Sex Pheromones of Summer Fruit Tortrix Moth Adoxophyes orana: Two Synergistic Isomers

Abstract. *Both* cis-*9- and* cis-*11-tetradecenyl acetate were isolated from 2500 virgin females of the summer fruit tortrix moth* Adoxophyes orana *(F.v.R.) by gel permeation, column chromatography on silica gel, and gas chromatography. Their chemical, physical, and biological properties were identical to those of the synthetic compounds. In contrast to the single compounds, mixtures of these pheromones were highly active in laboratory and field tests. Catches, in traps, with the pheromone mixture were comparable to those obtained when live females were used as bait. This is the first example of a member of the lepidoptera in which the presence of two isomers is an absolute requirement for activity in laboratory and in field experiments.*

The summer fruit tortrix moth, *Adoxophyes orana* (F.v.R.), is the main pest in pome fruit orchards in the Netherlands and is also a major pest in other European countries. Earlier work suggested that the insect females produce a potent sex pheromone, and crude extracts of virgin females have been used as a survey tool (*1*). Sex pheromone research on *A. orana* has also been performed in Japan, where the insect is known as the smaller tea tortrix, and where it is a major pest in tea cultures (*2*).

Experiments carried out by Roelofs (*3*) with another tortricid moth, the red-banded leaf roller *Argyrotaenia velutinana,* support the idea that, apart from its usefulness as a survey tool, a sex pheromone may also be used for direct control of moths in orchards. For such purposes pheromones have to be available in larger quantities. This demands elucidation of their chemical structure and their subsequent synthesis.

Adoxophyes orana were reared on an artificial diet (*4*), at 23° to 25°C and RH of 50 to 60 percent with a light regime of 17 hours light and 7 hours darkness. Pupae were collected and 3 to 4 days after their emergence, extracts from 2500 female moths were prepared in two batches: 1000 after homogenization of the whole insects, and 1500 after homogenization of the abdominal tips only. Both homogenates were then treated in a similar way and yielded comparable results. They were extracted with methylene chloride and, after evaporation of the solvent, the residue was taken up in acetone and subjected to gel filtration on Sephadex LH-20 (*5*).

The fractions collected were bioassayed as follows. A few drops of each were put on filter paper. This was inserted into a tube, and air was gently blown across the paper into bottles, each containing 10 or 15 male moths, usually 2 to 3 days old. The tests were always performed at the same time of the day, that is, from 8:30 to 9:30 a.m., immediately after the end of the dark period. The light intensity was about 5 lux. We considered a fraction to be active when it elicited the typical behavior of sexual excitement (wing fluttering, copulatory attempts).

The solvent was evaporated from the combined active fractions, the residue taken up in benzene and chromatographed on a silica gel column of about 2200 theoretical plates: samples were injected into the column, through which the mobile phase (benzene) was pumped at a constant velocity. Monitoring was continuous (differential refractometer). The column was capable of separating straight-chain alcohols with 12, 14, and 16 carbon atoms, as well as their acetates, and could be used repeatedly.

The active fractions were again combined, the solvent was evaporated and the residues were taken up in hexane. In subsequent gas chromatography, samples of 10 to 50 μl containing pheromone from 100 to 500 insects were injected and detection was carried out by flame ionization (with splitter), or thermal conductivity. Fractions were collected in glass capillaries cooled with liquid nitrogen (*6*). On polar as well as apolar columns (*7*), the biological activity was found in the fraction that contained the entire major peak. The Kovats retention index of this peak, on OV-101 (1780) as well as on diethylene glycol succinate (DEGS)(2125), appeared to be close to those of some synthetic tetradecenyl acetates. Kovats retention values of 2114, 2115, 2119, and 2143 were found on DEGS for the *cis*-2, *cis*-5, *cis*-7, and *cis*-11 compounds, respectively (*8*). In general, this value increases as the distance from double bond to acetate group increases (*9*). For infrared (IR) and ozonolysis studies, the active fraction from DEGS was freed from "bleeding" by gas chromatography on a 2-m, OV-17 column. The IR spectrum, taken from a sample of about 5 μg, was almost identical to those of the C_{14} acetates mentioned above: bands at 1735, 1235, and 1038 cm^{-1} (acetate) and 3000 cm^{-1} (-CH=CH-).

Table 1. Number of male moths caught from 1 August to 13 September 1971. Type of trap: Sectar insect trap (3 M Comp.). Dispenser: closed polyethylene cap.

Bait (μg of *cis*-9/μg of *cis*-11)	Orchard	
	I	II
400/ 0	0	6
300/100	135	60
200/200	13	9
100/300	3	1
0/400	0	0
Two virgin females	77	115

The absence of a strong band at 960 cm^{-1} that is characteristic of *trans* unsaturation (*10*) indirectly proves the existence of the *cis*-configuration. Moreover, weak absorption bands were found at 1630 to 1640 cm^{-1} and at 690 cm^{-1}, as is common in *cis*-compounds (*11*). The spectrum was quite similar to that of synthetic *cis*-9-tetradecenyl acetate (*12*). After hydrogenation by reaction gas chromatography (*13*), the saturated ester was identified as tetradecenyl acetate by retention and IR data.

The active fraction from the SiO_2 column was subjected to gas chromatography and mass spectroscopy (GC/MS) in an LKB 9000 apparatus, with SE 30 as the stationary phase. The mass spectrum showed a tetradecenyl acetate to be present: a parent peak was found at the mass to charge ratio (*m/e*) 254, and a strong peak at 254–60.

Microozonolysis (*14*) of the purified compound, collected from OV-17 and dissolved in 10 μl of CS_2, yielded pentanal (identified by gas chromatography comparison with synthetic aldehydes). This proved that the double bond was in the position 9.

These data are consistent for the structure of *cis*-9-tetradecenyl acetate. Synthetic samples of this compound (*15*) appeared to be identical in all physical and chemical properties to the compound isolated from *A. orana.* However, in the bioassay, the synthetic products did not show any activity.

An examination of the gas chromatogram on DEGS revealed that the main peak was always followed by a minor one that was not completely separated from the main peak. We de-

termined, by subfractionation, that the biological activity was confined to the region in which the two peaks overlapped. Fractions which contained the first part of the main peak or the last part of the minor peak were completely inactive.

The minor peak was collected separately by gas chromatography on the DEGS column, and analyzed with the GC-MS apparatus. The mass spectrum appeared to be identical to that of synthetic *cis*-11-tetradecenyl acetate, and to differ from those of the other tetradecenyl acetate available. The retention index (2139) of the compound indicated that it has the *cis*-configuration, which cannot be derived from its mass spectrum.

In the laboratory assays, as well as in field traps in apple orchards, mixtures of the *cis*-9- and *cis*-11-tetradecenyl acetates showed high activity, in contrast to the single compounds. For example, large catches of male moths were obtained with mixtures containing *cis*-9- and *cis*-11-tetradecenyl acetate in polyethylene caps (*16*). The ratio of the two compounds appeared to be very important, the largest catches being obtained when the ratio was 3 : 1 (Table 1). The catches with these mixtures were comparable to those obtained with traps baited with live females.

Electroantennogram studies of four tetradecenyl acetates, namely, those with a double bond at the 7-, 9-, 10-, and 11-positions, showed that only the *cis*-9 and *cis*-11 compounds give very strong responses (*17*).

The above results are not only of significance for the control of *A. orana* but also for chemical ecology (*18*) and for discussions on taxonomic and evolutionary aspects of sex pheromone specificity (*19*).

The *cis*-9-tetradecenyl acetate has been shown to be the sex pheromone of members of the family Noctuidae, such as the southern (*10*) and the fall (*12*) armyworm moths, and of one member of the family Gelechiidae (*19*). As far as we know, *A. orana* is the first member of the family Tortricinae for which *cis*-9-tetradecenyl acetate appears to be a sex pheromone.

The *cis*-11-tetradecenyl acetate had already been found as the sex pheromone of other tortricids, such as the red-banded (*20*) and the oblique-banded (*21*) leaf roller moths, which as *A. orana*, belong to the subfamily Tortricinae (*22*). In the oriental fruit moth, a tortricid of the subfamily Olethreutidae, the sex pheromone appeared to be a different compound, *cis*-8-dodecenyl acetate (*23*).

In all the insects mentioned above, except *A. orana*, the sex pheromones were active by themselves, although several other compounds, usually found by screening, could sometimes enhance their activity, and others sometimes inhibit it (*24*).

In the red-banded leaf roller, where *cis*-11-tetradecenyl acetate was found to be the sex pheromone, *cis*-9-tetradecenyl acetate strongly inhibits male response (*25*), whereas in *A. orana* it is a synergist.

The production of synergistic sex attractants by some insects may be important as a species-isolating mechanism. By combining a limited number of pheromones, the specificity of the sex attractants could be greatly enhanced.

The southern armyworm male is only attracted with a mixture of two compounds (*26*). These compounds, *cis*-9-tetradecenyl acetate and *cis*-9, *trans*-12-tetradecadienyl acetate, are not isomers, however. Moreover, each of them separately could also excite males in the laboratory.

In a survey of recent developments in the field of insect pheromones (*27*), in which the two sex pheromones of *A. orana* were also briefly mentioned, other examples were also given of insects in which more than one pheromone was present. However, *A. orana* appears to be the first example of a member of the Lepidoptera in which the presence of two isomers is an absolute requirement for attraction.

Note added in proof: When our report was in press, two papers appeared which showed that Japanese investigators have demonstrated independently *cis*-9- and *cis*-11-tetradecenyl acetate to be present in the smaller tea tortrix as well as in the summer fruit tortrix. Their results are in full agreement with ours (*28*). The first preliminary data on the structure of the sex pheromone of *A. orana* were given in a survey by Ritter (*27*) on 4 May 1971.

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7. Carrier gas: nitrogen, about 50 ml/min. Polar column: 5 percent DEGS on Chromosorb W(AW), 80 to 100 mesh, 6 mm diameter, 6 m length; 159°C. Apolar column: 5 percent OV-101 on Chromosorb G (AW, DMCS), 80 to 100 mesh, 6 mm diameter, 10 m length; 195°C.
8. The *cis*-2 and *cis*-7 compounds were obtained by reduction of the corresponding *yn* (acetylenic) compounds, which were synthesized. The *cis*-5 and *cis*-11 compounds were gifts from Dr. H. Pabon (Unilever Research Laboratory, Vlaarindgen) and from Dr. W. L. Roelofs (Cornell University, Geneva, N.Y.), respectively. The *cis*-11 compound was later also synthesized by reduction of the *yn*-compound, and purified by chromatography.
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