
Z11-16:OH	29.3	27
16:OH	9.3	9
16:OAc	9.0	8

was found to be similar to that reported in Japan (Takahashi *et al.*, 1979), the observed ratio was different. In Japan, the ratio be-

tween the main sex pheromone components, Z11-16:OAc and Z11-16:OH, of *P. separata* was 8:1. Actually, the Japanese pheromone composition had been observed in Taiwan rice fields, and additional trapping of *P. separata* males was reported (Lin *et al.*, 1983). Whether the difference in pheromone ratios found between Taiwan and Japan is due to pheromone polymorphism or simply individual variation will be further investigated. The classical case of pheromone poly- morphism was reported in the European corn borer *Ostrinia nubilalis*; Kochansky *et al.* (1975) verified the existence of two North American strains: one in New York (E strain) with a ratio of 4:96 of Z11-14:OAc to E11-14:OAc, and one in Ontario (Z strain) with a 97:3 ratio (Klun and Robinson, 1971). Recently, the turnip moth *Agrotis segetum* has been shown to be clearly polymorphic with respect to its sex pheromone communication system; studies of different populations imply that the European distribution of *A. segetum* is divided into at least three main pheromone populations: a French population prodcuing more Z5-10:OAc, a Swedish population producing less Z5-10:OAc, and an Armenian/Bulgarian population producing very little Z5-10:OAc (Lofstedt *et al.*, 1986; Hansson *et al.*, 1990). The present study further confirms that the pheromone composition of *P. separata* in Taiwan and Japan are different from that reported in mainland China (Zhu *et al.*, 1987). Whether these insects are different species or not will be further investigated.

Acknowledgements: The authors would like to thank the National Science Council, R.O.C. (NSC 80-0211-B-001-09) for its financial support.

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臺灣粟夜盜 *Pseudaletia separata* Walker 性費洛蒙腺體之化學組成

寇 融 周延鑫 賀孝雍

經由氣相色層分析儀與質譜儀之分析結果，(Z)-11-hexadecenyl acetate (Z11-16:OAc), (Z)-11-hexadecenol (Z11-16:OH), hexadecanol (16:OH) 與 hexadecanyl acetate (16:OAc) 為粟夜盜 *Pseudaletia separata* Walker 雌虫性費洛蒙腺體之主成份。此四種成份在每隻雌虫之平均含量分別為 60.0, 29.3, 9.3 與 9.0 ng，其比例為 56:27:9:8。