Sex Pheromone Components in the New Zealand Brownheaded Leafroller *Ctenopseustis obliquana* (Lepidoptera: Tortricidae)

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Z. Naturforsch. **40 c**, 262–265 (1985); received October 30, 1984

Sex Pheromone, (*Z*)-5-Tetradecenyl Acetate, (*Z*)-8-Tetradecenyl Acetate, *Ctenopseustis obliquana*, Tortricidae

The sex pheromone of *Ctenopseustis obliquana* was found to contain (*Z*)-8-tetradecenyl acetate and (*Z*)-5-tetradecenyl acetate in approximately 4:1 ratio. No Δ11-tetradecenyl compound was detected in *C. obliquana*, in contrast with the pheromones thus far reported from species of the tribe Archipini elsewhere in the world.

Introduction

The brownheaded leafroller *Ctenopseustis obliquana* (Walker) is a common pest of horticulture in New Zealand, being particularly important on kiwifruit, *Actinidia deliciosa* (formerly *A. chinensis* var. *hispida*) [1, 2]. The sex pheromones of this and other New Zealand leafrollers have been studied with a view to their use in pest control programmes. Of the three predominant leafroller pests, pheromone components have been identified in *Epiphyas postvittana* (Walker) [3] and *Planotortrix excessana* [4] and here we report the identification of two major components of the sex pheromone of *C. obliquana*.

Materials and Methods

A laboratory colony of *C. obliquana* was established from moths collected at Auckland. They were reared individually on a diet similar to that of Roelofs and Feng [5], substituting dried and powdered leaf of *Acmena smithii* (Poiret) for alfalfa leaf meal. Abdominal tips of virgin female moths were extracted as previously described [6]. Male moths were maintained separately under a natural light cycle until required for bioassay. Electroantennogram (EAG) responses of male moths were determined by the method of Roelofs [7] with standards presented as in [6]. An electroantennographic detector system for the gas chromatograph [8] was also used. Pheromonal activity of fractions was also tested by a field cage bioassay based on that of Tamaki *et al.* [9] following the procedure previously described [4]. For statistical comparison trap catches were transformed to $\sqrt{(\chi + 1/2)}$ and differences between means tested by analysis of variance and Duncan's new multiple range test. In the table, means of untransformed catches are given. Field tests of synthetic materials used sticky traps, rubber caps and statistical analysis as described above. Traps were hung 1.5 m above ground, 15 m or more apart, in areas

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0341-0382/85/0300-0262 $ 01.30/0

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infested with *C. obliquana* and the trap positions rotated weekly when the catches were recorded.

Gas chromatography (GC) and mass spectrometry (MS) instrumentation and procedures were as previously described [6] unless otherwise noted. GC columns used were: OV-1 (3%, 2 m × 5.3 mm), PDEAS (3%, 4 m × 2.1 mm) and SE-30 (3%; 2 m × 2.1 mm) all on Chromosorb W-HMDS 100/120 mesh, packed in stainless steel tubing; Carbowax 20M SCOT (50 m × 0.25 mm, glass; Scientific Glass Engineering Ltd., Australia) and DB-1 bonded phase WCOT (30 m × 0.3 mm, fused silica; J. and W. Scientific Inc., USA). Preparative thin layer chromatography (TLC) on silica gel G impregnated with silver nitrate (30% w/w), saponification and acetylation procedures, and bis-thiomethylation of the alkenyl acetates were carried out as previously described [4].

Results and Discussion

In initial tests in the field cage, traps containing either virgin female moths, or abdominal tip extracts from them, both caught male *C. obliquana*. Abdominal tip extract was then fractionated by GC on OV-1 with successive 1 min fractions collected as previously described [6]. When these fractions were screened for EAG activity using an antenna from a male *C. obliquana*, maximal response was obtained with the fraction corresponding to the retention time (R_T) of tetradecyl/tetradecenyl acetates. When the fractions of greatest EAG activity were washed out and fractionated again by GC on PDEAS to separate tetradecyl, tetradecenyl and tetradecadienyl acetates (14:Ac, U14:Ac, DU14:Ac), the maximum EAG response was obtained with the fractions with R_T's encompassing U14:Ac. The fractions with greatest EAG activity were saponified and then refractionated by GC on SE-30, and the fraction with R_T corresponding to tetradecenols collected. This had greatly reduced EAG activity. However, when it was reacetylated and again subjected to preparative GC on SE-30, the original EAG activity was restored and appeared in the fraction with R_T corresponding to U14:Ac. Thus the EAG-active material is probably one or more U14:Acs. When a series of all these from $\Delta 4$ to $\Delta 12$ was screened for EAG activity the maximum response was to 8*Z*14:Ac.

A parallel series of experiments was carried out using the field cage bioassay. When female abdominal tip extract was fractionated by column chromatography on Florisil [11] only the fractions eluted with 5% and 10% diethyl ether in pentane were active (Table I (a)). Increasing the elution volume by 50% restricted activity to the 5% ("esters") fraction (Table I (b)). Saponification of this fraction eliminated its activity, which was then restored by acetylation (Table I (c)), in accord with the principal pheromone components being acetates. When the "esters" fraction was further fractionated by silica gel/silver nitrate TLC, the Z-alkenyl acetate fraction was found to be active (Table I (d)).

The EAG and field cage bioassay results together indicated one or more U14:Acs as principal pheromone components in *C. obliquana*. Confirmation of their presence in female tip extract was then sought by GC-MS analysis using a Carbowax 20 M SCOT

Table I. Field cage bioassays: catches of male *C. obliquana* in traps containing fractions of female abdominal tip extract (20 female equivalents per trap): (a) fractionation by Florisil chromatography: elution volume = 2 × bed volume (2 replicates; 75 moths released, 56 trapped); (b) fractionation by Florisil chromatography: elution volume = 3 × bed volume (2 replicates; 130 moths released, 117 trapped); (c) saponification and acetylation of active fraction from (b). (2 replicates; 140 moths released, 75 trapped); (d) fractionation by silica gel/silver nitrate TLC (2 replicates; 80 moths released, 58 trapped).

	Test fraction	Mean catch [males/trap][a]
(a)	0% ether in pentane	0.5 b
	5%	8.5 a
	10%	12.0 a
	20%	2.5 b
	50%	1.5 b
	100%	2.0 b
	blank	1.0 b
(b)	5% ether in pentane	32.0 a
	10%	0.5 b
	5% + 10% recombined	25.0 a
	blank	1.0 b
(c)	5% ("esters") fraction from (b)	13.5 a
	saponified	1.5 b
	saponified and acetylated	21.0 a
	blank	1.5 b
(d)	saturated	0.0 b
	E	2.5 b
	Z	24.0 a
	blank	0.5 b

[a] Means within the same treatment followed by the same letter are not significantly different at the 5% level.

column and selected ion monitoring (SIM) for m/z 61 (corresponding to $CH_3CO_2H_2^+$ species from acetates) and m/z 196, 194 and 192 (corresponding to M-60 fragment ions from 14:Ac, U14:Ac and DU14:Ac respectively). Signals were observed with R_T's corresponding to traces of 14:Ac (m/z 61 and 196) and two U14:Acs in about 1:4 ratio (m/z 61 and 194). The amounts were small: the larger U14:Ac signal was estimated to represent about a nanogram per female moth. When the GC-MS analysis was repeated on fractions from tip extract previously subjected to silica gel/silver nitrate TLC the two U14:Acs were shown to be in the *Z*-alkenyl fraction. During this GC-MS analysis, EAG assay of the effluent gas at the exit port of the MS membrane separator showed activity corresponding to the two U14:Ac signals. The R_T relative to 14:Ac (R_T^{rel}) of the more abundant, and more EAG-active of these, matched that of 8*Z*14:Ac within experimental error. The R_T^{rel} of the minor component matched 5*Z*14:Ac or 6*Z*14:Ac, which we were unable to resolve under our GC conditions. This pair of positional isomers is one of the most difficult to separate in the tetradecenyl acetate series [12].

Double bond positions were determined by a bis-thiomethylation procedure [10] and GC-MS analysis using a DB-1 bonded phase capillary column. When abdominal tip extract from 100 female *C. obliquana* was derivatized, GC-MS analysis revealed spectra containing m/z 348 (M^+) at retention times corresponding to two derivatized tetradecenyl acetates. Scans of these spectra also showed the three major fragment ions expected from Δ5-tetradecenyl acetate (m/z 173, 175 and 115) in the tetradecenyl acetate of shorter R_T, and those expected from Δ8-tetradecenyl acetate (m/z 217, 157 and 131) in that of longer R_T. No such sets of fragment ions corresponding to 6-, 7-, 9-, 10- or 11-tetradecenyl acetates were found. Taken together with the silver nitrate TLC and other results, this established the presence of 8*Z*14:Ac and 5*Z*14:Ac in the female tip extract.

When these compounds were tested singly in traps in the field, 8*Z*14:Ac attracted few male *C. obliquana*, and 5*Z*14:Ac none at all. However, when mixtures were tested, significantly higher numbers were trapped with 8*Z*14:Ac and 5*Z*14:Ac at ratios near that observed in female abdominal tip extracts (Fig. 1). Of the two rates tested (10 or 50 µg 8*Z*14:Ac plus various amounts of 5*Z*14:Ac), the lower rate caught more *C. obliquana* and the maximum catch was with 8*Z*14:Ac and 5*Z*14:Ac in 4:1 ratio. We conclude that 5*Z*14:Ac and 8*Z*14:Ac are constituents of the sex pheromone of *C. obliquana*. The only other tortricines from which either of these compounds have been previously reported are the two pheromone-types of *Planotortrix excessana* [4]. Both *C. obliquana* and *P. excessana* are endemic to New Zealand [13, 14] and are regarded as generalized Archipini [15]. In neither species did we find any Δ11-tetradecenyl compound. This sets them apart from other Archipini whose pheromones have been reported [16].

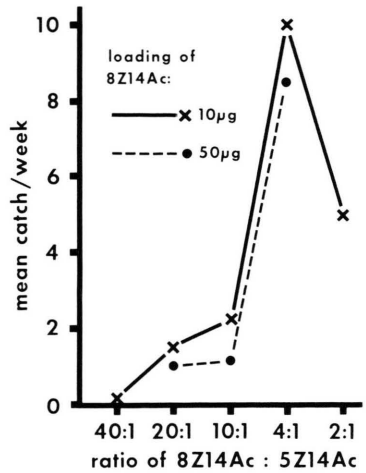

Fig. 1. Captures of male *Ctenopseustis obliquana* in traps baited with mixtures of 8*Z*14:Ac and 5*Z*14:Ac on rubber stoppers, at Havelock North, May 20 to September 20, 1983 (2 replicates).

Acknowledgements

We thank J. G. Charles for conducting field trials and J. S. Dugdale for taxomonic advice.

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