ISOLATION AND IDENTIFICATION OF A SUPPLEMENTARY COMPONENT OF THE SEX PHEROMONE OF Mamestra brassicae FEMALES

S. F. Nedopekina, B. G. Kovalev, and V. A. Khlebnikov

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Using gas–liquid chromatography and mass spectrometry, from an extract of the sex pheromone of females of the cabbage moth Mamestra brassicae L. an additional component of the pheromone has been isolated and identified – heptadec-cis-11-en-1-ol acetate.

It has been previously shown convincingly that the main component of the sex pheromone of females of the cabbage moth Mamestra brassicae L. (Lepidoptera: Noctuidae) is hexadec-cis-11-en-1-ol acetate [1-4]. However, literature information on the biological activity of synthetic hexadec-cis-11-en-1-ol alone under field conditions is contradictory [5-7], and its species specificity is fairly low [8]. We are therefore making a search for additional components in order to increase the activity and selectivity of the synthetic cabbage moth pheromone. A repeat analysis of extracts of the sex pheromone showed the additional presence, in an amount of 1-5% of the main component, of acetates of the saturated tetradecanol and hexadecanol, of hexadec-trans-11-en-1-ol [9], of hexadec-cis-9-en-1-ol acetate [10], and of heptadec-cis-11-en-1-ol acetate [4]. In field experiments, additions of these substances to the main component did not increase its power of attraction [5]. According to [11], 1% of hexadec-cis-11-enal substantially increases the activity of hexadec-cis-11-en-1-ol. The hypothesis has been expressed that the main component has been missed or that geographically remote races of the cabbage moth are not identical.

We have continued a study of the composition of a crude extract of the pheromone glands of cabbage moth females located in the south-western zone of the USSR with the aim of finding additional components.

To reveal the possible presence of heptadec-cis-11-en-1-ol acetate, the crude extract in an amount of 50 female-equivalents was separated by preparative GLC on a column containing the nonpolar liquid phase Apiezon L with the collection of one-minute fractions which were tested electroantennographically by a procedure described previously [12].

Figure 1 shows the responses of the antennae of cabbage moth males to the fractions obtained and a chromatogram of synthetic acetates of tetradec-, hexadec-, and heptadec-cis-11-en-1-ols. The most active fraction (11-12) contained the main component of the pheromone – hexadec-cis-11-enyl acetate (component 1). A small response was obtained to fraction 17-18, corresponding in retention time to heptadec-cis-11-enyl acetate.

Fraction 17-18 was isolated preparatively in an amount of 1000 female-equivalents. The main component present in this fraction (component 2) coincided in its retention time with synthetic heptadec-cis-11-en-1-ol acetate on columns with phases of different polarities: SE-30, Apiezon L, XE-60, and diethyleneglycol succinate.

The product of the alkaline hydrolysis of component 2 gave GLC peaks with the same retention indices as those of synthetic heptadec-cis-11-en-1-ol and its acetate with phases of different polarities:

Substance	Retention index, I	
	SE-30	*XE-60*
cis-11-C_{17}OAc	2075	2310
cis-11-C_{17}OH	1966	2267
Component 2	2075	2310
Product of the hydrolysis of component 2	1966	2267

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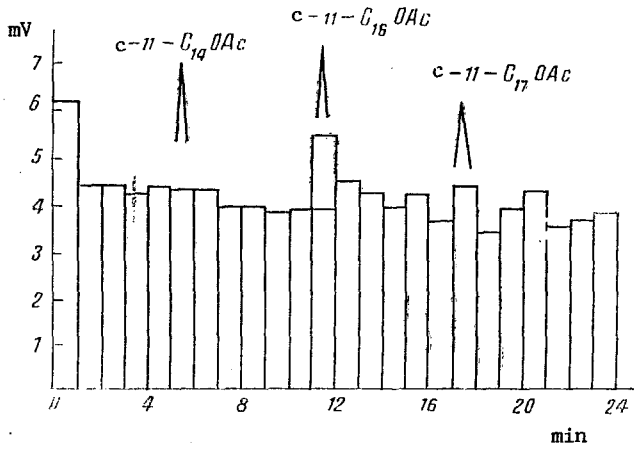

Fig. 1. Responses of the antennae of cabbage moth males to one-minute fractions of a crude extract and a chromatogram of the synthetic acetates of tetradec-, hexadec-, and heptadec-cis-11-en-1-ols. Column containing Apiezon L, 200°C, rate of flow of nitrogen 40 ml/min.

The position of the double bond in component 2 was determined by comparing the retention times of the acetoxy aldehydes obtained on the micro-ozonolysis of synthetic acetates with the authentic structures of the hexadec-cis-9-, cis-10, cis-11, and cis-12-en-1-ols and the products of the micro-ozonolysis of component 2.

The product of the micro-ozonolysis of component 2 coincided in its retention time in a column containing the stationary phase SE-30 with 11-acetoxyendecanal – the product of the micro-ozonolysis of hexadec-cis-11-enyl acetate, i.e., the double bond was present in position 11:

Compound	Ozonolysis product	Retention time, min
$CH_3(CH_2)_5CH=CH(CH_2)_8OAc$	$OHC(CH_2)_8OAc$	2,2
$CH_3(CH_2)_4CH=CH(CH_2)_9OAc$	$OHC(CH_2)_9OAc$	3,2
$CH_3(CH_2)_3CH=CH(CH_2)_{10}OAc$	$OHC(CH_2)_{10}OAc$	4,6
$CH_3(CH_2)_2CH=CH(CH_2)_{11}OAc$	$OHC(CH_2)_{11}OAc$	6,1
Component 2		4,6

The structure of component 2 was confirmed by mass spectrometry. The mass spectrum of the natural sample taken at 20 eV contained a peak with m/z 236 that is characteristic for acetates of monoenic alcohols and corresponds to the ejection of acetic acid from the molecular ion $[M - AcOH]^+$, and also the peaks of fragments corresponding to the hydrocarbon breakdown of this ion $[M - AcOH - nCH_2 = CH_2]^+$, and $[M - AcOH - nCH_2]^+$. The mass spectra of the natural sample and of the synthetic heptadec-cis-11-enyl acetate (at 20 eV) proved to be practically identical (I, %):

m/z	Natural sample	Synthetic hexadec-cis-11-enyl acetate
236	65	95
208	40	27
194	25	20
180	30	40
166	30	30
152	38	63
138	57	65
124	75	93
110	100	100

Thus, an additional component in an amount of 25% of the main component (according to GLC results) has been found in an extract of the pheromone glands of cabbage moth females, and this is heptadec-cis-11-en-1-ol acetate.

EXPERIMENTAL

Chromato-mass spectrometry was performed on a MKh-1321 instrument. Separation was carried out on a column 2 m long filled with 3% of OV-1 on the support Gas–Chrom with a grain size of 100/120 mesh.

Gas chromatography was performed on a Chrom-42 instrument using 3 mm × 2.5 m glass columns filled with 8% of Apiezon L on Chromaton N-AV-HMDS, 80/100 mesh, 3% of SE-20 on Chromaton Super, 80/100 mesh, 5% of XE-60 on Chromaton N-AW-HMDS, 80/100 mesh, and 6% of diethyleneglycol succinate on Chromaton N-AW-HMDS, 80/100 mesh.

The antennograms were recorded by the method and on the apparatus described in [12].

The synthetic samples used in the investigation were obtained via acetylenic alcohols.

Preparation of the Extract. The tips of the abdomens of 2- to 3-day-old females containing the gland producing the pheromone were cut off and extracted with methylene chloride at 5°C for 3-5 days. The extract was filtered, the residue was washed several times with the solvent, and the extract was made up to a definite volume.

Collection of One-Minute Fractions. To record analytical chromatograms and for the preparative collection of one-minute fractions we used an extract from 50 females. The fractions were collected in 1 mm × 30 cm glass capillaries which were attached to the outlet of the column.

Preparation of a Natural Sample for Mass Spectrometry. The fraction corresponding to the second component of the pheromone was isolated by preparative GLC on a column containing SE-30 from the crude extract obtained from 400 females.

Micro-Ozonolysis of the Pheromone. Micro-ozonolysis was performed by the method of Berozza and Bierl [13]. The ozonolysis products were chromatographed on a 3 mm × 2.5 m glass column filled wih 3% of SR-30 on Chromaton Super 80/100 mesh.

Hydrolysis of the Pheromone. The second component of the pheromone isolated from 100 females was saponified by being boiled with 2 ml of 10% aqueous caustic soda and 2 ml of ethanol for 25 h. Then the mixture was extracted with ether and the extract was dried with Na_2SO_4 and evaporated and the residue was used in GLC.

SUMMARY

The acetate of heptadec-cis-11-en-1-ol has been isolated from an extract of the sex pheromone of females of the cabbage moth Mamestra brassicae and identified.

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