A STUDY OF THE SEX PHEROMONE OF *Tortrix viridana*

B. G. Kovalev, S. F. Nedopekina,
M. I. Chekanov, B. I. Osetsimskii,
K. V. Lebedeva, and N. I. Bocharova

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cis-Tetradec-11-en-1-ol acetate has been isolated from an extract of the sex pheromone of the green oak tortrix *Tortrix viridana* L., and has proved to be attractive under sexual conditions for the males of this species.

The green oak tortrix *Tortrix viridana* L. (Lepidoptera: Tortricidae) — one of the main leaf-eating pests of the oak [1] — is distributed throughout the area of growth of this tree. The existence of a synthetic sex hormone will permit the control of this pest and the fight against it to be reorganized.

The scheme of investigation of a crude extract of the sex hormone of *T. viridana* consisted in detecting the electroantennographically (EAG) active components, isolating and identifying them, and determining their behavioral role by the laboratory olfactometer test or under field conditions.

For the detection of EAG-active components, a crude extract of the sex hormone in an amount of 20 female-equivalents was subjected to preparative gas—liquid chromatography by taking off one-minute fractions into glass capillaries using a column with a nonpolar liquid phase (OV-1), the contents of the capillaries were tested by the EAG method. The maximum response ($\sim$ 1.2 mV) was obtained with a capillary containing fractions with retention times of about 19.5 min. A comparison of the retention time with the retention times of standard C_{12} and C_{14} unbranched saturated alcohols and their acetates show that the activity coincided with the retention time of tetradecanol acetate (Fig. 1). The active fraction was eluted from the column and was chromatographed under the same conditions as previously with a nonpolar phase. it was represented mainly by a single peak, the retention time of which coincided with that for tetradecanol acetate, and also with a definite peak on the chromatogram of the crude extract. A similar investigation was carried out on a column with a polar liquid phase (Hi-Eff) (Fig. 2). On the basis of the results of gas—liquid chromatography and EAG, it may be concluded that the EAG-active component of the sex pheromone of the green oak tortrix is the acetate of an alcohol containing 14 carbon atoms.

The mass spectrum of the EAG-active component (Fig. 3) contains a peak with m/z 194 which is characteristic for the acetate of a monoenic alcohol, corresponding to the splitting out of acetic acid $[M - AcOH]^{+\cdot}$, and also the peaks of fragments corresponding to the hydrocarbon decomposition of this fragment $[M-AcOH-nCH_2=CH_2]^{+}$ and $[M-AcOH-nCH_2]^{+\cdot}$. The detection in the spectrum of a fairly strong peak with m/z 61, corresponding to the ion $[CH_3COOH_2]^{+}$ also confirms the presence of an acetate group in the molecule of the component.

Since we did not have a large amount of biomaterial available, the position of the double bond in the molecule of the tetradecen-1-ol was determined by a study using the EAG method of the responses of isolated antennae of green oak tortrix males to a number of standard acetates of cis-trans-isomeric C_{14} alcohols with different positions of the double bond. The greatest responses were obtained from the acetates of cis- and trans-tetradec-11-en-1-ols (Fig. 4), which shows the position of the double bond in the pheromone molecule in position 11.

The geometry of the double bond was established by comparing the retention time of the active component (20.7 min) with that for the acetate of cis-tetradec-11-en-1-ol (20.6 min) and the acetate of trans-tetradec-11-en-1-ol (18.8 min) in a column with the specific phase XF-1150.

All-Union Scientific-Research Institute of Biological Methods of Plant Protection, Kishinev, Moldles Moldavian Forestry Scientific-Production Amalgamation, Bendery, and All-Union Scientific-Research Institute of Chemical Means of Plant Protection, Moscow.
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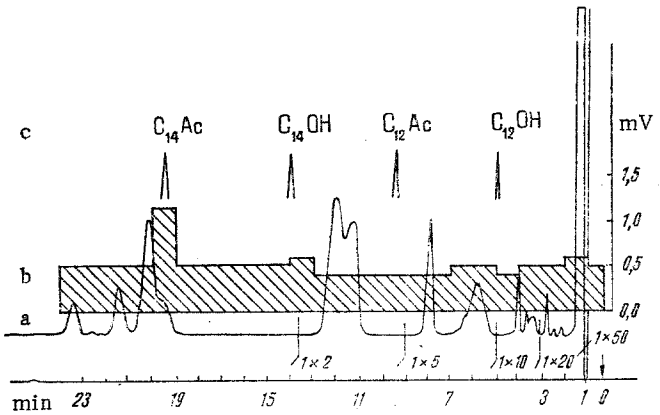

Fig. 1. Chromatograms of an extract of the sex pheromone of the female green oak tortrix (20 female-equivalents) (a) and of standard saturated alcohols and their acetates (c), and the responses of male antennae to the one-minute fractions (b). Column with OV-1, 167°C, rate of flow of nitrogen 40 ml/min.

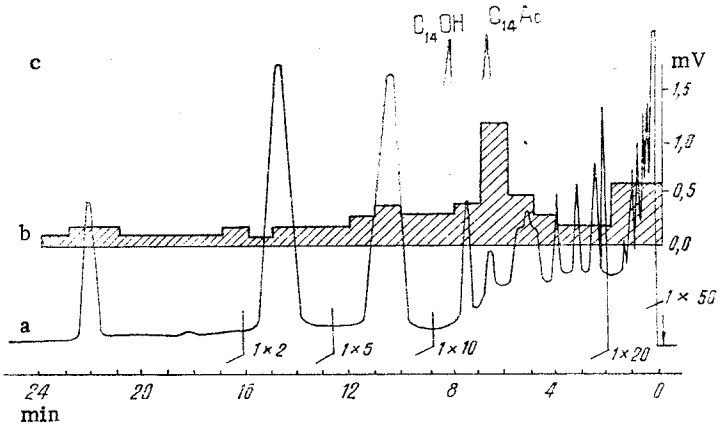

Fig. 2. Chromatogram of an extract of the sex pheromone of the green oak tortrix females (30 female-equivalents) (a) and of a standard saturated alcohol and its acetate (c), and the responses of male antennae to one-minute fractions (b). Column with Hi-Eff, 167°C, rate of flow of nitrogen 40 ml/min.

Thus, the EAG-active component can be ascribed the structure of cis-tetradec-11-en-1-ol.*

The sexual trials showed that cis-tetradec-11-en-1-ol acetate is not only an electroantennographically active but also a behaviorally active substance which exhibits a fairly high attractiveness with respect to green oak tortrix males under sexual conditions. Here the isomeric purity of the substance is of fundamental importance. The presence of trans-tetradec-11-en-1-ol acetate in amounts exceeding 2% almost completely inhibits the response of the males to the cis-tetradec-11-en-1-ol acetate (Table 1).

EXPERIMENTAL

Chromato—mass spectrometry was carried out on an LKB 2091 instrument at 20 V. Separation was performed on a capillary column, 25 m × 0.36 mm, containing SE-30. The initial

*During the preparation of this paper for publication, a communication appeared [5] the authors of which arrived at the same conclusion concerning the structure and composition of the sex pheromone of *Tortrix viridana* L.

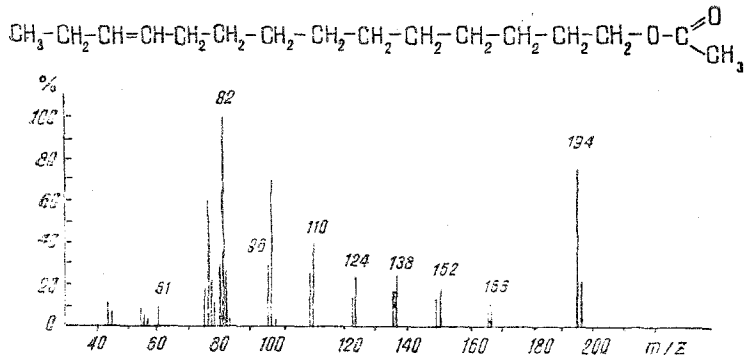

Fig. 3. Mass spectrum of the active component of the sex pheromone of the green oak tortrix at 20 eV.

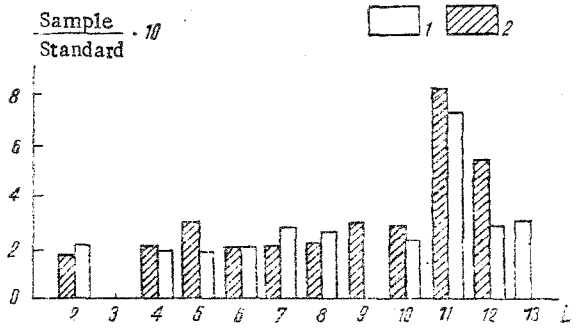

Fig. 4. Normalized responses of the antennae of green oak tortrix male to a series of synthetic tetradecen-1-ol acetates: 1) the trans isomer; 2) the cis isomer; L — position of the double bond.

temperature of 120°C was maintained for 5 min and it was then raised to 250°C at the rate of 5° per min. A Chrom-42 instrument was used for gas chromatography, with 2.5 m × 3 mm glass columns filled with 3% of OV-1 on Gas-Chrom Q, 100-120 mesh, and 3% of cyclohexanedimethanol succinate on Gas-Chrom Q, 100-120 mesh, at 167°C with a 40-ml/min flow of nitrogen. Antennograms were recorded by the method and on the apparatus described elsewhere [3]. The synthetic samples were obtained via acetylenic alcohols.

Young green oak tortrix caterpillars were collected and were fed in the laboratory at 18° for 20 days on bunches of oak flowers. The pupated insects were divided by sex and were kept in desiccators until the imagines hatched.

Preparation of the Extract. The tip of the abdomen (8-9 segments), where the glands producing the pheromone are located, were cut off from one- to two-day females and they were placed in methylene chloride (1.0 ml of solvent per 100 females) and steeped at 5-7°C for 3-4 days. The extract was filtered, the residue was washed several times with solvent, and the combined extract was made up to a definite volume.

Taking of One-Minute Fractions. To record analytical chromatograms and for the preparative taking of one-minute fractions we used an extract 20-30 green oak tortrix females. The samples were taken into glass capillaries, 30 cm × 1 mm, which were attached directly to the outlet of the column.

Preparation of the Sample for Mass spectrometry. A crude extract of 200 females was separated preparatively in a column with a nonpolar phase (OV-1) in portions corresponding to 50 females each.

The geometry of the double bond was determined with the aid of a 3.6 m × 3 mm metal column containing 10% of the liquid phase XF-1150 on Gas-Chrom Q, 100-120 mesh, at 178°C. The active fraction isolated preparatively in a column with a nonpolar phase (OV-1) from the crude extract corresponding to 100 females was studied.

TABLE 1. Attractiveness of Mixtures of the Acetates of cis,trans-Isomeric Tetradec-11-en-1-ol with respect to Males Collected during the Seven Days of the Mass Flight of the Tortrix

Ratio of cis and trans isomers. Dose 2000 μg	Number of males trapped in each repetition				Mean over the seven days
	1	2	3	4	
98:2	53	66	38	42	50.5
94:6	1	6	8	7	5.5
92:8	2	5	4	11	5.5
90:10	16	10	14	4	11.0
86.5:13.5	7	9	5	8	7.3
Control	2	14	10	13	9.8
NSR_{05} = 9.95					

SUMMARY

cis-Tetradec-11-en-1-ol has been isolated from an extract of the sex pheromone of the green oak tortrix and has been identified; under field conditions it actively attracts the males of this species.

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