IDENTIFICATION OF THE SEX PHEROMONE OF THE TORTRICID MOTH *Cacocimorpha pronubana*

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Four components have been isolated from an extract of the sex pheromone of *C. pronubana* by gas—liquid chromatography, electroantennography, and chromato-mass spectrometry: cis-tetradec-11-en-1-ol and its acetate and the acetates of trans-tetradec-11-en-1-ol and of cis-tetradec-9-en-1-ol.

The tortricid *Cacocimorpha pronubana* is a polyphage damaging the buds, flowers, and leaves of 140 species of plants from 42 families [1]. In the Crimea it has been reported as first-degree pest of the pomegranate. The use of the pesticides on the pomegranate for suppressing the pest is limited because of their phytoncidity [1]. The sex pheromone of *C. pronubana* is unknown, but its identification would open up new approaches for the development of promising methods of combating this pest.

The scheme of studying an extract of the sex pheromone of the tortricid moth *Cacocimorpha pronubana* HB. (Lepidoptera: Tortricidae) was similar to that which we have described previously [2, 3]. In order to reveal the electroantennographically (EAG) active components, a crude extract of the sex pheromone in an amount of 32 female equivalents was chromatographed preparatively on a column with the nonpolar liquid phase OV-1, 1-min fractions being taken in glass capillaries the contents of which were then tested by the EAG method [4]. In this way we detected two EAG-active fractions with retention times of 6-8 and 10-12 min, which correspond to the retention times of tetradecanol and its acetate (Fig. 1). A similar chromatogram of a crude extract of the sex attractant recorded on the same column from 20 female-equivalents showed the presence of strong peaks with retention times corresponding to tetradecanol and its acetate (see Fig. 1). To identify the components of the pheromone, the alcohol and acetate fractions were accumulated by the preparative gas-chromatographic separation of the crude extract on a column with the nonpolar OV-1 phase. From these fractions, on the same column, components corresponding in their retention times to standard cis-tetradec-11-en-1-ol and its acetate were isolated, and their mass spectra were recorded. The mass spectra of both components contained ions with m/z 194, corresponding to the splitting out of acetic acid and water from the molecular ions of the acetate and of the alcohol, respectively. In addition, they contained fragments corresponding to the hydrocarbon fragmentation of the 194 ion. The presence of an acetate group in the molecule of the acetate component was confirmed by the presence in its mass spectrum of a peak with m/z 61, corresponding to the ion $(CH_3COOH_2)^+$.

The position of the double bond in the acetate and of that in the alcohol was established by determining the EAG responses of the antennae of the male moths to a number of cis—trans isomeric tetradecan-1-ols and their acetates the greatest numbers of responses of the antennae were obtained only to cis-tetradec-11-en-1-ol and its acetate and trans-tetradec-11-en-1-ol acetate.

To determine its geometry and also its isomeric composition, the acetate component was subjected to gas—liquid chromatographic study on a column with the high-nitrile XF-1150 phase. It was found that it was represented by two peaks the retention times of which coincides with those of the acetates of cis-tetradec-11-enol (21 min) and trans-tetradec-11-en-1-ol (19 min) in a ratio of 4:1, respectively.

Priesner [5] has established the presence of four specialized types of receptor cells in the trichoid sensillae of the antennae of males of the tortricid moth *C. pronubana* responding

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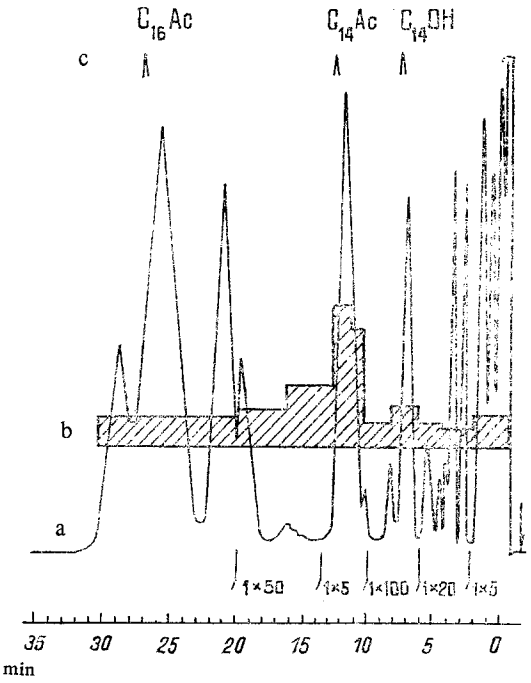

Fig. 1. Chromatogram of an extract of the sex pheromone of females of *C. pronubana* (32 female equivalents) (a); responses of the antennae of males to 1-min fractions (b); and positions of the chromatographic peaks of standard saturated hexadecanol and tetradecanol acetates and tetradecanol (c). Column with OV-1, 178°C, rate of flow of nitrogen 40 ml/min.

to cis-tetradec-11-en-1-ol and its acetate and the acetates of trans-tetradec-11-en-1-ol and of cis-tetradec-9-en-1-ol. In order to detect the last-mentioned substance in the composition of the sex pheromone of *C. pronubana*, the acetate component was studied by chromato-mass spectrometry using a capillary column with the nonpolar SE-30 phase on which, as was established beforehand, the acetates of cis-tetradec-11-en-1-ol and of cis-decadec-9-en-1-ol are well separated. In fact we detected a peak of low intensity with a retention time of 16.5 min (the retention time of cis-tetradec-11-en-1-ol acetate under these conditions is 17.0 min), the mass spectrum of which had a peak with m/z 194 corresponding to the fragment $(M - CH_3COOH)^+$, and also peaks corresponding to the hydrocarbon breakdown of this fragment. The amount of cis-tetradec-9-en-1-ol acetate, estimated from the capillary chromatogram, was about 75% of the amount of the acetate component.

EXPERIMENTAL

Chromato-mass spectrometry was carried out on a IV 2091 instrument at 70 eV. Separation was effected in a capillary column 25 m × 0.36 mm containing SE-30. Chromatographic conditions for the acetate: An initial temperature of 120°C was maintained for 1 min and was then raised to 250°C at 5°C per minute. Chromatographic conditions for the alcohol: An initial temperature of 50°C was maintained for 5 min and was then raised to 230°C at the rate of 5°C per minute. Gas—liquid chromatography was carried out on a Chrom-42 instrument. A glass column 2.5 m × 3 mm filled with 3% OV-1 on Gas Chrom Q 100-120 mesh were used at 178°C with a nitrogen flow of 40 ml/min. Antennograms were recorded by the method and on the apparatus described previously [4]. Synthetic samples were obtained via acetylenic alcohols.

Isolation of the Biomaterial. *Cacoecimorpha pronubara* was bred in the laboratory in a synthetic nutrient medium and under the conditions described in [6]. The pupated insects were separated according to sex and stored in desiccators until they hatched.

Preparation of the Extract. The tips of the abdomens (8-9 segments), where the gland producing the pheromone is located, was cut off from 2- to 3-day females and they were placed in methylene chloride (1.0 ml of solvent per 100 tips) and were steeped at 5-7°C for 3-4 days. The extract was filtered, the residue was washed several times with the solvent, and the extract was made up to a definite volume.

The Taking of 1-min Fractions. To record analytical chromatograms and for the preparative acquisition of 1-min fractions we used an extract from 20-30 *C. pronubana* females. Samples were taken in glass capillaries with dimensions of 30 cm × 1 mm which were attached directly to the outlet of the column.

Preparation of a Sample for Mass Spectrometry. The crude extract from 150 females was separated preparatively on a column with a nonpolar phase (OV-1) in 50-female portions. For final purification, the combined acetate and alcohol fractions isolated from the extract were introduced in a single portion into the same column, and the acetate and alcohol components were taken off in separate capillaries.

Determination of the Geometry of the Double Bond. The isomeric composition of the acetate fraction was determined on a metal column 3.6 m × 3 mm with 10% of the liquid phase XF-1150 on Gas Chrom Q, 100-120 mesh, at 178°C. The acetate component from 80 *C. pronubana* females that had been twice purified on a column with OV-1 was used.

The mass spectra were recorded by K. V. Lebedeva (VNIIKhSZR [All-Union Scientific-Research Institute of Chemical Means of Plant Protection]).

SUMMARY

Four components have been isolated from an extract of the sex pheromone of females of the tortricid moth *Cacoecimorpha pronubana*: cis-tetradec-11-en-1-ol and its acetate, and the acetates of trans-tetradec-11-en-1-ol and of cis-tetradec-9-en-1-ol.

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