(*Z*)-9-Dodecenyl Acetate, a Component of the Sex Pheromone of *Cnephasia longana* Haworth (Lepidoptera: Tortricidae)

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(*Z*)-9-Dodecenyl Acetate, Sex Pheromone, *Cnephasia longana,* Tortricidae

By means of electrophysiological investigations, GC- and GCMS-analysis of gland extracts and GC-analysis with EAG-detection (*Z*)-9-dodecenyl acetate was proven to be a component of the sex pheromone of female *Cnephasia longana* moths. (*E*)-9-dodecenyl acetate acts as a pheromone inhibitor, as shown by field trials.

Since the end of the seventies, in the Federal Republic of Germany, considerable damage caused by tortricid moths of the genus *Cnephasia* (Lepidoptera: Tortricidae) has been observed on a number of species of cereals. Pfalz and Rheinhessen were the main gradation areas for the omnivorous leaf tier, *Cnephasia longana,* and cereal leaf roller, *C. pumicana.* In these areas, the both insect species were almost equally responsible for the damage. The latter, for Germany, is a new species [2]. With the identification of the chemical composition of the female sex pheromone of *C. pumicana* by Biwer *et al.* [3], it was possible to investigate the distribution of this species in the FRG [4]. In the case of *C. longana,* only low numbers of trap catches have been reported in field trials conducted for other species using (*Z*)-9-dodecenyl acetate (*Z*-9-DDA) [5]. These low catches could have been casual events. However, in a screening performed in 1982, this substance proved to be extremely attractive for male leaf tier moths, therefore it was possible to take into consideration both *Cnephasia* species together in a pheromone trap survey conducted all over Germany [6].

Abbreviations: *Z*-9-DDA, (*Z*)-9-dodecenyl acetate; EAG, electroantennogram; GC, gas chromatography; GCMS, GC combined mass spectrometry; FID, flame ionisation detector.

Pheromones 50 [1]

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The analysis of the female *C. longana* glandular extracts should give information, whether *Z*-9-DDA actually is the species own sex attractant.

Materials and Methods

Insect material

The electrophysiological studies with male moths were carried out with a laboratory strain, bred in the Institut für Biologische Schädlingsbekämpfung (BBA Darmstadt). The first gaschromatographic investigations were done with pheromone gland extracts obtained from about 1.700 female moths, collected from walnut trees with an electric exhaustor in the night of 21.7.1983, near Dirmstein/Pfalz.

For the further gaschromatographic and mass spectrometric analysis, only pheromone glands from virgin females obtained from the laboratory colony were used. For this purpose female pupae were incubated for hatching in a thermostat at 24 °C maintained on a 16 h light/8 h dark cycle. Since the female moths emit their sex attractant only before sun rise, it was possible to take them out from the thermostat before the light was switched on, and remove the abdominal tips with the pheromone glands. The glands were collected in hexane and stored at −20 °C.

Electroantennogram EAG

Using the electroantennogram technique (EAG) [7], with male *Cnephasia* antennae the stimulus potentials of several test compounds and fractions of

the gland extract were measured (filter paper strips loaded with test compounds, airstream of 1 sec, 1 cm/sec air velocity), and the electrophysiological activities thus obtained were compared with each other.

Gas chromatography with electroantennogram detection

The effluent of the GC column 1 (UCON HB 5500) was split, and one part was passed to the FID and the other part was mixed with humidified, warm air and blown over a male insect antenna [8]. By means of implanted capillary electrodes thus the retention times of the biologically active compounds could be determined.

Gas chromatography

1. Packard 437, 25 m glass capillary UCON HB 5500, isothermal 160 °C, 10 psig N_2 (for electroantennogram detection);
2. Perkin Elmer Sigma 1, 25 m fused silica capillary column SE-54, inj. 240 °C, det. 260 °C, splitless injection at room temperature, isothermal 155 °C, 12 psig N_2, FID;
3. Packard 438 A with Shimadzu Data System CR3A, 12 m × 0.23 mm fused silica capillary column CPM-19 (≙ OV-17); inj. 240 °C, det. 260 °C, splitless, temp. progr. 60–260 °C, 10 °C/min, initial 2 min, 14 psig N_2.

Gas chromatography – mass spectrometry

Finnigan 3200E quadrupole spectrometer and Data System 6000, 38 m × 0.23 mm fused silica capillary column CPM-19, splitless injection, direct coupling, 70 eV EI-spectra, 2 sec/scan.

LC-Fractionation of the gland extract

The hexane extract of the pheromone glands from wild female moths was separated into 15 fractions by liquid chromatography on silica gel (0.06–0.2 mm, 30 cm × 15 mm), using an eluent system of increasing polarity [9]. The fractions were concentrated with a stream of nitrogen and the solutions thus obtained were used for further analyses.

Microfractionation by means of a syringe

A "micro-column" of LiChrosorb SI 60 (Merck, medium grain size 5 µm) was made between two glass wool plugs at the bottom of a 100 µl syringe (SGE). The length of the column was 5 mm (volume 5 µl). By means of a second syringe, the concentrated extract was charged on top of this "micro-column", and subsequently eluted with 5 µl pentane, 5 µl pentane/ether (8:2) and 5 µl ether directly into the GC injection port [10] (see Fig. 1).

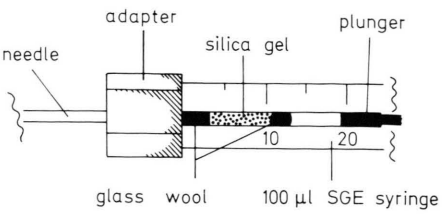

Fig. 1. A schematic drawing of the micro-fractionation method by means of a syringe.

Results and Discussion

In the determination of EAG amplitudes of male *Cnephasia* antennae for a series of test compounds known as components of tortricid pheromones [11], (*Z*)-9-dodecenyl acetate showed the highest electrophysiological activity.

The extract of the wild females was separated by liquid chromatography into 15 fractions and by means of the EAG technique the physiological activity of each fraction was determined. Only the first 20% ether/pentane fraction, which corresponded to the elution volume of acetates, showed a significant EAG response. Thereby, the response amplitude of 10 female equivalents corresponded to the response of about 1 ng (*Z*)-9-dodecenyl acetate, under similar conditions.

The GC analysis of this LC-fraction, using a male antenna as the biological detector [8] showed a component with the EAG-activity and retention time of (*Z*)-9-dodecenyl acetate. A subsequent analysis on a GC-phase of different polarity (SE-54) also yielded a signal (FID) with the expected retention time. This peak was confirmed as (*Z*)-9-dodecenyl acetate by cochromatography.

Since the extract obtained from the wild females contained only a minute amount of the pheromone, we reared 430 female insects in the laboratory *ab ovo*, removed the pheromone glands from the virgin females and extracted them with hexane. This extract was separated by a novel microfractionation

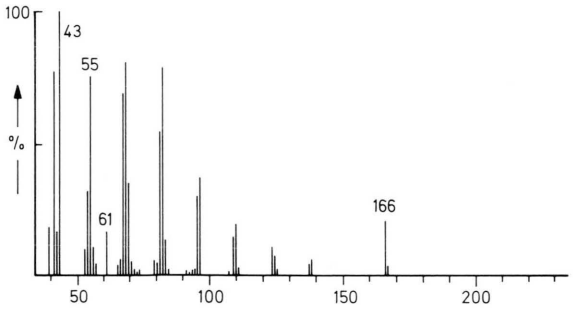

Fig. 2. Mass spectrum of (*Z*)-9-dodecenyl acetate, the sex pheromone of *C. longana* females.

technique, similar to ref. [10], adapted for the first time in pheromone analysis. The four fractions thus obtained were gaschromatographically investigated (CPM-19). In the second fraction (20% ether/pentane) (*Z*)-9-dodecenyl acetate was identified and confirmed by cochromatography.

The same fraction in a second separation was analysed by GCMS. Again a compound with the retention time same as that of (*Z*)-9-dodecenyl acetate, eluted from the column, and its mass spectrum was identical with that of the authentic acetate (Fig. 2).

Mass chromatograms of ions characteristic for spectra of alkenyl acetates did not yield any proof for the presence of a second acetate pheromone component in any appreciable concentration. The quantitative evaluation of the total ion current signal established the content of dodecenyl acetate to be less than 1 ng per *Cnephasia* female gland.

In the course of the determination of the distribution of pest lepidoptera all over Germany, using pheromone traps [4, 6], also the attractancy and the trap catch efficacy of (*Z*)-9-dodecenyl acetate (*Z*-9-DDA) was tested for the omnivorous leaf tier, *Cnephasia longana*. Pheromone traps of the type Biotrap® (Hoechst AG) were baited with lures, each loaded with 1 mg of attractant, and hung in wood thickets near cereal fields. The traps were monitored each week. Dodecyl acetate, known to act synergistically with many sex attractants [12], did not increase the catch rate when added to (*Z*)-9-dodecenyl acetate (Table I). Lures containing the corresponding (*E*)-isomer besides *Z*-9-DDA, used for trapping the cereal leaf roller, *C. pumicana*, were not attractive for the omnivorous leaf tier, *C. longana*. This means that (*E*)-9-dodecenyl acetate is an inhibitor for the sex attractant of the species investigated (Table I).

Table I. Field trapping of males of *C. longana* with (*Z*)-9-dodecenyl acetate in the Pfalz, FRG [6].

Attractant* Composition	Bait loading µg	Total no. of ♂♂ trapped Haßloch	Dirmstein
Z-9-DDA	1,000	433	503
Z-9-DDA 12Ac	500 500	554	460
Z-9-DDA *E*-9-DDA	500 500	0	1
Z-9-DDA *E*-9-DDA 12Ac	250 250 500	0	3

* *Z*-9-DDA = (*Z*)-9-dodecenyl acetate, *E*-9-DDA = (*E*)-9-dodecenyl acetate, 12Ac = dodecyl acetate.

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