ISOLATION AND IDENTIFICATION OF THE SEX PHEROMONE OF FEMALES OF Heliothis maritima (Lepidoptera, Noctuidae)

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Four components have been isolated from an extract of the sex pheromone of the moth Heliothis maritima and have been identified: hexadecanal, hexadec-cis-11-enal, hexadec-cis-9-enal, and hexadec-cis-11-en-1-ol.

The moth Heliothis maritima Grasl. is a polyphagous insect that is widely distributed in the southern regions of the RSFSR, in the Ukraine, and in Modavia, attacking more than 70 plant species from 22 families; fodder crops, vegetables, essential-oil crops, maize, and tobacco [1].

Its sex pheromone is unknown even though it is necessary for the development of new methods of combating this pest. In the present communication we describe the identification of the sex pheromone of this moth.

To reveal the active components of the pheromone, a crude extract in an amount of 10 female-equivalents was chromatographed on a column with the stationary phase SE-30. Under the same conditions we performed the preparative taking of two-minute fractions (30 female-equivalents) followed by electroantennographic (EAG) testing. A chromatogram of the extract and the distribution of biological activity in it are shown in Fig. 1. As can be seen, the greatest response of the antennae of males was obtained to fractions 6-7 and 7-8, which, from their retention times, fell into the region of aldehydes with 16 carbon atoms.

By capillary chromatography on columns with nonpolar (DV-1) and moderately polar (DV-WAX) phases using synthetic standards in the region of retention times corresponding to aldehydes with 16 carbon atoms in the chain we detected three components (Table 1), one of which corresponded to hexadecanal (component 1), another (component 2) to hexadec-cis-11-enal, and the following one (component 3) to an unsaturated aldehyde with an unknown position of the double bond. Simultaneously, a fourth component was identified from its retention times on the same capillary columns – hexadec-cis-11-en-1-ol (component 4).

The information obtained by capillary chromatography was confirmed mass-spectrometrically. The spectrum of component 1 contained characteristic peaks with m/z 196 and 222 corresponding to the splitting out of acetaldehyde (McLafferty rearrangement) and water from an ion with a molecular mass of 240, as for hexadecanal. Fragments with m/z 166, 152, 124, and 110 from the hydrocarbon fragmentation of the ion with m/z 222 also corresponded to a saturated aliphatic chain.

The mass spectrum of component 2 contained a fairly intense peak of the molecular ion with m/z 238, a characteristic $[M - H_2O]^+$ peak with m/z 220, and hydrocarbon-breakdown fragments with m/z 164, 150, 136, 122, and 108, corresponding to the molecule of an unsaturated aldehyde with one double bond.

The mass spectrum of component 3 was similar to that of component 2: It contained the peak of the molecular ion with m/z 238, the characteristic $[M - H_2O]^+$ peak with m/z 220, and the fragments from the hydrocarbon breakdown of a monounsaturated aldehyde. The retention times on columns with different polarities of component 3 coincided with those of hexadec-cis-9-enal (see Table 1).

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TABLE 1. Retention Times of Some Synthetic Standards and of Components of the Pheromone of the Clover Moth on Columns of Different Polarities

Name	Retention time, min	
	DV-1	DV-WAX
Hexadecanal	24,287	16,059
Hexadec-cis-11-enal	23,962	16,510
Hexadec-cis-9-enal	23,768	16,368
Hexadec-cis-11-en-1-ol	25,387	18,970
Component 1	24,290	16,069
Component 2	23,958	16,512
Component 3	23,746	16,375
Component 4	25,405	18,975

TABLE 2. Retention Times of the Dimethyl Disulfide Adducts of Some Synthetic Standards and of Component 2 of the Sex Pheromone on a Column with a Polar Phase

Name	Retention time, min (DV-WAX)
Component 2 CH_3SSCH_3	41,75
$C_4H_9CH(SCH_3)-CH(SCH_3)-(CH_2)_5CHO$	41,75
$C_6H_{13}CH(SCH_3)-CH(SCH_3)-(CH_2)_7CHO$	40,20
$C_8H_{17}CH(SCH_3)-CH(SCH_3)-(CH_2)_5CHO$	39,75

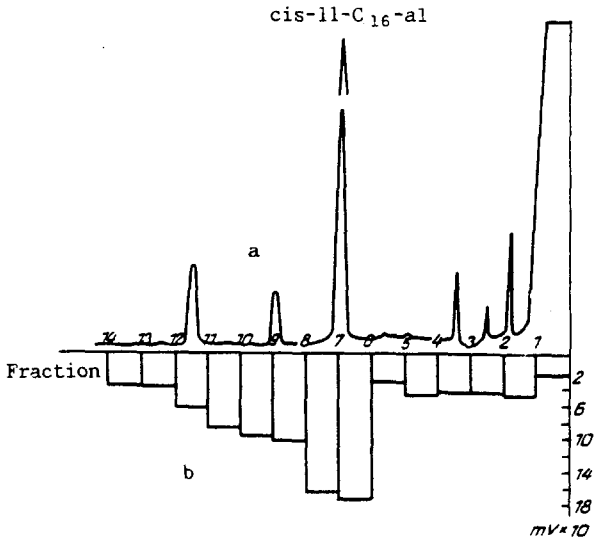

Fig. 1. Chromatogram of a crude extract of H. maritima females (a) and the response of the antennae of males to two-minute fractions of the extract (b). Column with SE-30, 2.5 m, 185°C, rate of flow of nitrogen 40 ml/min.

The mass spectrum of component 4 contained the peak of the molecular ion with m/z 240, the peak of a characteristic fragment with m/z 222 corresponding to $[M - H_2O]^+$, and the peaks of the hydrocarbon breakdown fragments $[M - H_2O{-}CH_2{=}CH_2]^+$ and $[M - H_2O{-}CH_2]^+$.

The positions and geometries of the double bonds in components 2 and 4 were confirmed by the EAG testing of a series of cis-trans-isomeric hexadecenals and hexadecenols. It was found that the greatest response of the antennae of the males of the H. maritima was evoked by hexadec-cis-11-enal and hexadec-cis-11-en-1-ol. Additionally, the position of the double bond in component 2 was shown by a gas-chromatographic comparison (Table 2) of the retention times of the dimethyl disulfide adduct of component 2 and of its mass spectrum with those for the adducts of authentic hexadec-cis-7-, -cis-9-, and -cis-11-enals. As can be seen from Table 2, the retention time of the dimethyl disulfide adduct of component 2 coincided with that for the dimethyl disulfide adduct of hexadec-cis-11-enal. The mass spectra of the two substances were identical and contained the molecular ion with m/z 332 and intense characteristic ions with m/z 117 and 215 corresponding to the fragments $[C_4H_9CH{=}S{-}CH_3]^+$ and $[CH_3S{=}CH(CH_2)_9CHO]^+$, respectively, and showing the 11-position of the double bond in the molecule of component 2.

Thus, four components of the pheromone of the moth Heliothis maritima have been identified: hexadecanal, hexadec-cis-11-enal, hexadec-cis-9-enal, and hexadec-cis-11-en-1-ol, in a ratio of 4:74:1:7.

EXPERIMENTAL

Gas–liquid chromatography was conducted on Chrom-5 and HP 5890 instruments. A 3 mm × 2.5 m glass column filled with 3% of SE-30 on Chromaton N-AW-HMDS, 80-100 mesh, was used at 185°C with a 40 ml/min flow of nitrogen and also a 0.53 mm × 5 m column of the SCOT type coated with methylsilicone, at 160°C with a 10 ml/min flow of helium.

Capillary chromatography was conducted on HP 5880 A and HP 5890 instruments. Columns with dimensions of 0.25 mm × 30 m containing DV-1 (nonpolar) and DV-WAX (polar) were used under conditions of programmed heating from 80 to 250°C at the rate of 10°C/min. Chromatomass spectrometry was carried out on the HP 5890 instrument with a HP 5970 mass-selective detector. Separation was achieved on a 0.25 mm × 30 m column containing DV-WAX with heating from 80 to 230°C at the rate of 10°C/min.

Antennograms were recorded by the procedure and on the apparatus that we have described previously [2]. The synthetic specimens used in the investigation were obtained from acetylenic alcohols and by means of the Wittig reaction.

Acquisition of the Biomaterial. Some clover moths were collected in nature in the form of pupae, which were brought to the imago state in the laboratory, and some were bred on a synthetic nutrient medium in the laboratory for the mass breeding of phytophagous insects of VNII BMZR, for which the authors express their gratitude to V. F. Bozhenko and T. N. Stengach.

Preparation of the Extract. The tips of the abdomens of 2- to 3-day females present in the attractive attitude were carefully cut off under a binocular viewer and were placed for 10 min in twice-redistilled hexane, and then the solution with the eluted pheromone was transferred to another vessel and was used for GLC.

Taking of Two-Minute Fractions. The two-minute fractions were collected to 1 mm × 30 cm capillaries cooled with liquid nitrogen that were attached to the outlet from the column.

Synthesis of the Dimethyl Disulfide Adducts of Hexadec-cis-7, cis-9-, and -cis-11-enals [3, 4]. A solution of 1.0 mg of one of the aldehydes in 50 µl of hexane was treated with 50 µl of dimethyl disulfide and 5 µl of a solution of iodine in ether (60 mg of iodine in 1 ml of absolute ether), after which the mixture was heated at 50°C for 12 h. Then it was cooled, 200 µl of hexane was added, and the iodine was eliminated by shaking with 70 µl of a 5% aqueous solution of sodium thiosulfate. The organic layer was separated off and was concentrated to 20 µl, and 2 µl of this solution was introduced into a gas–liquid chromatograph with a mass-spectrometric detector.

Synthesis of the Dimethyl Disulfide Adduct of the Natural Pheromone. A solution of 150 female-equivalents of the crude extract of the sex pheromone in 20 µl of hexane was treated with 30 µl of dimethyl disulfide and 5 µl of iodine solution. The mixture was heated at 50°C

for 12 h. Then it was cooled, and, after the addition of 100 μl of hexane, it was washed with 70 μl of a 50% aqueous solution of sodium thiosulfate, and the organic layer was separated off, concentrated to 3 μl, and introduced into a gas–liquid chromatograph with a mass-spectrometric detector.

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