ISOLATION AND IDENTIFICATION OF A COMPONENT OF THE SEX PHEROMONE OF Aegeria apiformis

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An electroantennographically active substance has been isolated from an extract of the sex pheromone of the hornet clearwing moth Aegeria apiformis C. and has been identified by gas-liquid chromatography and mass spectrometry as octadeca-cis-3,cis-13-dien-1-ol.

The hornet clearwing moth Aegeria apiformis C. (Lepidoptera, Sesiiidae) is a serious trunk pest of the poplar, the fight against which is difficult because of its concealed mode of life [1, 2]. Its sex pheromone is unknown, but it is necessary for the development of new approaches to the fight against this pest.

To isolate the components of the sex pheromone, a crude extract from 370 abdominal tips of the clearwing moth females was subjected to column chromatography on silica gel with elution of this substance by a mixture of ether and hexane. Fractions with a volume of 3 ml were collected and were tested by electroantennography (EAG) [3]. The EAG-active fractions were eluted with mixtures of 15, 20, and 25% of ether in hexane, which showed the polar nature of the components. The combined fractions were evaporated and an aliquot of 50 female-equivalents was subjected to micropreparative gas-liquid chromatography on a column with the nonpolar OV-101 phase, 1-min fractions being collected in glass capillaries, the contents of which were then tested by the EAG method. The greatest response was obtained to a fraction with a retention time of 9-10 min. The retention times of the standard substances octadecanol and its acetate under these conditions were 9.9 and 13.6 min, respectively (Fig. 1). Octadecanol and its acetate were selected as the standard substances in view of the fact that only isomers of octadeca-3,13-dien-1-ol and its acetate have been found as components of the sex pheromones of insects of the family Sesiidae [4] or been found by sexual screening [5-7]. As can be seen from Fig. 1, the EAG active fraction coincided well in retention time with octadecan-1-ol.

The EAG-active fractions were subjected to preliminary purification on silica gel, then additionally purified by micropreparative GLC on a column with the OV-101 phase under the conditions described in Fig. 1 and 1-min fractions with retention times of 9-10 min were collected.

A chromato-mass spectrometric study of this GLC-purified alcoholic fraction showed the presence of a peak corresponding in its retention time on a column with the nonpolar OV-1 phase to octadeca-cis-3,cis-13-dien-1-ol. The chemical-ionization mass spectrum contained strong peaks with m/z 265 and 267, corresponding to $(M^+ \pm 1)$ and also fragments with m/z 247 and 249 corresponding to the fragmentation $(M^+ \pm 1) - 18$ and differing little from that of an authentic sample of octadeca-cis-3,cis-13-diene-1-ol. On the basis of these facts the component of the sex pheromone can be ascribed to the structure of a dienic alcohol with 18 carbon atoms.

The positions and geometry of the double bonds were established by the GLC method using a capillary column 25 m long with the liquid phase SP-2340 (a silicone polymer containing 75% of cyanopropyl groups) [8] in the light of the fact that the double bonds in the molecules of sex pheromones are present in positions 3 and 13 or, as a rare exception, in positions 2 and 13 [9, 10]. On comparing the retention times of all four isomers of octadeca 3,13-dien-1-ol with that of the component of the sex pheromone clearwing moth (12.0 min), the lat-

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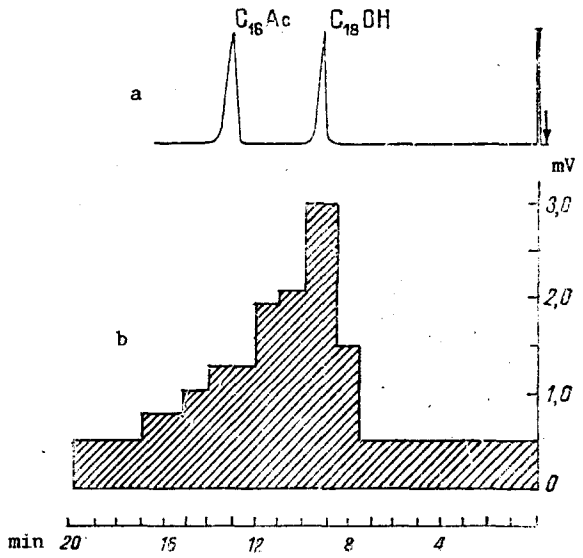

Fig. 1. Chromatogram of standard saturated octadecanol and its acetate (a) and the responses of the antennae of males to 1-min fractions of an extract of the sex pheromone of hornet clearwing moth females (b). Column with OV-101, 223°C; rate of nitrogen 30 ml/min.

ter was found to coincide with the retention time of octadeca-cis-3,cis-13-dien-1-ol (12.0 min).

EXPERIMENTAL

Chemical-ionization mass spectra were obtained on a Finnigan model 1015 C instrument with methane as the carrier gas and the reagent. A 2 mm × 1.8 m column filled with 3% of OV-1 on Gas Chrom 100-120 mesh was used. Capillary chromatography was performed on a Varian 1400 instrument using a 0.25-0.30 mm × 25 m column with the phase SP-2340 at a thermostat temperature of 175°C and a linear velocity of helium of 18 cm/sec. The micropreparative GLC purification and the taking of 1-min fractions were carried out on a Chrom-5 instrument with 3 mm × 2.5 m column filled with 3% of OV-101 on Gas Chrom 100-120 mesh at a rate of flow of nitrogen of 30 ml/min.

Acquisition of the Biomaterial. Cocoons of the hornet clearwing moth were obtained by excavations in the trunks of the poplars in April-May. The cocoons were placed in a single layer in desiccators on sawdust which was periodically wetted with water. The desiccators were kept at a temperature of 20-25°C. The caterpillars that hatched out were sorted according to sexual characteristics.

Preparation of the Extract. To prepare an extract of the sex pheromone we used 1- to 2-day virgin females. The extract was prepared in the morning (7-9 o'clock) using for this purpose females presenting the appeal pose. Abdominal tips (8-9 segments) were cut off and placed in methylene chloride. The amount of methylene chloride was 5 ml per 100 tips, and steeping was carried out at 5-7°C for 3-4 days. Then the extract was filtered, the residue was washed with the solvent several times, and the extract was brought to a definite volume.

CONCLUSIONS

Octadeca-cis-3,cis-13-dien-1-ol has been isolated from an extract of abdomens of females of the hornet clearwing moth (Aegeria apiformis C.) and has been identified as a sex pheromone.

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