Sex Pheromone of *Eupoecilia ambiguella: cis*-9-Dodecenyl Acetate as a Major Component

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The electroantennogram-active compound in abdominal tip extracts of *E. ambiguella* females was identified as *cis*-9-dodecenyl actetate. The compound was present at a level of 25 ng per female and absent in a male extract. The synthetic chemical shows prominent electroantennogram activity, elicits a mating dance response in the males, and is attractive in the field. The results indicate that *cis*-9-dodecenyl acetate is a major component of the *E. ambiguella* sex pheromone.

The tortricoid moths *Eupoecilia (Clysia) ambiguella* Hb., (Einbindiger Traubenwickler in German, *Cochylis* in French) and *Lobesia botrana* Den. et Schiff. (Bekreuzter Traubenwickler or *Eudémis*) are the two main insect pests in European viticulture. The two species co-exist in some areas, but from their climatic preferences and geographical distribution, the former is considered a Northern and the latter a Southern grape pest [1]. The sexual behaviour of the two species was studied in the late 1930's by Götz [2, 3] who demonstrated the existence of specific sex attractants and diurnal activity rhythms for each insect. Synthetic sex pheromones are now being developed for practical use in pest monitoring and control. In *L. botrana*, the sex pheromone has been identified as *trans*-7, *cis*-9-dodecadienyl acetate [4–6]. We report here chemical and biological evidence for *cis*-9-dodecenyl acetate as a pheromone component of *E. ambiguella*.

Materials and Methods

A continuous culture of *E. ambiguella* is maintained on the wheat germ-sawdust diet used for mass rearing of the codling moth by Mani at Wädenswil (*cf.* [7]). Male pupae from this culture or collected in vineyards in Switzerland, and methylene chloride extracts of excised female abdominal tips, were sent to Geneva, N.Y., for studies with the electroantennogram (EAG) technique [8, 9].

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High-resolution gas chromatography (GC) on glass capillary columns (50 m × 0.3 mm, Ucon 50 HB 5100 or silicone SF-96), was carried out on Carlo Erba 2200 gas chromatographs provided with inlet splitter and flame ionization detector. The electroantennographic detector (EAD) [6] was equipped with an *E. ambiguella* male antenna. Methods involving mass spectrometry were the same as described previously [10]. They included extraction of abdominal tips with ethyl ether, chromatography of extracts on silica gel, sweep codistillation, and GC using a splitless injection technique.

The pheromone bioassay with *E. ambiguella* males has been described [11]. In brief, males are kept individually in glass vials, and a stream of air from a pipette containing a film of test sample is directed at each insect. Response is regarded as positive if the mating dance begins within 1 or 2 seconds and continues after removal of the pipette. The test is carried out during the third night after emergence of the males, three hours after dark. In tests with synthetic chemical, 10^{-2} female equivalents (FE) of a crude extract were included as a standard.

Results and Discussion

Electroantennograms of male *E. ambiguella* antennae were recorded with isomer series of the 10-, 12- and 14-carbon *n*-alkenols and their acetic acid esters [8]. EAG amplitudes were found to be highest with dodecenyl acetates. The EAG profile of this series is shown in Fig. 1. Responses are normalized with *cis*-6-dodecenyl acetate which was used as a standard. Prominent activity was found with the double bond in 9-position; the *cis* compound was the more active of the two geometrical isomers. First

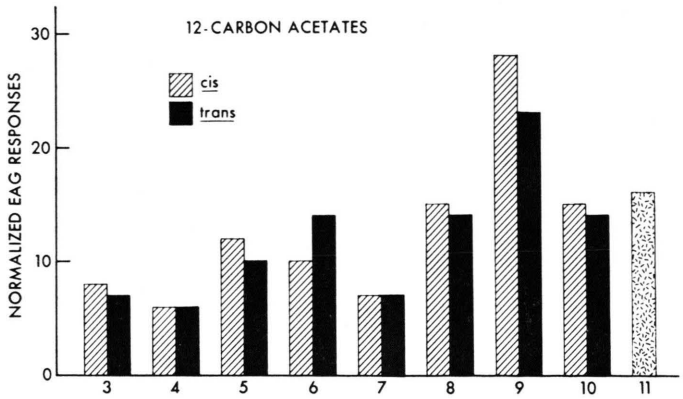

Fig. 1. EAG profile of dodecenyl acetates in *E. ambiguella* males. Responses are normalized with *cis*-6-dodecenyl acetate used as a standard.

evidence for the identity of the EAG active component of female extracts was obtained from gas chromatograms on packed columns. By collecting fractions from a polar (cyclohexane dimethanol succinate) and a nonpolar (silicone OV-1) phase, EAG activity was recovered each time at the retention time expected for a dodecenyl acetate. These data suggested *cis*-9-dodecenyl acetate as a possible pheromone structure.

Analysis on glass capillary columns with electroantennographic detection gave more precise information on position and configuration of the double bond in the EAG-active female component. Fig. 2 shows the separation of dodecyl acetate and seven dodecenyl acetates on Ucon, and an EAD chromatogram of a female extract obtained under the same conditions. The signal obtained from the female extract

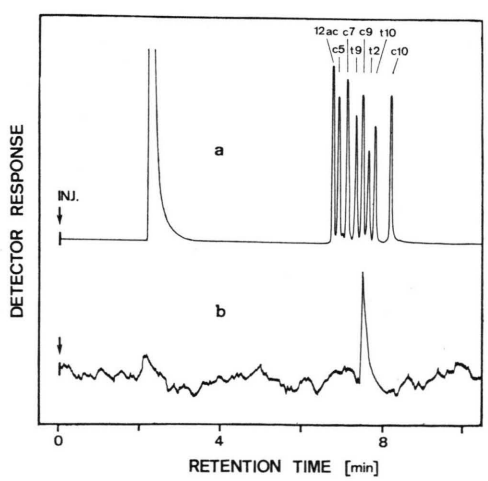

Fig. 2. Gas chromatograms of Ucon 50 HB 5100 (50 m × 0.3 mm glass capillary columns at 140°. a: Mixture of dodecyl acetate (12ac) and seven dodecenyl acetate isomers, flame ionization detection. b: Female extract of *E. ambiguella*, electroantennographic detection.

had the same retention time as *cis*-9-dodecenyl acetate on both polar (Ucon) and nonpolar (SF-96) columns. As seen in Fig. 3, *cis*-9-dodecenyl acetate could be separated on Ucon from all isomers investigated except the 11-isomer. The latter separation was accomplished on SF-96. The *trans*-3 isomer was not available for this study. No other component could be seen in EAD chromatograms of the female extract. Under the conditions of the experiment, peaks of comparable size with retention times up to those of C-14 acetates or higher would have been detected.

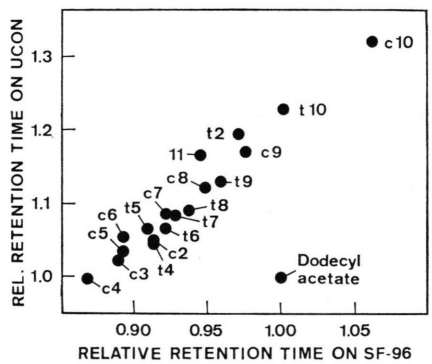

Fig. 3. Retention times of dodecenyl acetates on a polar (Ucon 50 HB 5100) and a nonpolar (silicone SF-96) capillary column, relative to dodecyl acetate.

The presence of *cis*-9-dodecenyl acetate in the female was confirmed with mass spectrometric techniques. An extract of 500 female tips (age 3 days) was fractionated on a silica gel column [10], and the 8% ethyl ether fraction containing the acetates was subjected to mass fragmentography at *m/e* 166 ($M^+ - CH_3COOH$) on the Ucon capillary GC column. The chromatogram (Fig. 4) showed the

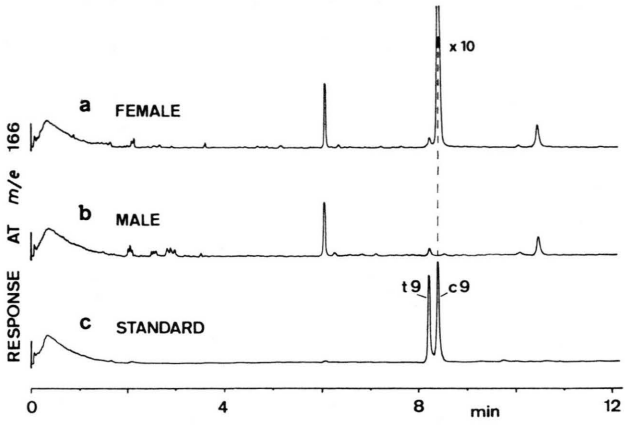

Fig. 4. Mass fragmentograms (*m/e* 166) of 8% ethyl ether fractions of *E. ambiguella*. a: female moth extract (10-moth equivalent); b: male moth extract (10-moth equivalent); c: synthetic *trans*-9- and *cis*-9-dodecenyl acetate.

presence of a compound in the females that was absent in male extracts and co-chromatographed with *cis*-9-dodecenyl acetate. A quantity of 25 ng/female was determined by using the *trans* isomer as an internal standard. The proportion of *trans* isomer naturally present in female extracts was less than 0.5%. No other female component could be detected in this mass fragmentogram that also was not present in the male extract.

Removal of non-volatile material from the 8% ethyl ether fraction by sweep codistillation permitted injection of enough material (100 FE) to obtain a mass spectrum at the retention time of the female component (Fig. 5). While mass spectra of geometrical isomers are identical, positional isomers were found to exhibit small but significant differences in ion intensities [12]. The spectrum of the female component was identical in all respects with *cis*-9-dodecenyl acetate.

Bioassay data obtained with fractions and synthetic standards were in agreement with *cis*-9-dodecenyl acetate as a pheromone component. The fractions obtained from female extract on the silica gel column were all inactive except the one eluting with 8% ethyl ether which elicited full response. Activity was lost when the female extract was saponified with methanolic KOH, and restored when the hydrolyzed mixture was treated with acetyl chloride. With the exception of the *trans*-3 isomer, all dodecenyl acetates were tested with 20 *E. ambiguella* males at 1 ng per pipette. Samples which did not give a fully negative result in the first test were re-tested. Only *cis*-9-dodecenyl acetate elicited the same mating dance response that was observed with a female extract. No more than 2 males in each test showed a response to any other isomer while 11 to 17 males responded to the *cis*-9-dodecenyl acetate standard. Purity of the samples was 93% or better by capillary gas chromatography.

Responses to various amounts of *cis*-9-dodecenyl acetate (0.2% *trans*) per pipette are shown in Fig. 6. Numbers of males responding are given as

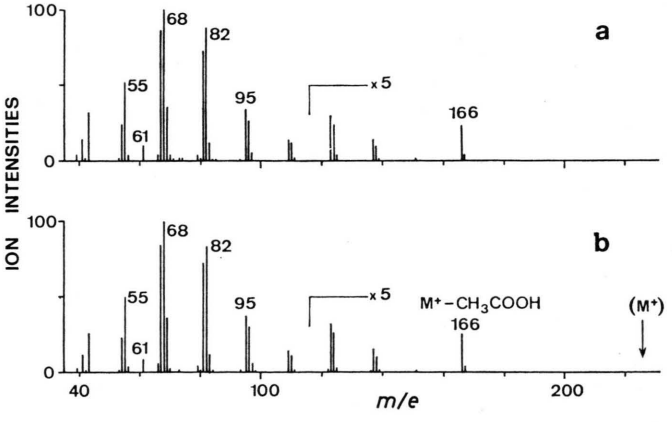

Fig. 5. Mass spectra (*m/e* 35–240) at 23 eV. a: female component; b: synthetic *cis*-9-dodecenyl acetate.

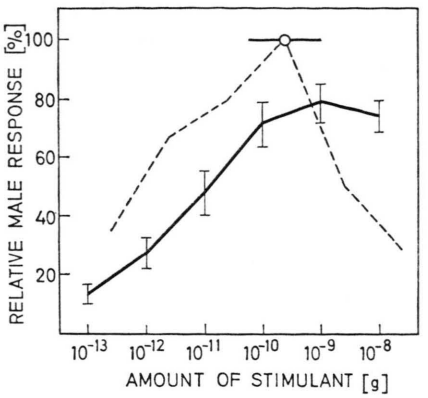

Fig. 6. Bioassay response (± standard error) of *E. ambiguella* males to various amounts of stimulant per pipette. Solid line: *cis*-9-dodecenyl acetate. Broken line: Female extract, for comparison [11] (adjusted for 1 FE = 25 ng).

percent of the standard (10^{-2} FE). Each point of the curve was replicated 8 times with 20 males. The synthetic chemical showed nearly the same effect as an extract when presented at the optimum amount of ca. 1 ng. The response curve of a female extract from an earlier paper [11] is superimposed for reference according to the analytical data of 1 FE = 25 ng *cis*-9-dodecenyl acetate. Position of the maximum and slope at low doses are similar in both curves, but the female extract shows a decrease of activity at high values which is not observed with the synthetic product and may be caused by impurities acting as inhibitors. A further comparison is not possible because the two curves were recorded at different occasions and the bioassay and analytical data were obtained from different batches of extract. A horizontal displacement of the curves could indicate that the amount of *cis*-9-dodecenyl acetate present does not fully account for the bioassay activity of the female extract and that additional compounds are involved. A dose-response curve obtained with a preparation containing 2% *trans* isomer was in essence identical to the one shown, but higher amounts of *trans* isomer were clearly inhibitory. With doses of 1 ng, no bioassay activity was found when the proportion of *trans* was 30% or higher.

In the field, *cis*-9-dodecenyl acetate is attractive to *E. ambiguella* males. Some trapping data are given in Table I. Captures increased with 1 to 100 µg *cis*-9-dodecenyl acetate (2% *trans*) per rubber cap and seemed to level off at higher dosage without reaching those of virgin females. Attraction is again

Table I. Catches of *E. ambiguella* males with *cis*-9-dodecenyl acetate (2% *trans*, on rubber caps) and virgin females. Traps: Pherocon 1C.

Treatment	Mean number per trap	
	Test 1	Test 2
1 µg	1.5 a	
10 µg	11.5 b	3.1 a
100 µg	26.1 c	5.4 a b
1000 µg		9.1 b
5 ♀♀		29.9 c

Means in the same test followed by the same letter are not significantly different at the 5% level as determined by Duncan's multiple range test.

dependent on isomeric purity; no moths were captured at *trans* contents of 8% or more. In one field test with a natural field population, highest captures were made with the two lowest *trans* contents of 0.2 and 1.1%. This would be in accordance with the high isomeric purity of the female product found by GC-MS analysis and would raise the question whether presence of the *trans* isomer is necessary for attraction at all. Results of other tests with laboratory-reared males were not as conclusive with regard to the optimum isomer ratio. It remains open, at this point, whether full attractivity is only a question of proper isomer ratio and release rate, or if additional compounds are involved which have so far escaped our attention. Lepidopteran sex pheromones often contain small amounts of secondary compounds which are essential for attraction but show little EAG activity.

Conclusions

In this paper, we present evidence that *cis*-9-dodecenyl acetate is present in the females of *E. ambiguella* and absent from the males. Identification is based on capillary gas chromatography in combination with electroantennographic detection and mass spectrometry. Positional and geometrical isomers can be excluded from differences in retention times and ion intensities. *E. ambiguella* males react specifically to synthetic *cis*-9-dodecenyl acetate in electroantennography and laboratory bioassay, and are attracted to it in the field. From this evidence, the compound can be considered a sex pheromone component of *E. ambiguella*. The absence of other EAG-active components, the presence of comparably large amounts in the females (*cf.* [5, 10]), and the field attractivity of the chemical by itself indicate that *cis*-

9-dodecenyl acetate is a major component of the pheromone. While the search for the complete pheromone blend continues, we have decided to publish this information because preliminary tests have shown that seasonal catches with *cis*-9-dodecenyl acetate parallel those obtained with female-baited traps and exceed catches in lure-pots. This indicates that the chemical already may be useful for monitoring the occurrence of *E. ambiguella* in vineyards.

cis-9-Dodecenyl acetate is involved in sex attraction of a number of other Lepidoptera. It has been identified as the sex pheromone of the American grape berry moth, *Paralobesia viteana*[13]. It has been found to be attractive to *Episimus argutanus*[14] and *Spodoptera frugiperda*[15] and, in combination with the *trans* isomer, to *Enarmonia formosana*[16]. It also has been reported to be an inhibitor of the *Rhyacionia buoliana* sex pheromone[17, 18].

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