ISOLATION AND DETERMINATION OF THE STRUCTURES OF COMPONENTS OF THE SEX PHEROMONE OF *Pandemis ribeana*

V. P. Konyukhov

UDC 547.99

It has been shown with the aid of gas-liquid chromatography, mass spectrometry electroantennographic testing, and some microchemical reactions that the sex pheromone of females of the tortricid moth *Pandemis ribeana* HB is a mixture of the acetates of tetradec-cis-11-en-1-ol (I) and tetradec-trans-11-en-1-ol (II) in a ratio of 1:1. In the tests it was found that a mixture of (I) and (II) in a ratio of 7.5:92.5, respectively, was the most attractive for females of *P. ribeana*.

The torticid moth *Pandemis ribeana* HB. is a dangerous pest of orchards with a wide area; it causes considerable losses of yield, damaging the leaves and sometimes the fruit as well [2]. A study of the sex, pheromone of this pest was begun for practical application in a system of protecting orchards from it.

We have previously reported on the identification of the components of the sex pheromone of females of *P. ribeana* as tetradec-tis-11-enyl acetate (I) and tetradec-trans-11-enyl acetate (II). Under field conditions, the most attractive mixture of (I) and (II) for males of this species had a ratio of 1:1 [1].

In the present paper we give the results of a study of the structure and components of the sex pheromone of the *P. ribeana* and further field trials of them.

At the present time, the sex pheromones of a number of tortricid moths have been isolated and identified. In the structural respect they are most frequently cis and trans isomers of tetradecenyl acetates with double bonds in positions 11 and 9 [3, 4]. It was to be expected that such compounds should also be present in the sex pheromone of *P. ribeana* females.

When monoenic C_{12}, C_{14}, and C_{16} alcohols and their acetates were tested by the electroantennogram (EAG) method, the largest number of responses in the antennas of *P. ribeana* males was obtained to the acetates of tetradec-cis-11-en-1-ol (1.27) and tetradec-trans-11-en-1-ol (1.07) (Fig. 1).

GLC analysis on columns with the phases OV-101 and XE-60 of the initial extract from the tips of the abdomens of females of *P. ribeana* revealed substances identical, with respect to their retention times, with the C_{12} and C_{14} alcohols and their acetates. The micropreparative sampling of one-minute fractions of an extract of the females on column of the phases OV-101 and testing of the fractions with the aid of EAG on antennas of *P. ribeana* males found one with the greatest response at 14-15 min (0.74 mV) the retention time of which was identical with that of a monoenic tetradecenyl acetate (Fig. 2).

To identify the EAG-active component it was isolated by the micropreparative method on a column with the phase OV-101. The nature of the functional group of this component was determined by its hydrolysis with alkali and then by the acetylation of part of the component after hydrolysis. When GLC was performed on columns with phases of different polarities, on chromatograms of the products of the hydrolysis of the component a peak was detected coinciding in its retention time with the C_{14} alcohol, while the products of the acetylation of the component after hydrolysis showed a peak identical in its retention time with the C_{14} acetate (see Table 1). The products of the hydrolysis of the component and the reacetylated component were tested by the EAG method on antennas of *P. ribeana* males. As can be seen from Fig. 3, the reacetylated component had EAG responses at the level of the EAG-active component.

All-Union Scientific-Research Institute of Biological methods of Plant Protection, Kishenev. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 434-439, May-June, 1987. Original article submitted Ocrober 24, 1986.

0009-3130/87/2303-0361$12.50 © 1987 Plenum Publishing Corporation

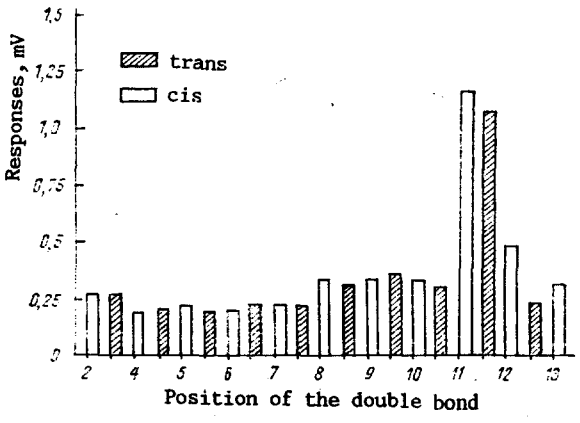

Fig. 1

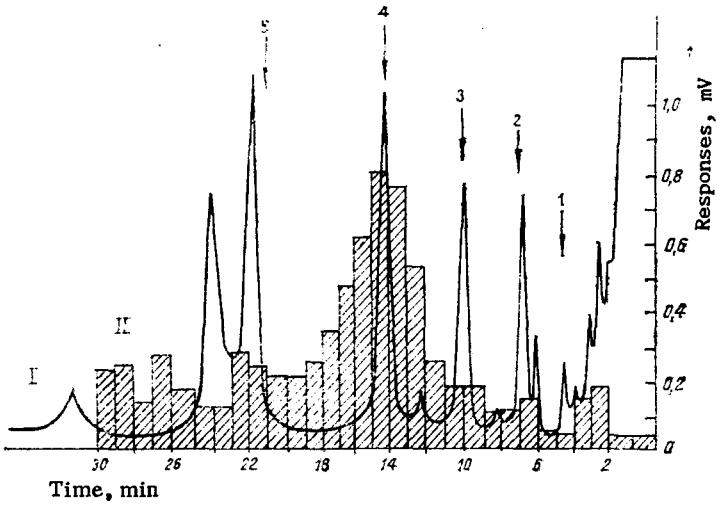

Fig. 2

Fig. 1. Graph of the EAG responses of the antennas of *P. ribeana* males to monoenic tetradecenyl acetates.

Fig. 2. I. Chromatograph of an extract of 10 *P. ribeana* females on a column (3 mm × 2.5 mm) with the phase OV-101 on Chromaton AW-NMDS (60-80 mesh); rate of flow of carrier gas (nitrogen) 45 ml/min; thermostat temperature 180°C; 1) dodecan-1-ol; 2) dodecan-1-yl acetate; 3) tetradecan-1-ol; 4) tetradecan-1-yl acetate; 5) hexadecan-1-ol. II. Graph of the EAG response of the antennas of *P. ribeana* males to one-minute fractions of an extract of 30 abdominal tips from females of the same species.

Then the structure of the component was studied with the aid of mass spectrometry. The mass spectra of the EAG-active component and of cis-tetradec-11-enyl acetate were identical. The presence in the mass spectra of the component of ions with m/z 239 ($[M]^+ - 15$), 211 ($[M]^+ - 43$); 195 ($[M]^+ - 59$) and 194 ($[M]^+ - 60$) and others characterized the EAG-active component as a C_{14} monoenic acetate.

To determine the position of the double bond, the component and authentic samples of C_{14} acetates were subjected to microozonolysis. GLC on columns with the phase XE-60 and OV-101 showed that with respect to their chromatographic behavior the products of the microoxonolysis of the component proved to be identical with the products of the microozonolysis of tetradec-cis-11-enyl acetate and differed from those of tetradec-cis-10-enyl acetate (Table 2).

GLC on a column with the specific phase XF-1150 and in a glass capillary column (GCC) with the phase SP-2340 showed that the EAG-active component consisted of two substances the retention times of which coincided with those of tetradec-cis-11-enyl acetate and tetradec-trans-11-enyl acetate in a ratio of 1:1 (Table 1). Compounds (I) and (II) were tested in the field on *P. ribeana* males separately and in a mixture with one another (Table 3).

Variant 4 of the mixtures of these substances proved to be the most attractive. The results of the field trials agree with a report [5] that an increase in the amount of tetradec-trans-11-enyl acetates improves the attractiveness of the lure. Doses of the sex pheromone of 10, 100, 500, 1000, and 2000 μg were also tested in the field. The most attractive proved to be a mixture of (I) and (II) in a ratio of 7.5:92.5, respectively in an amount of 500 μg, which can be recommended as a sex pheromone for use in a system of protecting orchards from *P. ribeana*.

EXPERIMENTAL

Imagoes of *P. ribeana* were obtained by feeding caterpillars collected in nature on bouquets of apple blossom. Gas-chromatographic investigations were performed on Chrom-41 (Czechoslovakia) and LKh-M8MD ("Khromatograf") instruments. Flame-ionization detectors were used. For analytical and preparative purposes 3 mm × 2.5 m glass columns containing the phases OV-101, XE-60, and SF-1150 on Chromaton AW-NMDS (60-80 mesh) were used. The column containing the phase XF-1150 had dimensions of 2 mm × 4.5 m. A 0.25 mm × 39 m glass capillary column was prepared by a method described in the literature [6].

*We express our thanks to É. A. Mistryukov for assistance in coating the GCC.

TABLE 1. Retention Times of the EAG-Active Component, of the Reacetylated Component, of the Component after Hydrolysis, and after Some Substances of Known Structure

Substance	Retention time, min			
	OV-101; 2,5 m; 180°	XE-60; 2,5 m; 170°	XF-1150; 4.5 m; 170°	GCC; 40 m × 0,25 mm, 124°
1. EAG-Active component	14,5	7,4	10,5 8,5	13,4 12,5
2. Tetradec-cis-11-enyl acetate	14,5	7,4	10,5	13,4
3. Tetradec-trans-11-enyl acetate	14,0 10,5	6,9 6,6	8,5 —	12,4 —
4. Component after hydrolysis	10,5	6,6	12,0	—
5. Tetradec-cis-en-1-ol	10,0	6,4	9,0	—
6. Tetradec-trans-en-1-ol				
7. Reacetylated component	14,5	7,4	—	—

TABLE 2. Retention Times of the Products of the Microozonolysis of the EAG-Active Component and Samples of Substances of Known Structure

Initial substance	Product of microozonolysis	Retention time, min	
		OV-101; 2,5 m; 180° C	XE-60; 2,5m 120° C
EAG-Active component		12,5	15,9
Tetradec-cis-11-en-1-yl acetate	$OHC(CH_2)_{10}OCOCH_3$	12,5	15,9
Tetradec-cis-10-en-1-yl acetate	$OHC(CH_2)_9OCOCH_3$	9,6	13,8
Tetradec-cis-12-en-1-yl acetate	$OHC(CH_2)_{11}OCOCH_3$	14,6	17,8
Tetradec-cis-11-en-1-ol	$OHC(CH_2)_{10}OH$	8,1	15,0

Mass spectra were recorded on a MAT-111 chromato-mass spectrometer (Varian) using a 3 mm × 1 m column filled with Varoport-30, 100-200 mesh, impregnated with the phase SE-30. The mass spectra were recorded at a thermostat temperature of 170°C with a rate of flow of carrier gas (helium) of 15 ml/min, and an ionization energy of 70 eV. Testing by the EAG method was performed on the instrument and by the procedure described in [7]. The preparative sampling of the fractions was carried out by a known method [8]. Before use, the solvents were purified in packed columns with silica gel and were then redistilled.

Preparation of the Extract. Sexually mature virgin females were used for obtaining the extract on the third day after hatching. The last (8-9th) segments of the abdomens with the gland producing the sex pheromone were cut off and they were placed in methylene chloride (1 ml of solvent was used for each 100 tips). They were steeped at 4-5°C for 5-6 days and were filtered off, and the residue was washed with the solvent.

Determination of the Amount of EAG-Active Components in an Extract of *P. ribeana* Females. An extract of 45 females was added in single portion to the evaporator of the chromatograph and was separated on a column containing the phase OV-101 at 160°C with a rate of flow of carrier gas of 65 ml/min, and fractions were taken in glass capillaries cooled with dry ice, which were connected with the outlet of the column. One-minute fractions were taken for 30 min and were tested by the EAG method on the antennas of *P. ribeana* males.

Preparative Isolation of the EAG-Active Component. An extract from 50 females was injected in a single portion into the column of a chromatograph with the phase OV-101, and a fraction was collected in cooled capillaries that coincided in its retention time with a sample of tetradec-cis-11-enyl acetate under the conditions described above. The EAG-active component from 500 females was isolated similarly.

Alkaline Hydrolysis of the Component and Acetylation of the Products of its Hydrolysis. An aliquot of the component from 200 females was dissolved in 3 ml of 5% sodium hydroxide in 50% ethanol, and the solution was kept at 100°C for 4 h. The reaction products were extracted with ether (3 × 0.5 ml). The combined ethereal extracts were dried with sodium sulfate and divided into two parts. One of them was subdivided into 5 parts. The fractions of the component after hydrolysis were chromatographed on columns with the phases OV-101 and XE-60.

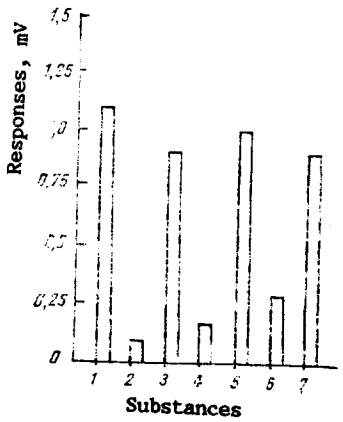

Fig. 3. Graph of the EAG responses of the antennas of *P. ribeana* males to substances in the EAG-active component known to possess structural isomerism: 1) EAG-active component; 2) component after hydrolysis; 3) reacetylated component; 4) tetradec-cis-11-en-1-ol; 5) tetradec-cis-5-en-1-yl acetate; 6) tetradec-cis-11-enyl acetate; 7) tetradec-trans-11-enyl acetate.

TABLE 3. Field trials of Mixtures of the Components of the Sex Pheromone of *P. ribeana* in Various Ratios (July, 1981, "Gidigich" sovkhoz [communal farm], Moldavian SSR)

Variant	Substance	Dose, mg	Number of traps	Total number trapped from June 4 to 22	Average number/trap
1	Tetradec-cis-11-enyl acetate	0,5			
	Tetradec-trans-11-enyl acetate	1,5	5	75	15
2	Tetradec-cis-11-enyl acetate	0,4			
	Tetradec-trans-11-enyl acetate	1,6	5	96	19,2
3	Tetradec-cis-11-enyl acetate	0,2			
	Tetradec-trans-11-enyl acetate	1,8	5	110	22
4	Tetradec-cis-11-enyl acetate	0,15			
	Tetradec-trans-11-enyl acetate	1,85	5	127	25,4

To the product of the hydrolysis of the EAG-active component equivalent to 100 females, in 2 ml of ether, was added 1 ml of acetyl chloride, and the mixture was left to stand for 12 h. Then the solution was concentrated by the passage of nitrogen, the residue was dissolved in 1 ml of hexane, and the solution was divided into 5 portions. The portions of the acetylated component were chromatographed on columns with the phases OV-1 1 and XE-60. The results of identification by the GLC method of the substances of the component after hydrolysis and of the reacetylated components are given in Table 1.

Portions of the extract of the component, of the component after hydrolysis and of the reacetylated component equivalent to 20 females were deposited on strips of filter paper (5 × 0.4 cm) which were placed in glass tubes and were tested by the EAG method on the antennas of *P. ribeana* males (Fig. 3).

Microozonolysis of the EAG-Active Component. The EAG-active component from 50 females was dissolved in 0.1 ml of carbon disulfide and, with cooling, a gentle current of ozone was passed through the solution. The ozonides formed were decomposed by the addition of small amounts of triphenylphosphine. Simultaneously, 1-mg portions of tetradec-cis-11-enyl acetate, tetradec-cis-10-enyl acetate, and tetradec-cis-12-enyl acetate were each dissolved in 0.1 ml of carbon disulfide and subjected to mircoozonolysis similarly. The microozonolysis products were analyzed with the aid of GLC on columns with the phases OV-101 and XE-60 at 180 and 120°C at rates of flow of the carrier gas (nitrogen) of 41 and 42 ml/min, respectively (Table 2).

Determination of the Isomeric Composition of the EAG-Active Component. The component from 20 females and the acetates of tetradec-cis-11-en-1-ol and of tetradec-trans-11-en-1-ol were chromatographed on a 2 mm × 4.5 m column containing the phase XF-1150 on Chromaton AW-NMDS 80-100 mesh at 170°C with a rate flow of carrier gas (nitrogen) of 45 ml/min. For this purpose we also used a 0.25 mm × 39 m GCC with the phase SP-2340. Chromatography was performed at 124°C with a rate of flow of carrier gas (helium) of 1.5 ml/min. The substances of the component were analyzed by comparing their retention times with the retention times of samples of substances with known structures (Table 1).

SUMMARY

The components of the sex pheromone have been isolated from an extract of sexually mature virgin females of the moth *Pandemis ribeana* and have been identified as tetradec-cis-11-enyl

acetate in a ratio of 1:1. Field trials have shown that the most attractive mixture contains these substances in a ratio of 7.5:92.5.

LITERATURE CITED

1. T. I. Bichina, V. P. Konyukhov, and V. N. Golovatyuk, New Methods in Plant Protection [in Russian], Shtiintsa, Kishinev (1978), p. 24.
2. B. G. Kovalev, S. F. Nedopekina, V. P. Konyukhov, F. S. Rivilis, K. V. Lebedeva, and A. Kost, in: Abstracts of the 5th Indo-Soviet Symposium on the Chemistry of Natural Compounds [in Russian], Erevan (1978), p. 38.
3. J. P. Tette, in: Pheromones, M. C. Birch (ed.), North-Holland/Elsevier, New York (1978), p. 309.
4. W. Roelofs, A. Hill, J. Tette, and A. Mansen, Environ. Entomol, 3, No. 5, 747 (1974).
5. S. Maini, P. Edison, A. Bortolotti, and L. P. Castellar, Boll. Inst. Entomol. Univ. Stud., Bologna, 37, 101 (1983).
6. E. L. Ilkova and E. A. Mistryukov, Chromatographia, 11, 77 (1971).
7. V. A. Minyailo, B. G. Kovalev, and V. D. Bednyi, Khemoretseptsiya Nasekomykh, No. 3, 25 (1978).
8. W. L. Roelofs, Ecol. Bull, No. 31, 25 (1980).

INSECT PHEROMONES AND THEIR ANALOGS.

XVII. SYNTHESIS OF (Z)-HEXADEC-11-ENAL AND (Z)-HEXADEC-11-EN-1-YL ACETATE

V. N. Odinokov, G. Yu. Ishmuratov, I. M. Ladenkova, and G. A. Tolstikov

UDC 542.943.5+547: 313+632.936.2

(Z)-Hexadec-11-enal and (Z)-hexadec-11-1-yl acetate — components of the sex pheromone of insects of the genera *Heliothis* and *Manestra*, respectively — have been synthesized by the condensation of undec-10-enal with hex-1-yne, deoxygenation of the heptadec-1-en-12-yn-11-ol formed via the corresponding tosylate to heptadec-1-en-12-yne, and the selective oxonolysis of the latter.

(Z)-Hexadec-11-enal and (Z)-hexadec-11-en-1-yl acetate are components of the sex pheromones of many species of insects of the order *Lepidoptera* [1, 2]. The above-mentioned aldehyde has been identified as the main component of these pheromones of moths of the genus *Heliothis* [2-5], and the acetate as a component of the pheromones of moths of the genus *Manestra* [6-8]. These compounds have been synthesized by modifying the carbon skeleton of (Z)-unsaturated compounds [9] by Wittig's method for the olefination of carbonyl compounds [10-12], or via the corresponding alkynes obtained by the interaction of alkali-metal acetylides with alkyl halides [8, 13-16] (see following page).

We have found a route to the synthesis of the hexadec-11-enal (VII) and the hexadec-11-en-1-yl acetate (X) with the (Z)-configuration that is based on the interaction of the readily available undec-10-enal (I) with hex-1-yn-1-ylmagnesium bromide. The deoxygenation of the heptadec-1-en-12-yn-11-ol (II) so formed via the corresponding tosylate (III) gave the key synthon — heptadec-1-en-12-yne (IV). As was to be expected, the ozonolysis of the enyne (IV) took place selectively at the double bond and, depending on the subsequent treatment of the peroxide product of ozonolysis, led either to 1,1-dimethoxyhexadec-11-yne (V) or to hexadec-11-yn-1-ol (VIII). The acetylenic acetal (V) was smoothly converted into the desired (Z)-alkenic aldehyde (VII) by hydrogenation over Lindlar catalyst in the presence of quinoline to 1,1-dimethoxyhexadec-11Z-ene (VI) followed by the elimination of the acetyl protection in an acid medium. To convert the acetylenic alcohol (VIII) into the other desired product —

Institute of Chemistry, Bashkir Branch, Academy of Sciences of the USSR, Ufa.

Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 440-444, May-June, 1987. Original article submitted December 29, 1986.

© 1987 Plenum Publishing Corporation