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SEX PHEROMONE COMPONENTS ISOLATED FROM CHINA CORN BORER, *Ostrinia furnacalis* GUENÉE (LEPIDOPTERA: PYRALIDAE), (*E*)- AND (*Z*)-12-TETRADECENYL ACETATES

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Abstract—The sex pheromone components from the corn borer spreading widely in China, *Ostrinia furnacalis* Guenée, have been identified as (*E*)-and (*Z*)-12-tetradecenyl acetates (*E* and *Z* 12–14: Ac). The ratio of *E* isomer to *Z* isomer was 53:47. Traps containing 1×10^{-7}–1×10^{-5} g of these compounds captured more males than did live females or their tip extract (3–6 female equivalents). Tetradecyl acetate (14: Ac) was also identified in the tip extract. Its quantity was about 1.8 times the sum of the other two isomers. However, including this compound in its natural ratio in pheromone traps resulted in a decrease in trap catches ($P < 0.05$).

Key Words—Lepidoptera, Pyralidae, *Ostrinia furnacalis*, sex pheromone, corn borer, (*E*)-12-tetradecenyl acetate, (*Z*)-12-tetradecenyl acetate, tetradecyl acetate, gas chromatography–mass spectrometry, selected ion monitoring technique.

INTRODUCTION

From external morphology, the corn borer spreading widely in China is almost indistinguishable from the European corn borer, *Ostrinia nubilalis* Hübner. Therefore, some authors (e.g., Cai, 1973) described the China corn borer as *O. nubilalis*. However, Mutuura and Munroe (1970) identified this species as *O. furnacalis* Guenée.

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It has been known for several years that two species of the genus *Ostrinia*, *O. nubilalis* and *O. obumbratalis* Lederer, use the same compounds, namely, (*Z*)- and (*E*)-11-tetradecenyl acetates (*Z* and *E*11-14:Ac) in their sex pheromone communication systems (Klun and Junk, 1977; Klun and Robinson, 1972). However, the sex pheromone of *O. furnacalis* has not yet been identified.

During the period August 1974 to March 1975, we carried out field screening tests with synthetic unsaturated and saturated straight-chain acetates in corn fields in Yangshan County and Hainan Island, Kwangtung Province, of South China. Neither pure *Z*11-14:Ac, *E*11-14:Ac, nor their mixtures in different ratios attracted male corn borer moths in our field experiments.

The preliminary experiments on female tip extracts started in the summer of 1977. According to the results of chemical reactions and GC-MS experiments, we concluded that the pheromone components of *O. furnacalis* were tetradecenyl acetates, but the position of the double bond was not known (Yang, 1978). At the end of 1978, the sex pheromone system was finally characterized qualitatively and quantitatively. Field tests on synthetic pheromonal acetates were carried out during the period 1979–1980. In the present paper the chemical composition of the sex pheromone of *O. furnacalis* and the pheromonal activity of the synthetic compounds are described.

METHODS AND MATERIALS

Collection and Extraction. All corn borers used in this study were collected in corn fields in Yangshan County. They were mainly collected in the form of pupae, but some of them were obtained in the final larval stage. The larvae collected in such a way were allowed to stay until pupation. The pupae were sexed, and the females were allowed to emerge under room conditions. The healthy female moths were transferred into a screened cage (60 cm high × 40 cm diameter), sprayed with water twice a day, and held at room temperature for 1–2 days. Then the moths were narcotized by treatment with methylene chloride or diethyl ether vapor at 3–5 AM (mating time for the corn borer). As was proved by bioassay, the pheromone gland of *O. furnacalis* was located on the surface of the intersegmental membrane between the 8th and 9th abdominal segments. These segments were normally retracted in the 7th segment. In order to obtain the gland, the females were forced to extrude the 8th and 9th abdominal segments by gentle squeezing of the base of the abdomen. The abdominal tip, consisting of the 8th and 9th segments, was snipped into methylene chloride solvent. The crude preparations were stored at 0° C in a refrigerator before use. After removal of solvent from the filtrate, a

yellow oil was obtained in amounts corresponding to about $3\text{–}5 \times 10^{-5}$ g per female tip.

TLC and Column Chromatography. The thin-layer chromatography was performed on silica gel G (about 0.25 mm thick), using petroleum ether (60–90° C) and diethyl ether (95:5, v/v) as a solvent system. This procedure was used to check the purity of fractions eluted from the silica gel column before they were injected into GC columns.

A glass column (1 cm ID) packed with 30 g of 100–140 mesh silica gel was used to clean up the crude extracts utilizing the same solvent system as above. Extract from 2000 female tips was loaded onto a column each time.

Microchemical Reactions. A detailed report on microchemical reactions for sex pheromone has been published separately (Yang, 1979). For each reaction a purified fraction or crude extract corresponding to about 70–150 FE was usually used. Generally, the reactions were carried out in a volume as small as 1 ml or less. Transfer of material from one vessel to another was avoided in order to reduce loss.

The oxidation reaction of crude or purified extract was carried out at room temperature with $KMnO_4$ in acetone, acidified by H_2SO_4.

Addition reactions of pheromone-active fractions eluted from a silica gel column were performed with bromine solution in CCl_4. Elimination of bromine from brominated products were accomplished by grinding the dibromides with zinc powder in ethanol.

Saponification of pheromone-active fractions was carried out in a 4% KOH–ethanol solution under reflux for an hour. Reacetylation was performed by addition of acetyl chloride to the alcoholic constituents, which were obtained by extraction of the saponified mixture with petroleum ether or diethyl ether.

The microozonolysis reaction procedure has been described elsewhere (Beroza and Bierl, 1967).

GC-MS Experiments. All chromatographic data in the present work were obtained on a Varian Aerograph 2740 GC-MAT 311 A MS system fitted with a two-stage Watson-Biemann separator using the selected ion monitoring (SIM) technique at 70 eV. The advantages of this technique over the conventional total ion current monitoring method are its good selectivity and high sensitivity. We have successfully detected as little as 2×10^{-11} g of (*Z*)-9-dodecenyl acetate, while researching the sex pheromone of the sugarcane borer, *Argyroploce schistaceana* Snellen, utilizing m/z 61 $(CH_3COOH_2)^+$ as a characteristic ion for straight-chain acetates (Laboratory No. 1, Kwangtung Institute of Analysis, 1978).

It is most important to choose the proper ions for the SIM technique. As shown above, the ion at m/z 61 $(CH_3COOH_2)^+$ is suitable for detection of straight-chain acetates. Its intensity is always high, and in most cases the

interference from impurities is negligible. On the other hand, in some cases the intensity of the molecular ion M^+ is too weak to be detected, but fortunately the intense ion $(M- CH_3COOH)^+$ may be used to calculate the molecular weight of the original compound.

Both Carbowax 20 M support-coated open tubular (SCOT) glass capillary columns and a 5% DEGS (on 80–100 Gas Chrom Q) stainless-steel column (3.5 m × 2 mm ID) were used in the present study.

Field Trapping Tests. All steps in purification and identification were monitored for pheromonal activity by field trapping tests. The synthetic pheromone components were also tested by the same technique. A simple water trap was used for this purpose. It was a 30-cm-diameter vessel filled with water, with detergent added to reduce the surface tension of water. A paper roll impregnated with pheromonal solution and supported 1–1.5 cm above the water surface was used as bait. The height of the trap above the ground depended on the height of the plants. Usually, it was about 80–100 cm. The lures in the traps were changed every night.

In 1979 no pure *Z* 12–14: Ac was available, so a pure *E* 12–14: Ac and a crude *Z* isomer sample (*Z* 12–14: Ac/*E* 12–14: Ac/ 14: Ac = 92: 1: 7) were used to make lures for trapping tests (Tables 2 and 3). In 1980 all three acetates were obtained in pure form; therefore, more accurate field experiments could be done (Tables 4 and 5).

Since the numbers of replicates changed from sample to sample, we could not use the statistical test especially designed for comparison of several samples of equal size. We had to compare every pair of samples using the *t* table (Anderson, 1978; Fryer, 1966), and then summarize the results (Tables 2–5).

All field tests were carried out in Yangshan County, Kwangtung.

RESULTS AND DISCUSSION

Identification of Pheromone Components. The pheromone-active components from female corn borers were completely destroyed by saponification and could be restored by reacetylation. The activity was also destroyed by bromination or oxidation by $KMnO_4$ and restored in the first case by debromination. The results of these microchemical reactions suggest that the pheromone of *O. furnacalis* consists of acetates of unsaturated alcohols.

Only a narrow band on the TLC plate was found to be pheromone-active. Its R_f value (about 0.5–0.6) coincided with that of straight-chain acetate esters and was slightly higher than that of glycerides.

Further support for esters was also obtained when a crude extract was purified by chromatography on silica gel. During the clean-up procedure one to two 6-ml (usually 13th and/or 14th) fractions showed biological activity.

The eluted volume of active fractions was also coincident with that of synthetic long straight-chain acetates.

The purity of all active fractions from the silica gel column was checked separately by TLC. Only the highly pure fractions were injected onto GC columns.

As discussed above the acetate character of the pheromone compounds was indicated by preliminary experiments, including microchemical reactions and thin-layer and column chromatography. The next step involved detection and identification of the straight-chain acetates present in female tip extracts using GC-MS and the SIM technique.

When the ion at m/z 61 was monitored, three well-separated GC peaks (retention times for peaks I, II, and III were 20.43, 26.70, and 28.85 min, respectively, Table 1) were found in the pheromone-active fractions on a Carbowax 20 M SCOT capillary column (36 m × 0.29 mm ID) under the following conditions: the carrier gas (helium) was adjusted to a flow rate of 3.6 ml/min with 30 ml/min makeup helium; the column was heated isothermally at 150° C. Only peak I at 20.43 min could be found when monitoring m/z 196; peaks II and III at 26.70 and 28.85 min could be found when monitoring m/z 194; and finally none of these peaks appeared when monitoring m/z 192 (Figure 1). These results indicate that I was a peak from tetradecyl acetate, and II and III were peaks from tetradecenyl acetates.

According to the data of synthetic samples on Carbowax 20 M SCOT capillary columns (Table 1), the retention times of GC peaks were positively correlated with increasing distance between the acetate group and the carbon–carbon double bond. In addition, the retention times of *Z* isomers were longer than those for corresponding *E* isomers. The compounds represented by GC peaks I, II, and III were identical with 14:Ac (I), *E*12-14:Ac (II), and

TABLE 1. RETENTION TIMES OF GC PEAKS OF SEX PHEROMONE COMPONENTS AND SOME STANDARD STRAIGHT-CHAIN ACETATES (OCTOBER 5–17, 1978)

	Retention time (min)						
Column number[a]	Peak I	*E*9-14:Ac	*Z*9-14:Ac	*E*11-14:Ac	*Z*11-14:Ac	Peak II	Peak III
1	10.38	12.30	12.85		13.16	13.63	14.80
2	20.43		23.90	24.52	25.63	26.70	28.85
3	35.62			41.30	43.85	45.73	49.23

[a]GC conditions: SCOT glass capillary columns, coated with Carbowax 20 M, column temperature 150° C, makeup helium 30 ml/min, two-stage Watson-Biemann type separator. Column 1: 37 m × 0.32 mm ID, helium flow 1.5 ml/min; column 2: 36 m × 0.29 mm ID, helium flow 3.6 ml/min; column 3: 50 m × 0.31 mm ID, helium flow 3.6 ml/min.

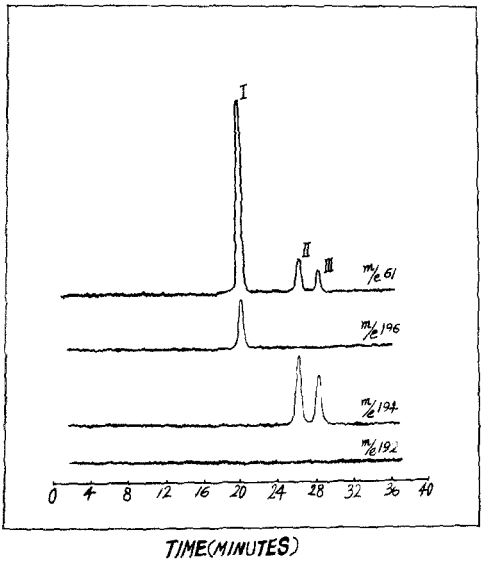

FIG. 1. Mass chromatograms of sex pheromone components of *Ostrinia furnacalis* (GC conditions: see Table 1, column 2).

Z 12–14:Ac (III), respectively, in retention times and mass fragmentation patterns. These three components were eluted in the same order from a DEGS-packed column, operating at 160° C and a flow rate (helium) of 20 ml/min. Their retention times were 6.78, 8.93, and 9.70 min, respectively. They showed the same spectral characteristics as the components eluted from capillary columns. Coinjection of active fractions with authentic compounds was also conducted. Upon coinjection, the intensities of the GC peaks increased, but their retention times and widths remained unchanged. These enhancement experiments further confirmed the above-mentioned assignments.

The unsaturated acetates were ozonized to the corresponding aldehydes by Beroza's method. A peak at 14.23 min was detected in the ozonized products on the DEGS column, operating at 180° C and a helium flow of 30 ml/min, when the ion of *m/z* 61 was monitored using the SIM technique. This compound was identical with the authentic 12-acetoxydodecanal in retention time and mass spectral characteristics (*m/z* 61 and 139); thus, the ozonolysis experiment further supported the conclusion that 12-tetradecenyl acetates were present in the pheromone system of *O. furnacalis*.

The *E*-to-*Z* ratio for 12-tetradecenyl acetates determined by gas chromatographic peak areas was 53:47. At the same time, the quantity of tetradecyl acetate was about 1.8 times the sum of the other two compounds.

Field Trapping Tests. The preliminary field tests showed that mixtures of

E and *Z* 12–14: Ac in nearly all possible ratios were attractive to males, but the most efficient lures were the mixtures with ratios like the natural one (Table 2). On the basis of data listed in Table 2, we came to the conclusion that synthetic mixtures at microgram levels were more attractive to males than three live virgin females. For example, the mean catches for the artificial mixture in natural *E*/*Z* ratio were nearly six times higher than those for live female traps (9.3 against 1.6).

The mixture with the natural *E*/*Z* ratio which showed the highest attractiveness in field tests (trap No. 6 in Table 2) was used to investigate the relationship between lure doses and mean catches (July 29–August 1, August 15–September 5, 1979). The maximum sexual attractiveness was reached within a rather broad range of doses (1×10^{-7}–1×10^{-5} g per trap). Again the efficiency for traps at this dose level was higher than that for three virgin females. At the same time, no dependence of mean catches on dose could be found within this dose range, but mean catches diminished significantly as the lure dose either decreased or increased from this range (Table 3). The males were not trapped with lure dose higher than 1×10^{-4} g per trap.

Although populations of corn borer adults were relatively low in Yangshan County in late August and early September 1979, mean catches for artificial mixtures reached two figures, and maximum catches were 44 males per trap per night.

TABLE 2. BIOASSAY RESULTS FOR SYNTHETIC MIXTURES (JULY 22–AUGUST 1, 1979)

Trap number	Mixture composition (%)[a]			Number of replicates	Mean trap catches (males/trap/night)[b]
	*E*12–14: Ac	*Z*12–14: Ac	14: Ac		
1	1	92	7	10	4.2bcd
2	11	83	6	6	3.5bcde
3	21	74	5	8	5.0abc
4	31	64	5	6	5.2abc
5	41	55	4	8	8.0ab
6	51	46	3	19	9.3a
7	60	37	3	8	5.8abc
8	70	28	2	6	3.7cde
9	80	19	1	8	4.1cd
10	90	9	1	6	3.7cde
11	100	0	0	12	2.3de
12	3 Virgin females			7	1.6e
13	Unbaited			13	0.08f

[a] Total dose of lure was 2×10^{-6}–1×10^{-5} g per trap.
[b] Means followed by the same letter in each experiment were not significantly different from each other at 5% level.

TABLE 3. RELATIONSHIP BETWEEN MEAN CATCHES AND DOSES OF LURE (JULY 29–AUGUST 1, AUGUST 15–SEPTEMBER 5, 1979)

Dose (g/trap)[a]	Number of replicates	Mean catches (males/trap/night)[b]
5×10^{-9}	15	4.3b
1×10^{-8}	17	2.2bc
2×10^{-8}	12	1.0c
1×10^{-7}	16	9.6a
2×10^{-7}	44	11.4a
5×10^{-7}	22	10.7a
1×10^{-6}	58	12.3a
2×10^{-6}	32	12.2a
5×10^{-6}	12	10.6a
1×10^{-5}	10	8.7a
2×10^{-5}	10	2.7b
1×10^{-4}	8	0d
2.6×10^{-4}	4	0.25cd
1×10^{-3}	10	0d
Unbaited	35	0.1d
1 Virgin female	2	1.0
2 Virgin females	2	4.0
3 Virgin females	4	4.7

[a]Percentage composition of synthetic mixture: *E*12–14:Ac/*Z*12–14:Ac/14:Ac = 51:46:3.
[b]Means followed by the same letter in each experiment were not significantly different from each other at 5% level. Mean catches for virgin females were not included in the statistical calculation, because only a few live females were available for tests.

As mentioned above, there were three long-chain acetates in tip extract from female *O. furnacalis*, and the saturated tetradecyl acetate was the major constituent in the mixture, so it was imperative to learn what role this compound could play in the pheromonal communication system for this species. During a period of 1977–1978, we attempted to use pure 14:Ac as a bait in field screening experiments, but these experiments failed. Moreover, after the carbon–carbon double bond in female extract had been destroyed by treatment with bromine or $KMnO_4$, the resultant mixtures, still containing unchanged 14:Ac, became unattractive to males.

As the role of 14:Ac in the reproductive behavior of *O. furnacalis* was quite unclear, a special study was designed to evaluate its synergetic or inhibitory effects on the attractiveness of *E* and *Z* 12–14:Ac mixture in the field. Side-by-side comparison of a three-component lure (*E*12–14:Ac/*Z*12–14:Ac/14:Ac = 17:15:68) with a two-component lure (*E*12–14:Ac/*Z*12–14:Ac/14:Ac = 51:46:3) showed that the mean catch for the first was 4.7 males/trap/night vs. 11.7 for the last ($P < 0.05$). The ratio of *E*/*Z* in three-component lure was 53:47, which was identical with that for the two-

component lure and natural extract from female tips. As mentioned above, the percentage composition of the natural extract was *E*12–14:Ac/*Z*12–14:Ac/14:Ac = 19:17:64 with a *E*/*Z* ratio of 53:47. The total dose of (*E*)-and (*Z*)-12-tetradecenyl acetates in the three-component lure was the same as in the two-component one ($2\text{–}2.5 \times 10^{-6}$ g per trap). The only difference for these two kinds of lures was in the amount of saturated tetradecyl acetate.

Results from additional field experiments were obtained in July 1980. Most of these data are presented here. As shown in Table 4, pure 14:Ac and *Z*12–14:Ac did not have attractivity significantly different from zero (unbaited traps). The pure *E*12–14:Ac, however, showed real activity which was significantly above zero (unbaited traps). A comparison between the attractiveness of pure and mixed acetates revealed the mixture of *E* and *Z*12–14:Ac in their natural ratio to be about 4.5 times more attractive than the pure *E* isomer. Nevertheless, it is difficult to explain the difference between pure *Z* and *E* isomers in attractive behavior. These results are similar to those obtained in the summer of 1979 (Table 2).

At present, the major purpose of field trapping tests is to research the role of 14:Ac in sex attraction. On the basis of data in Table 5, we can conclude that tetradecyl acetate, even though it is present in female tip extract, is not a sex pheromone component for *O. furnacalis*. As a matter of fact it acts as an inhibitor in the sex communication system. A small amount of 14:Ac added to the artificial sex pheromone mixture significantly diminishes the mean trap catches. The inhibitory effect of 14:Ac on sex attractiveness increases as its content in the mixture rises.

Since the role of 14:Ac in the pheromonal behavior for *O. furnacalis* is

TABLE 4. BIOASSAY RESULTS FOR SYNTHETIC ACETATES (JULY 6–11, 1980)

Composition of lures (μg)			Number of replicates	Mean catches (males/trap/night)[a]
*E*12–14:Ac	*Z*12–14:Ac	14:Ac		
1	1	0	44	2.89a
2	0	0	40	0.65b
0	2	0	38	0.08c
0	0	2	85	0.07c
	Unbaited		35	0c
	3 Virgin females		9	0.22

[a]Means followed by the same letter in each experiment are not significantly different from each other at 5% level. Mean catches for virgin females are not included in statistical calculation, because only a few live female moths were available for tests.

TABLE 5. TEST OF ROLE OF 14:AC IN SEX ATTRACTION (JULY 13–17, 1980)

Composition of lures (μg)			
*E*12–14:Ac	*Z*12–14:Ac	14:Ac	Mean catches (males/trap/night)[a]
2	2	0	8.3a
2	2	0.2	4.2b
2	2	0.4	2.9b
2	2	0.8	3.6b
2	2	1.2	2.1bc
2	2	1.6	1.7bc
2	2	2.0	1.5bc
2	2	2.4	2.0bc
2	2	2.8	0.2de
2	2	3.2	0.9bcd
2	2	3.6	0.7cd
2	2	4.0	0.9bcd
2	2	6.0	0.7cd
2	2	8.0	0.4cd
2	2	10.0	0.5cd
2	2	12.0	0.5cd
2	2	14.0	0.3cde
2	2	16.0	0.7cd
	Unbaited		0.05e

[a]Mean catches in 4 replicates (for unbaited traps and traps without 14:Ac) or 2 replicates (for all other traps) over 6 successive nights of trapping. Means followed by the same letter in each experiment are not significantly different from each other at 5% level.

not that of a sex pheromone constituent, we are planning to determine: (1) whether or not this compound is present in the other parts of the female moth body apart from the sex pheromone gland or tissues near to it; (2) if it is, whether or not it is also present in the male moth body; and (3) whether or not this compound is actually released together with the other two acetates by the female moth during the mating time.

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EDITORS' NOTE

The same compounds in a ca. 1:1 ratio have been reported as the sex pheromone components of *Ostrinia furnacalis* (Guenée) (called the Asian corn borer moth) from the

Philippines (Klun, J.A., Bierl-Leonhardt, B.A., Schwarz, M., Litsinger, J.A., Barrion, A.T., Chiang, H.C., and Jiang, Zhungxie. 1980. Sex pheromone of the Asian corn borer moth. *Life Sci.* 27:1603–1606).

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