Sex Pheromone of the Apple Leafminer Moth, *Phyllonorycter ringoniella* (MATSUMURA) (Lepidoptera: Gracillariidae):
Activity of Geometrical Isomers of Tetradecadienyl Acetates

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All geometrical isomers of (3, 10)-, (4, 10)-, (5, 10)- and (6, 10)-tetradecadienyl acetates were synthesized as candidate compounds of the apple leafminer moth's pheromone component. The activities of combinations of 16 dienes with 2 monoenes were tested under field conditions. The combination of (*Z*)-10-tetradecenyl acetate and *E*4,*Z*10-tetradecadienyl acetate was as active as virgin females and it is strongly suggested that the sex pheromone of the apple leafminer moth is this mixture. (*E*)-10-Tetradecenyl acetate and the other 2 isomers of *E*4,*Z*10-tetradecadienyl acetate showed weak activity.

The apple leafminer moth is one of the most important pests in apple orchards in Japan. The larvae injure mature apple leaves by making so-called lower surface mines resulting in a characteristically wrinkled epidermal layer at the tissue feeding instars. A few mines hasten defoliation, causing deterioration of fruits and inhibition of the growth of new buds. Each insecticide is effective only for a limited stage of this pest, making insecticidal control difficult without monitoring pest occurrence (UJIE, 1969). Pheromone traps are useful for such monitoring and, furthermore, mass trapping or communication disruption using pheromone may be applied to control this species. UJIE et al. (1986) reported the isolation of the pheromone but the chemical structure was not determined. We synthesized pheromone candidates and estimated the chemical structure of the sex pheromone of this species.

MATERIALS AND METHODS

Florisil column chromatography. Chromatography was carried out according to the method of class separation of lipids (CARROLL, 1961). Hexane, 5%, 15%, 25% and 50% ether in hexane were used successively as a solvent system.

Silver nitrate impregnated (16.7%) silica gel chromatography. 1%, 3%, 5%,

Fig. 1. Syntheses of all isomers of (4, 10)-, (5, 10)- and (6, 10)-tetradecadienyl acetates. (4, 10): $n=6$; (5, 10): $n=5$; (6, 10): $n=4$. 1: HBr; 2: $(\text{C}_6\text{H}_5)_3\text{P}$; 3: WITTIG reaction, $\text{C}_3\text{H}_7\text{CHO}$; 4: chromic acid oxidation; 5: silver nitrate silica gel chromatography; 6: WITTIG reaction, $[(\text{C}_6\text{H}_5)_3\text{P}(\text{C}_6\text{H}_{12}\text{OH})]\text{Br}$; 7: acetylation; 8: silver nitrate silica gel chromatography.

Fig. 2. Syntheses of all isomers of (3, 10)-tetradecadienyl acetates. 1: HBr; 2: $(\text{C}_6\text{H}_5)_3\text{P}$; 3: WITTIG reaction, $\text{C}_3\text{H}_7\text{CHO}$; 4: silver nitrate silica gel TLC; 5: CBr_4 , $(\text{C}_6\text{H}_5)_3\text{P}$; 6: $\text{HC}\equiv\text{CC}_2\text{H}_4\text{OTHP}$, buthyl lithium; 7: hydrogenation, Lindlar, H_2 ; 8: acetylation; 9: hydrogenation, Na, NH_3 ; 10: acetylation.

10%, and 50% ether in hexane were used for stepwise elution. Fifteen milliliters each eluate was collected in a test tube using a fraction collector, and checked with a gas chromatograph.

Gas chromatography (GC). To determine the purity of synthesized chemicals, a capillary G-SCOT column (0.28 mm $\times$ 50 m, SP-2340 coated) was used under programmed conditions (80 to 160°C, 10°C/min). Samples were introduced to the column with the splitless mode for 30 sec.

Field tests. Hexane solution of samples was impregnated to plastic capsules (10 $\times$ 20 mm, Yasumoto Kasei, YK-1). Two entrance type traps with sticky plate (25 $\times$ 25 cm) were used and test capsules were placed on the plates. These were set 1.5 m above the ground 3 m apart in apple orchards in Iwate Prefecture.

Synthesis. All geometrical isomers of (3, 10)-, (4, 10)-, (5, 10)- and (6, 10)-tetradecadienyl acetates were synthesized as shown in the schemes of Fig. 1 and 2. All

Table 1. Field attraction of apple leafminer moth by synthesized pheromone candidates

Lure	No. of males caught/trap/night
1983, July 29–Aug. 1	
E10-TDA+E3, E10-TDDA	73
+E3, Z10-TDDA	58
+Z3, E10-TDDA	76
+Z3, Z10-TDDA	64
Z10-TDA+E3, E10-TDDA	54
+E3, Z10-TDDA	62
+Z3, E10-TDDA	59
+Z3, Z10-TDDA	72
Without lure	56
3 VF	3,106
1984, Aug. 31–Sept. 4	
E10-TDA+E5, E10-TDDA	8
+E5, Z10-TDDA	15
+Z5, E10-TDDA	13
+Z5, Z10-TDDA	11
Z10-TDA+E5, E10-TDDA	10
+E5, Z10-TDDA	16
+Z5, E10-TDDA	11
+Z5, Z10-TDDA	12
Without lure	10
2–3 VF	1,183
1984, Aug. 28–Sept. 2	
E10-TDA+E6, E10-TDDA	8
+E6, Z10-TDDA	2
+Z6, E10-TDDA	6
+Z6, Z10-TDDA	4
Z10-TDA+E6, E10-TDDA	5
+E6, Z10-TDDA	4
+Z6, E10-TDDA	4
+Z6, Z10-TDDA	4
E10-TDA+E4, E10-TDDA	46
+E4, Z10-TDDA	60
+Z4, E10-TDDA	4
+Z4, Z10-TDDA	40
Z10-TDA+E4, E10-TDDA	20
+E4, Z10-TDDA	368
+Z4, E10-TDDA	5
+Z4, Z10-TDDA	4
Without lure	5
2–3 VF	444

The ratio of monoene to diene is 100 μg to 10 μg . VF: caged virgin females. TDA: tetradecenyl acetate. TDDA: tetradecadienyl acetate.

isomers were purified by Florisil and silver nitrate silica gel chromatography. Wittig reaction was carried out according to the method of HORIIKE et al. (1980).

All the isomeric purity of dienes and 10-tetradecenyl acetates were estimated to be 99+ % by capillary GC. All chemicals used for synthesis were purchased from Tokyo Kasei, Ltd..

RESULTS AND DISCUSSION

The diene component of the pheromone of the apple leafminer moth was estimated to be a nonconjugated diene with one double bond in the 10 position and the other in the vicinity of the acetate functional group (UJIE et al., 1986). From the chemical data on isolated pheromones of lepidopterous species (TAMAKI, 1985), we thought the double bond positions of 3, 4, 5, or 6 usual and the possibility of positions 1 and 2 very low. Then we synthesized all isomers of (3, 10)-, (4, 10)-, (5, 10)- and (6, 10)- tetradecadienyl acetates as pheromone candidate compounds. The activities of all combinations of 16 dienes with 2 monoenes were tested under field conditions and the results are shown in Table 1. It is very clear that only the 5 combinations of 10-tetradecenyl acetate and (4, 10)-tetradecadienyl acetate are active. Especially, the combination of (*Z*)-10-tetradecenyl acetate and *E*4, *Z*10-tetradecadienyl acetate is as active as virgin females. From this result, the sex pheromone of the apple leafminer moth is strongly suggested to be the mixture of (*Z*)-10-tetradecenyl acetate and *E*4, *Z*10-tetradecadienyl acetate. To our knowledge, these compounds have not been reported as pheromone components of Lepidoptera. (*E*)-10-Tetradecenyl acetate and the other 2 isomers of *E*4, *Z*10-tetradecadienyl acetate showed weak activity and further investigation on the pheromonal activity of these compounds is desirable.

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