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ISOLATION, IDENTIFICATION, AND SYNTHESIS OF SEX PHEROMONE COMPONENTS OF FEMALE TEA CLUSTER CATERPILLAR, *Andraca bipunctata* WALKER (LEPIDOPTERA: BOMBYCIDAE) IN TAIWAN

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Abstract—Octadecanal (18:Ald), (*E*)-11-octadecenal (*E*11-18:Ald), (*E*)-14-octadecenal (*E*14-18:Ald) and (*E*,*E*)-11,14-octadecadienal (*E*11,*E*14-18:Ald) were isolated and identified as major components from the pheromone glands of the tea cluster caterpillar, *Andraca bipunctata*, in Taiwan by analyzing the mass spectra of gland components and their DMDS adducts. GC retention times and mass spectra of the components were in agreement with those of authentic synthetic compounds. The average amount of 18:Ald, *E*11-18:Ald, *E*14-18:Ald and *E*11,*E*14-18:Ald per female gland (1 to 3 days old) was 121 ± 76, 50 ± 20, 187 ± 75, and 237 ± 110 ng, respectively, in a ratio of 20:8:31:41. Synthetic *E*11,*E*14-18:Ald caught more males than each of the other three components or blank control in field trapping tests. *E*11,*E*14-18:Ald is reported as an insect sex pheromone for the first time. Male antenna responded to *E*11,*E*14-18:Ald strongly in an EAG analysis. Furthermore, 4 hr after the injection of PBAN (pheromone biosynthetic activating neuropeptide) into decapitated female moths (2 days old), the percentage of the *E*11,*E*14-C18 Ald in the gland extract increased from 0% to 75.5%, which was also significantly more than that of unligated and uninjected control at 55.1%. All these data indicated that *E*11,*E*14-18:Ald is the sex pheromone of the *Andraca bipunctata* in Taiwan.

Key Words—Decapitated female moth, octadecanal, (*E*)-11-octadecenal, (*E*)-14-octadecenal, (*E*,*E*)-11,14-octadecadienal.

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INTRODUCTION

The tea cluster caterpillar, *Andraca bipunctata*, distributed mostly in Sumatra, Java, India, southern China and Taiwan, causes severe damage to tea plants, often resulting in abandoning of whole tea plantations. Current method of controlling this pest with pesticides is ineffective (Banerjee, 1982; Tseng, 1994). In previous work, we demonstrated that the use of the female sex pheromone of the smaller tea tortrix moth, *Adoxophyes* sp., for monitoring population and mating disruption was very successful in tea plantations in Taiwan (Kou et al., 1990; Kou and Chow, 1991; Tsai and Chow, 1992). It is contemplated that the same strategy may be applicable to the control of the tea cluster caterpillar. The sex pheromone of the tea cluster caterpillar, *Andraca bipunctata*, however, has not been described. To facilitate possible future utilization, we characterized and identified the chemical constituents of the sex pheromone gland of the tea cluster caterpillar by analyzing the mass spectra of components in the glandular extracts and their DMDS adducts. The double-bond configuration was determined by comparing the natural products with authentic synthetic compounds on several GC columns. The effectiveness of each of the synthetic compounds was tested by the EAG method. Synthetic compounds or their mixtures were used to catch males in field-trapping tests. Results showed that *E*11,*E*14-18:Ald, or mixtures that contained *E*11,*E*14-18:Ald effectively caught more males than the blank control under field conditions. We concluded that (*E*,*E*)-11,14-octadecadienal is the sex attractant of *A. bipunctata* in Taiwan.

Previously sex pheromone has been identified for only two species in the family Bombycidae, *Bombyx mori* (Linnaeus) and *B. mandarina* Moore (Butenandt et al., 1959; Kuwahara and Yen, 1979; Mayer and McLaughlin, 1991). Both species use (*E*,*Z*)-10,12-hexadecadien-1-ol (bombykol) as the principal component of their sex pheromone. To our knowledge, this is the third member of Bombycidae whose pheromone has been identified. It is also the first identification of (*E*,*E*)-11,14-octadecadienal as an insect sex pheromone.

METHODS AND MATERIALS

Insects. The experimental colonies were set up with pupae supplied by the Taiwan Tea Experiment Station, Hsinchu, Taiwan. Hatched larvae were reared on fresh tea leaves. Male and female pupae were held in separate rooms at 20°C under a 14L:10D regime.

Preparation of Pheromone Gland Extracts. Ovipositors from 1- to 3-day-old virgin females were excised 2 hr into the scotophase and immersed in 10 μl hexane per gland for 2 min. The extracts were stored at 4°C until bioassay and structure analysis (Kou and Chow, 1991).

Derivatization. Dimethyldisulfide (DMDS) derivatives of monounsaturated and diunsaturated compounds were prepared as described previously (Buser et al., 1983; Vincenti et al., 1987).

Chemical Analysis. Gas Chromatography (GC) of the extract was performed on a Varian 3700 GC equipped with a flame ionization detector. Three different capillary columns were used: a 30-m × 0.25-mm-ID fused silica capillary column of DB-Wax phase, a 30-m × 0.25-mm fused silica capillary column of DB-23 phase, and a 30-m × 0.25-mm fused silica capillary column of DB-225 phase. Chromatographic conditions included nitrogen carrier at 0.5 kg/cm^2, isothermal column temperature at 200°C for the DB-Wax column; a temperature program from 100°C to 250°C at 5°C/min for the DB-23 column, and a program from 100°C to 220°C at 4°C/min for the DB-225 column.

Gas chromatographic–mass spectrometric (GC-MS) analysis was conducted using a Finnigan MAT Incos 50 spectrometer. Samples were introduced through a 30-m × 0.25-mm DB-23 capillary column programmed from 80° to 250°C at 5°C/min and held at 250°C for 5 min. Helium was used as the carrier gas. For the analysis of the DMDS adducts of the unsaturated compounds a 30-m × 0.25-mm DB-5 capillary column was used and the temperature was programmed from 150°C to 320°C at 1°C/min. Electron impact (EI) mass spectra were collected at 70 eV with the separator at 250°C and the source at 180°C.

Chemical Synthesis: (*E*)-11-Octadecenol was obtained from NU-Chek-Prep. Inc. Elysian, Minnesota. (*E*)-2-Hexenol, (*Z*)-2-hexenol, 1-pentyne, and 1-octyne were purchased from Tokyo Chemical Industry Co. Pyridinium dichromate (PDC), 10-bromodecanol, and 11-bromoundecanol were obtained from Aldrich Chemical Company, Inc. (Milwaukee, Wisconsin). 7-Dodecynol was purchased from Lancaster Synthesis Ltd. (Lancashire, United Kingdom). Octadecanol and dimethyl disulfide were obtained from Merck Chemical Company.

Octadecanal and (*E*)-11-octadecenal were prepared from octadecanol and (*E*)-11-octadecenol, respectively, using PDC as an oxidizing reagent according to Corey and Schmidt (1979). (*Z*)-11-Octadecenal, (*Z*)-14-octadecenal, (*E*)-14-octadecenal, (*E,E*)-11,14-octadecadienal, and (*Z,E*)-11,14-octadecadienal were synthesized according to the procedures outlined in Figure 3 below. (*E*)-2-Bromohexene was prepared from (*E*)-2-hexenol by reacting with HBr vapor. For the synthesis of (*Z,Z*)-11,14-octadecadienal, the same strategy was used in the preparation of (*Z,E*)- and (*E,E*)-octadecadienal, except that (*Z*)-2-hexenol was used as starting material.

Pheromone Titer Determination. The extracts were prepared as described above and subsequently analyzed with the external standard method of quantitative analysis. Pheromone biosynthesis activating neuropeptide (PBAN), a 33-amino acid peptide, was obtained from Dr. A. Raina (USDA INHL, Bldg 306, Barc-East, Beltsville, Maryland 20705) as a gift. For PBAN analysis, virgin female moths were ligated between the head and thorax and decapitated. Forty-

eight hours after decapitation, 5 pmol of PBAN was injected into the abdomen, and 4 hr later the sex pheromone gland was excised and extracted with hexane. Saline was injected into the abdomen of controls. The extract was stored at 4°C for later analysis. Quantitative analysis was performed by the external standard method as before.

EAG Analysis. Chemicals of different dosage were loaded in a 5-ml glass syringe and puffed over an antennae. The EAG response from the chemicals was compared to the response of the antennae to an air blank. Each chemical was tested using EAG with at least six different antennae. The EAG response was preamplified (100× or 1000×) and recorded with a V1000 video, 4035 digital storage oscilloscope and ES1000 recorder (Gould Co. Ltd.) (Yang et al., 1992).

Field Testing. Field testing of the synthetic chemical formulation was carried out first for released males in a tea plantation in Taiwan Tea Experiment Station, Puehsin, Hsinchu, Taiwan, during November 1994, and subsequently in a local open tea plantation near Academia Sinica for wild males in April and May 1995. Synthetic chemicals were used separately and as a mixture. A total of 0.1 mg synthetic compounds was dissolved in hexane and loaded in plastic tubes, 5 cm × 1 mm ID. The plastic tube was suspended on the inside at the top of a wing-shaped sticky trap (Kou et al., 1992). Blank traps and traps baited with two female extracts were used for comparison. Traps were placed 10 m apart and 1.5 m above ground. For the capture of released males, a total of 120 laboratory-reared 1- to 3-day-old unmated males were released. Each trap was checked every afternoon for eight days. For the wild male field tests, traps were checked every week.

RESULTS AND DISCUSSION

Chemical Analysis. GC analysis showed a total of four major peaks, labeled A–D, in the chromatogram of a gland extract (Figure 1). Component A, with a molecular weight of 268, had the same mass spectrum as octadecanal (Table 1). Components B and C, both having a molecular weight of 266, had virtually identical mass spectra, with only a slight difference in relative intensities. The mass fragmentation patterns suggested isomeric monounsaturated octadecenals. Component D had a molecular weight of 264, and the mass spectral fragmentation pattern suggested an octadecadienal.

Synthetic octadecanal matched component A from gland extract in retention times on three GC columns and by mass spectrometry (Table 2), and we concluded that component A is octadecanal (Figure 1). The double-bond position of the monounsaturated aldehydes, components B and C, were inferred by the DMDS derivatization method, which was previously used for the determination

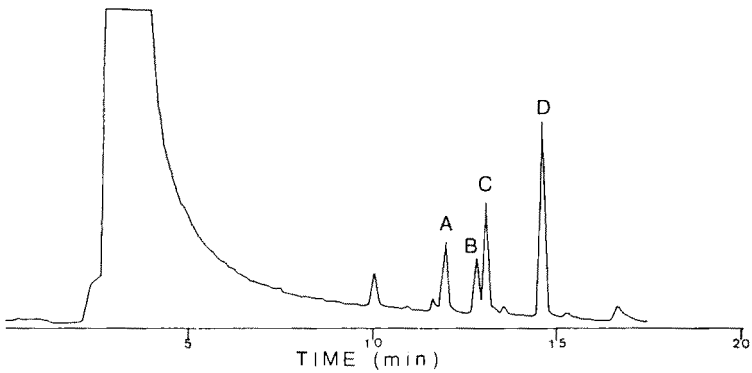


FIG. 1. Gas chromatogram of female sex pheromone gland crude extract. The gas chromatography was performed on a 30-m × 0.25-mm DB-Wax column and the oven temperature was isothermal at 200°C.

TABLE 1. MASS SPECTRAL DATA, AVERAGE QUANTITIES, AND PERCENTAGE OF FOUR COMPOUNDS IDENTIFIED FROM SEX PHEROMONE GLAND OF 1- TO 3-DAY-OLD *A. bipunctata* VIRGIN FEMALES (N = 22)

Compound	Mass spectral data, *m/z* (intensity, %)	Mean quantity (ng/female) (mean ± SD)	Percentage (mean ± SD)
18:Ald (A)	250(M^+-18, 4), 222(5), 194(4), 166(2), 138(10), 124(15), 110(18), 96(63), 82(90), 57(100)	121 ± 76	20 ± 3
*E*11-18:Ald (B)	266(M^+, 4), 248(18), 166(8), 135(20), 121(28), 111(32), 95(53), 83(60), 69(68), 55(100)	50 ± 20	8 ± 1
*E*14-18:Ald (C)	266(M^+, 4), 248(10), 166(4), 135(15), 121(20), 111(22), 95(43), 83(45), 69(49), 55(100)	187 ± 75	31 ± 7
*E*11,*E*14-18:Ald (D)	264(M^+, 3), 219(2), 165(1), 151(3), 123(3), 109(18), 95(33), 81(73), 67(100), 55(45)	237 ± 110	41 ± 4

TABLE 2. RETENTION TIMES OF 18:Ald, *E*11-18:Ald, *E*14-18:Ald, *E*11,*E*14-18:Ald, *Z*11-18:Ald, *Z*14-18:Ald, *Z*11,*E*14-18:Ald, *E*11,*Z*14-18:Ald, *Z*11,*Z*14-18:Ald AND PHEROMONE GLAND EXTRACT OF FEMALE *A. bipunctata* ON THREE GAS CHROMATOGRAPHIC COLUMNS

Compound	Source	Retention time (min)		
		DB-Wax	DB-23	DB-225
18:Ald	Natural	11.73	19.98	29.30
	Synthetic	11.79	20.01	29.344
*E*11-18:Ald	Natural	12.63	20.32	29.734
	Synthetic	12.67	20.34	29.760
*E*14-18:Ald	Natural	12.89	20.46	29.875
	Synthetic	12.93	20.48	29.903
*E*11,*E*14-18:Ald	Natural	14.39	20.94	30.483
	Synthetic	14.42	20.98	30.538
*Z*11-18:Ald	Synthetic		20.73	
*Z*14-18:Ald	Synthetic		20.89	
*Z*11,*E*14-18:Ald	Synthetic		21.16	
*E*11,*Z*14-18:Ald	Synthetic		21.34	
*Z*11,*Z*14-18:Ald	Synthetic		21.73	

of the double-bond position in monounsaturated acetates (Buser et al., 1983). DMDS adducts of the aldehydes were prepared, and the relevant mass spectral data are shown in Table 3. The mass spectrum of the DMDS derivative of component B showed a molecular ion at *m/z* 360 and two intense ions at *m/z* 145 and 215, presumably arising from a cleavage between the vicinal thiomethyl groups. Two possible aldehydes could yield the observed ions. The parent compound may be 11-octadecenal and the ion at *m/z* 145 could represent $CH_3-(CH_2)_5-CH=S^+CH_3$, which arises from the distal end of the molecule, whereas the ion *m/z* 215 would represent the functional-group-bearing end of the molecule (Figure 2A). Alternatively, the parent compound may be 6-octadecenal, with the ion at *m/z* 215 representing $CH_3-(CH_2)_{10}-CH=S^+CH_3$, arising from the distal end of the molecule, and the ion *m/z* 145 representing the functional-group-bearing end of the molecule. In order to distinguish between 11- and 6-octadecenal, the compounds in the gland extract were reduced to alcohols by $LiAlH_4$ and then derivatized to the DMDS adduct. The mass spectrum of the DMDS adduct of the alcohol of component B (Table 3) showed that the major fragments are at *m/z* 217 and 145, and we concluded that the component B is 11-octadecenal. The DMDS adduct of 6-octadecenol would give major fragments at *m/z* 147 and 215. Both (*E*)-11- and (*Z*)-11-octadecenal

TABLE 3. MASS SPECTRAL DATA OF DMDS DERIVATIVES OF MONOUNSATURATED AND DIUNSATURATED ALDEHYDES AND ALCOHOLS

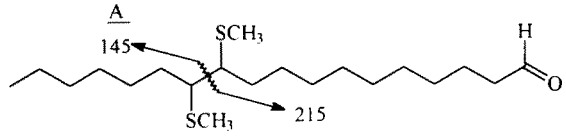
Compound	Mass spectral data of the DMDS derivatives (m/z, %)
*E*11-18:Ald (B)	360(M^+, 10), 243(12), 215(66), 145(95), 97(38), 81(33), 67(50), 61(85), 55(100)
*E*14-18:Ald (C)	360(M^+, 10) 257(72), 109(20), 103(60), 95(30), 81(30), 67(30), 61(100), 55(68)
*E*11-18:Alc	362(M^+, 0.25), 217(14), 145(21), 109(7), 95(17), 85(24), 71(34), 61(29), 57(91), 55(63)
*E*14-18:Alc	362(M^+, 0.36), 259(18), 109(8), 103(37), 95(16), 81(20), 67(24), 61(67), 55(61)
*E*11,*E*14-18:Ald[a] (D)	390(M^+, 3), 342(9-16), 239(48-100), 215(26-35), 175(20), 127(100), 103(19-38), 55(37-53)
*E*11,*E*14-18:Alc[a]	392(M^+, 2), 344(5-8), 241(12), 217(16-24), 175(10-24), 127(48-100), 103(19), 55(45-65)

[a]The ranges of abundance are due to the presence of chromatographically separated DMDS adduct isomers with slight differences in their mass spectra.

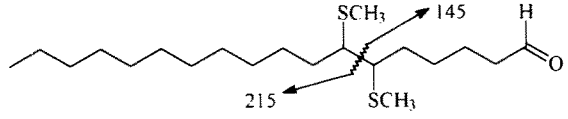
(Figure 3A) were synthesized and retention times on the DB-23 column were compared (Table 2). (*E*)-11-Octadecenal cochromatographed with component B of the gland extract, so it was concluded that component B is (*E*)-11-octadecenal.

Similar analysis of the mass spectrum of the DMDS derivative of component C (Table 3) revealed that the parent compound might be 14-octadecenal or 3-octadecenal (Figure 2B). Component C was reduced to the alcohol by $LiAlH_4$, as above, and the DMDS adduct of the corresponding alcohol was prepared and analyzed by GC-MS. The mass spectrum (Table 3) showed that the major fragments were at *m/z* 259 and 103, suggesting that the component C is 14-octadecenal, since the DMDS adduct of 3-octadecenol will give major fragments at *m/z* 105 and 257. Both *E* and *Z* isomers of 14-octadecenal were synthesized (Figure 3B) and compared with compound C on different GC columns (Table 2). The results indicated that (*E*)-14-octadecenal cochromatographed with component C of the gland extract and component C was identified as (*E*)-14-octadecenal.

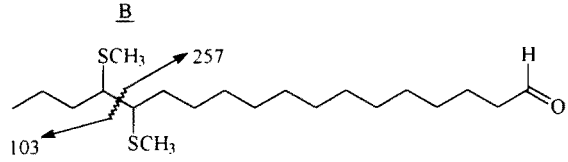
The diunsaturated octadecadienal was derivatized according to the procedure described by Vincenti et al. (1987). GC-MS analysis of the DMDS adduct showed fragments at *m/z* 215 and 103 (Table 3). From this fragmentation pattern, component D in the gland extract could be 11,14-octadecadienal or 3,6-octadecadienal (Figure 2C). Component D in the gland extract was reduced to an alcohol by $LiAlH_4$ and derivatized to its DMDS adduct. GC-MS analysis of



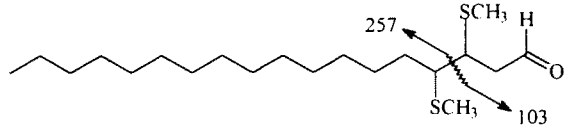
E- or Z-11-octadecenal DMDS adduct



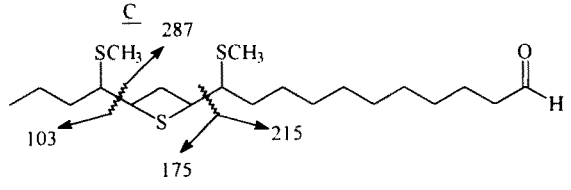
E- or Z-6-octadecenal DMDS adducts



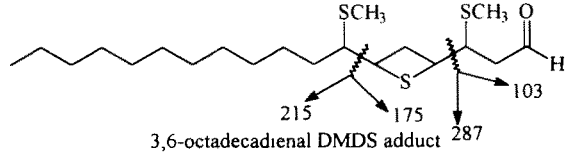
E- or Z14-octadecenal DMDS adduct



E- or Z-3-octadecenal DMDS adduct



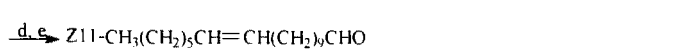
11,14-octadecadienal DMDS adduct



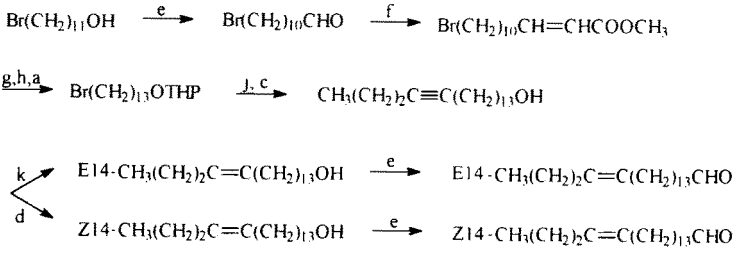
3,6-octadecadienal DMDS adduct

FIG. 2. Fragmentation patterns expected from DMDS adducts of candidate aldehydes in the gland extracts.

(A) Preparation of (Z)-11-octadecenal



(B) Preparation of (E)- and (Z)-14-octadecenal



(C) Preparation of (E,E)- and (Z,E)-11,14-octadecadienal

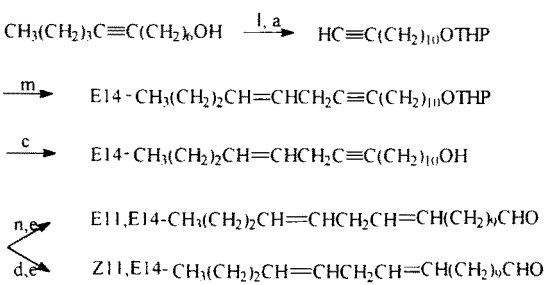


FIG. 3. Preparation of (*Z*)-11-octadecenal, (*E*)-14-octadecenal, (*Z*)-14-octadecenal, (*E,E*)-11,14-octadecadienal, and (*Z,E*)-11,14-octadecadienal. Reagents: (a) dihydropyrane/*p*-TSA (*p*-toluenesulfonic acid); (b) 1-octyne/*n*-BuLi (Schwarz and Waters, 1972); (c) MeOH/*p*-TSA; (d) H_2/Pd/$BaSO_4$; (e) PDC (pyridinium dichromate); (f) Wittig reaction, $Ph_3P{=}CHCO_2CH_3/CH_2Cl_2$; (g) H_2/Pd; (h) DIBAL (diisobutylaluminum hydride) (Yoon and Gyoung, 1985); (j) pentyne/*n*-BuLi (Schwarz and Waters, 1972); (k) $LiAlH_4$/diglyme (diethyleneglycol dimethyl ether) (Rossi and Carpita, 1977); (l) potassium 3-aminopropylamide (KAPA) (Brown and Yamashita, 1976); (m) (*E*)-2-bromohexene/*n*-BuLi (Schwarz and Waters, 1972); (n) Na/NH_3 (Schwarz and Waters, 1972). *Abbreviation:* THP, 2-tetrahydropyranyl.

the adduct showed a molecular weight of 392 and fragments at *m/z* 217 and 103 (Table 3). These results indicated that the alcohol was 11,14-octadecadienol, since 3,6-octadecadienol would give major fragments at *m/z* 215 and 105. Thus, it was concluded that component D in the gland extract was 11,14-octadecadienal. The four geometric isomers of 11,14-octadecadienal, i.e., (*E*,*E*)-, (*Z*,*E*)-, (*Z*,*Z*)-, and (*Z*,*E*)-11,14-octadecadienal, were synthesized and their retention times on GC were compared with that of the component D (Table 2). Cochromatography of (*E*,*E*)-11,14-octadecadienal with the natural product showed agreement on different columns. Thus, component D in the sex pheromone gland extract was identified as (*E*,*E*)-11,14-octadecadienal.

Pheromone Titer Determination and PBAN Stimulation Tests. Recently, Raina et al. (1989) reported that pheromone biosynthesis activating neuropeptide (PBAN) regulates pheromone production in some female insects. We found that the amount of the four components was zero if the adult female moth of the tea cluster caterpillar was decapitated and saline was injected into the abdomen (Table 4). In contrast, if PBAN was injected instead of saline into the neck-ligated and decapitated moth, the four components we have identified were found in the gland extract, and the percentage of *E*11,*E*14-18:Ald in the gland extract was as high as 75.5%. This indicated that all four compounds were probably related to pheromone production. The fact that the percentage content of *E*11,*E*14-18:Ald in the gland extract of the PBAN treated females was more than that of normal virgin females (55.1%) suggests that *E*11,*E*14-18:Ald is the sex pheromone of *Andraca bipunctata*.

EAG Analysis. The EAG analysis of octadecanal, (*E*)-11-octadecenal, (*E*)-14-octadecenal, and (*E*,*E*)-11,14-octadecadienal of different dosage showed that the antennae of the male strongly responded to (*E*,*E*)-11,14-octadecadienal and only slightly to the other three components (Figure 4). The EAG response of each component was measured only at doses of 200 and 300 ng since the response induced by 200 or 300 ng of synthetic chemicals was comparable to

TABLE 4. EFFECT OF PBAN ON PERCENTAGE OF FOUR COMPONENTS IN FEMALE PHEROMONE GLAND EXTRACT OF *A. bipunctata* WALKER

	18:Ald[a]	*E*11-18:Ald[a]	*E*14-18:Ald[a]	*E*11,*E*14-18:Ald[a]
Normal female (8)[b]	18.5 ± 4.3a	6.9 ± 1.2a	19.4 ± 2.4a	55.1 ± 4.9b
PBAN injected female (8)[b]	9.9 ± 2.3b	3.9 ± 1.1b	10.5 ± 1.3b	75.5 ± 3.3a
Ligated female (11)[b]	0	0	0	0

[a]Means followed by a different roman letter in the same column are significantly different ($P = 0.05$).

[b]Number of replicates indicated in parentheses.

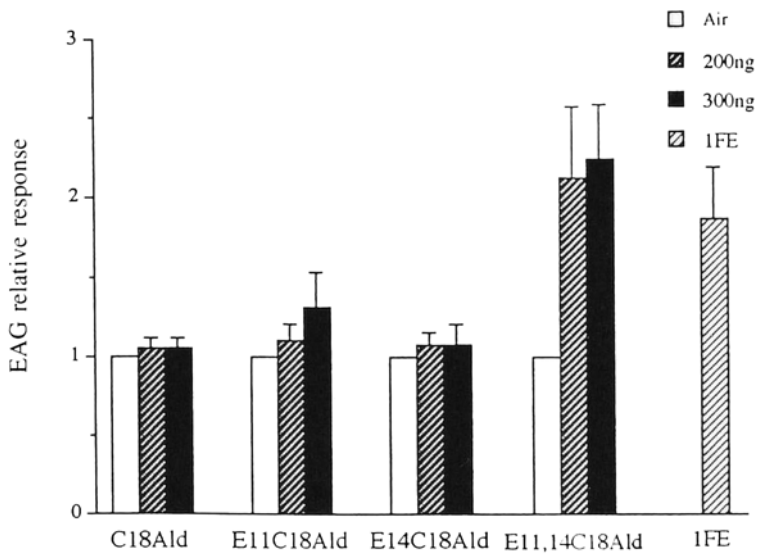


FIG. 4. EAG response of male *Andraca bipunctata* to 18:Ald, *E*11-18:Ald, *E*14-18:Ald, *E*11,*E*14-18:Ald, and 1 FE. The vertical bar indicates the standard error of the mean.

TABLE 5. NUMBER OF MALE *A. bipunctata* ATTRACTED TO SYNTHETIC COMPOUNDS, 18:Ald/*E*11-18:Ald/*E*14-18:Ald/*E*11, *E*14-18:Ald (20:8:31:41) AND VIRGIN FEMALE EXTRACT DURING NOVEMBER 6-16, 1994 AT TAIWAN TEA EXPERIMENT STATION, PUESHIN[a]

Date	18:Ald/*E*11-18:Ald/*E*14-18:Ald/*E*11,*E*14-18:Ald (20:8:31:41)		2 virgin female equivalent	Control
	0.1 mg	0.5 mg		
1994 Nov. 6	0	0	0	0
7	3	2	0	0
8	1	2	0	0
9	0	0	0	0
10	0	0	0	0
14	1	4	0	0
15	0	0	0	0
16	0	0	0	0
Total	5	8	0	0

[a]The lure dispenser was a plastic tube.

that of one female equivalent. The EAG results provided support for (*E*,*E*)-11,14-octadecadienal as the sex pheromone of the insect.

Field Trapping of Released Males. The results of field trapping of released males with mixtures of the synthetic compounds in a ratio of 20:8:31:41 and virgin-female extract are shown in Table 5. A total of 120 males were released and only 13 were caught. The reason for the poor recapture of males in this experiment was probably due to the low winter temperature when the trapping took place.

Field Trapping of Wild Males. The results of field trapping of wild males with each of the synthetic compounds and mixtures of the synthetic compounds are shown in Tables 6 and 7. The results in Table 6 showed that component D (*E*11,*E*14-18:Ald) alone, two-component treatments (A + D, B + D, C + D), and mixtures of four synthetic compounds (mixed in the same ratio as in the virgin female extract) caught more males than treatments without component D. The numbers of moths caught by the different treatments were not significantly different as long as component D was present. Table 7 shows the comparison of the number of males caught by three-component and four-component treatments. Although the adult season was almost over in May, and only a few male moths were caught in this test, the results also showed no difference between the three-component treatments and four-component treatments. The fact that the number of males captured by *E*11,*E*14-18:Ald alone and by mixtures of two, three, or four components were not significantly different from each other support EAG data and PBAN results, and we conclude that *E*11,*E*14-18:Ald is the attractive component of the sex pheromone gland of *Andraca bipunctata*. Although a single pheromonal component is rare in insect literature, it is not unprecedented. For example, female gypsy moths use only (7*R*,8*S*)-*cis*-7,8-epoxy-12-methyloctadecane to attract males. To see if there is any synergetic effect of the other components found in the gland extract, more extensive field tests will be conducted in the near future.

From our results of PBAN stimulation, EAG analysis, and field trapping tests, we tentatively conclude that the sex pheromone of *Andraca bipunctata* is *E*11,*E*14-18:Ald. If the additional three components in the gland extract do not have any function in male attraction, the reason they are present may be that they are precursors in the biosynthesis of (*E*,*E*)-11,14-octadecadienal. The moth may synthesize *E*11,*E*14-18:Ald from 18:Ald via *E*11-18:Ald or via *E*14-18:Ald in the pheromone gland. The Δ11 desaturase enzyme identified from some Lepidoptera may produce *E*11-18:Ald from the saturated 18:Ald (Roelofs and Wolf, 1988). A Δ14 desaturase is also reported to be involved in the biosynthesis of pheromone in the Asian corn borer (Zhao et al. 1990). Thus the sex pheromone *E*11,*E*14-18:Ald reported here could be produced by catalytic activities of the Δ11 and Δ14 desaturases in its biosynthetic pathway as reported in other insects.

TABLE 6. NUMBER OF MALE *Andraca bipunctata* ATTRACTED TO SYNTHETIC SEX PHEROMONE 18:Ald (A), *E*11-18:Ald (B), *E*14-18:Ald (C), *E*11,*E*14-18:Ald (D) DURING APRIL 1995 AT NANKANG TEA PLANTATION NEAR ACADEMIA SINICA[a]

	Treatment																										
	A			B			C			D			A+D			B+D			C+D			A+B+C+D			Control		
Date	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1995 Apr. 5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	3	0	1	1	3	1	0	3	5	0	0	0
8	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	2	1	1	4	1	4	9	0	0	0
15	0	0	0	0	0	0	0	0	0	0	1	2	0	2	3	1	3	4	0	1	3	5	1	2	0	0	0
20	0	0	0	0	0	0	0	0	0	1	4	3	2	3	1	2	1	4	3	2	4	2	3	3	0	0	0
29	0	0	0	0	0	0	0	0	0	4	11	24	17	8	14	17	14	12	15	10	12	9	9	9	0	0	0
Total		0			0			0		6	17	29	21	14	20	24	19	23	20	17	24	17	20	28	0	0	0
Average[c]		0b			0b			0b			17.3a			18.3a			22a			20.3a			21.6a			0b	

[a]The lure dispenser was a plastic tube. Total amount of the chemicals was 0.1 mg. I, II, III indicate replicates.
[b]The ratio of the four chemicals was 20:8:31:41.
[c]Averages followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

TABLE 7. NUMBER OF MALE *Andraca bipunctata* ATTRACTED TO SYNTHETIC SEX PHEROMONE 18:Ald (A), *E*11-18:Ald (B), *E*14-18:Ald (C), *E*11,*E*14-18:Ald (D) DURING MAY 1995 AT NANKANG TEA PLANTATION NEAR ACADEMIA SINICA[a]

	A+B+D			A+C+D			B+C+D			A+B+C+D			Control		
Date	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1995 May 8	0	2	2	2	0	1	3	1	2	1	2	2	0	0	0
15	1	0	0	1	1	0	1	1	2	0	1	1	0	0	0
25	3	1	5	2	1	0	0	1	4	2	4	5	0	0	0
30	0	1	0	0	1	2	2	0	1	1	3	0	0	0	0
June 5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Total	4	4	7	5	3	3	6	3	9	4	10	9	0	0	0
Average[c]		5.0a			3.6ab			5.3a			7.6a			0c	

[a]The lure dispenser was a plastic tube. Total amount of the chemicals was 0.1 mg. I, II, III are replicates.
[b]The ratio of the four chemicals was 20:8:31:41.
[c]Averages followed by the same letter are not significantly different by Duncan's multiple range test ($P = 0.05$).

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