Chemical ecology of astigmatid mites XLIX

β -Acaridial (2(E)-(4-methyl-3-pentenylidene)-butanedial), the alarm pheromone of *Tyrophagus longior* Gervais (Acarina: Acaridae)¹

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Abstract

Alarm pheromone activity was detected in the hexane extract of *Tyrophagus longior*. The GLC profile of the extract revealed six components; α -acaridial {2(E)-(4-methyl-3-pentenyl)-butanedial}, tridecane, β -acaridial {2(E)-(4-methyl-3-pentenylidene)-butanedial}, an unknown (M^+ ; m/z 166), *n*-hexyl linolate and *n*-hexyl stearate, among which β -acaridial was the major component, followed by tridecane. These compounds were each identified by GC/MS spectra and GLC retention times by comparing with those of authentic compounds. The active eluate from a SiO_2 column by 50% ether in hexane consisted of a peak of β -acaridial. The compound isolated from the mite was active at 50 ng, and its activity was the same as the authentic β -acaridial derived from *T. putrescentiae*.

Key words: Alarm pheromone, *Tyrophagus longior*, *Tyrophagus putrescentiae*, β -acaridial, α -acaridial

INTRODUCTION

Since the first identification of the alarm pheromone from the mold mite, *Tyrophagus putrescentiae*, as neryl formate {(Z)-3,7-dimethylocta-2,6-dien-1-ol formate} (Kuwahara et al., 1975), alarm pheromones of a total of 15 species belonging to 7 families of Astigmata have so far been identified (Kuwahara, 1990, 1995), including recent studies of four *Rhizoglyphus* mites (Akiyama et al., 1997). A total of 11 compounds were found to be responsible for alarm pheromone activities. Neral {(Z)-3,7-dimethylocta-2,6-dienal} and neryl formate are both the most widely distributed pheromones, and both function in 5 species (Kuwahara, 1990, 1995). Four species belonging to the genus *Tyrophagus* all use different chemicals as their pheromones, that is, the chemicals are species-specific (Kuwahara et al., 1989a). On the other hand, neryl formate is a common

component of the pheromone among four *Rhizoglyphus* mites which belong to the same family, and no species specificities are demonstrated. Furthermore, no chemical characteristics specific to any of the families have been detected among seven families, in which at least one species contains alarm pheromone (Kuwahara, 1990, 1995). At present, therefore, we have no explanation for why each species has chosen a certain compound as its pheromone among a set of opisthonotal gland components.

T. longior is known as one of the common *Tyrophagus* found in China. The presence of the alarm pheromone in this species was noticed by observing their escape behavior after disturbance of a mite among a group in a culture using a needle.

It is, therefore, interesting to clarify whether or not the present species possesses a compound responsible for its alarm pheromone activity that differs from those of the 15 species

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reported, and whether or not the species-specificity among the genus *Tyrophagus* is still retained. It is also desirable to accumulate more knowledge of secretory compounds from the mite and of their function from a chemical ecology viewpoint, and also for a possible application of chemotaxonomy to identify these mites.

MATERIALS AND METHODS

Tyrophagus longior Gervais (Acarina: Acaridae) was collected in the winter of 1993 from the decaying surface of okra, which was purchased at a grocery in Kyoto City as a product transported from Okinawa Prefecture. The species was maintained in the laboratory by feeding dry yeast at 25°C, 70–80% R.H. in a rearing vial (13 cm in diameter, 7 cm in height) as reported (Kuwahara et al., 1979).

The mold mite, *T. putrescentiae* (Schrank) was a strain collected from stored garlic in Aomori prefecture, and used for isolating the standard β -acaridial {2(*E*)-(4-methyl-3-pentenylidene)-butanedial} (Leal et al., 1989b). The species was also reared as mentioned above.

Instrument. Gas liquid chromatography (GLC) was carried out on an HP-5890 series II plus equipped with an FID using an HP-5 capillary column (0.32 mm $\times$ 30 m, 0.33 μ m in film thickness) at a temperature condition programmed from 60°C to 290°C at 10°C/min with an initial 2 min hold. Samples were analyzed at a split-less mode using He as a carrier gas, and the chromatogram was processed by an HP 3396 series II Integrator. GC-mass spectrometry (GC/MS) was performed by a Hewlett Packard 5989B mass spectrometer coupled with an HP-5890 series II plus gas chromatograph with the split-less mode, using the same capillary column under the same conditions as those mentioned above.

Chemicals. α -Acaridial {2(*E*)-(4-methyl-3-pentenyl)-butenedial} (Leal et al., 1989a) was prepared following the method reported (Suzuki et al., 1992). Although the synthetic method of β -acaridial {2(*E*)-(4-methyl-3-pentenylidene)-butanedial} has been reported (Leal et al., 1989b), the compound used as the standard was prepared from the hexane rinse of *T. putrescentiae* by the same isolation pro-

cedure. The hexane rinse from *T. putrescentiae* bodies (1.6 g), after concentration to 2 ml, was subjected to an SiO₂ column, and the column was successively eluted with a mixture of hexane and ether. The pure β -acaridial was obtained in a fraction of the 50% ether-in-hexane eluate. The quantity of isolated β -acaridial dissolved in 2 ml of hexane was determined to be 544 μ g by comparing the area of the GLC peak with that of the standard α -acaridial. The solution was then diluted successively with hexane to 1, 5, 10, 50, 100 ng/ μ l solution and used for bioassay by impregnating each 1 μ l aliquot onto a piece of filter paper (2 mm $\times$ 2 mm).

Bioassay method. Bioassay for the alarm pheromone activity was conducted by the modified method (Akiyama et al., 1997), based on the absolute quantity of a candidate compound on each filter paper (2 mm $\times$ 2 mm), as stated above.

Monitoring method of the opisthonotal gland secretion by GLC and GC/MS. A group of mites, each consisting of five live females or males, was separately collected by a needle into a small conical-bottomed tube (handmade, 8 mm o.d. $\times$ 30 mm ht). Two microliters of hexane was added to the tube and the tube was tapped several times to drop all mites into the solvent. A stereoscopic microscope was conveniently used to verify that all mites were trapped in the solvent portion. At 3 min (exact) after introduction of the solvent, the hexane rinse was collected by a micro-syringe, and then all portions of the rinse were subjected to analysis by either GLC or GC/MS.

Extraction and purification for bioassay and identification. *T. longior* (1.5 g) collected from the culture medium by the saturated saline flotation method were extracted by dipping mites for 3 min in hexane (15 ml $\times$ 2) to extract the opisthonotal gland secretion with body surface components (two times). The extract, after being evaporated to ca. 2 ml under reduced pressure, was transferred onto a silica gel (Wako-gel C200, 0.4 g) column, and the column was eluted and fractionated with each 4 ml of hexane, ether-hexane mixture (1% ether in hexane, 3%-, 5%-, 10%-, 20%- and 50%-) and ether, respectively. All fractions were then subjected to the bioassay described above.

RESULTS AND DISCUSSION

A typical GLC profile of the hexane rinse from *Tyrophagus longior* gave the following six major peaks; peak 1: t_R 10.09 min (4.7%), peak 2: 10.31 min (46.1%), peak 3: 11.08 min (35.8%), peak 4: 15.02 min (1.6%), peak 5: 22.97 min (9.5%) and peak 6: 23.17 min (2.3%), along with squalene which was derived from dietary yeast (Fig. 1). Peaks 2 and 3 were the most abundant components in the preparation. The profile differed from those of the related four species belonging to the same genus *Tyrophagus* (Kuwahara et al., 1989a) and the feasibility of the GLC profile for chemotaxonomic application was demonstrated in the present results.

Peak 1 (t_R 10.09 min) gave M^+ ion at m/z 166 (16%) and a base ion at m/z 69 (100%) with diagnostic ions at m/z 133 (36%), 123 (29%), 119 (21%), 55 (25%) and 41 (63%). The MS and GLC retention times were both identical to those derived from the standard α -acaridial (2(*E*)-(4-methyl-3-pentenyl)-butenedial) (Leal et al., 1989a), which was prepared as reported (Suzuki et al., 1992).

Peak 2 (t_R 10.31 min) gave M^+ ion at m/z 184 and the base ion at m/z 57 (100%) with diagnostic ions at m/z 127 (12%), 113 (12%), 99 (17%), 85 (54%), 71 (82%) and 43 (51%). The mass spectrum was identical with that of tridecane.

Peak 3 (11.08 min) gave M^+ ion at m/z 166 (30%) and the base ion at m/z 67 (100%) with

diagnostic ions at m/z 133 (88%), 123 (75%), 95 (87%), 91 (65%) and 41 (77%). The MS was identical to the one derived from β -acaridial (2(*E*)-(4-methyl-3-pentenylidene)-butanedial) (Leal et al., 1989b). Retention time of the peak was the same as that of the mold mite *T. putrescentiae*, from which β -acaridial was purified, as stated in Materials and Methods.

Although peak 4 (15.02 min) gave M^+ ion at m/z 164 (81%) and the base ion at m/z 105 (100%) with diagnostic ions at m/z 121 (26%), 91 (62%) and 78 (32%), its structure remained obscure.

Peak 5 (22.97 min) gave M^+ ion at m/z 364 (10%) and the base ion at m/z 81 (100%) with diagnostic ions at m/z 279 (14%), 262 (43%), 220 (10%), 178 (24%), 164 (22%), 150 (26%), 135 (22%), 123 (33%), 109 (48%), 95 (79%), 67 (91%), 55 (64%) and 43 (71%). The MS spectrum and t_R were both identical to those of *n*-hexyl linolate, and identified as *n*-hexyl linolate (Kuwahara et al., 1988). The compound is commonly found in all species of the genus *Tyrophagus* thus far examined.

Peak 6 (23.17 min) indicated an identical mass spectrum to that of *n*-hexyl stearate, presenting M^+ ion at m/z 368 (38%) and the base ion at m/z 285 (100%) with diagnostic ions at m/z 267 (21%), 145 (11%), 129 (17%), 84 (64%), 61 (29%) and 43 (32%).

The hexane extract (30 ml) from *T. longior* bodies, after being concentrated to ca. 2 ml, was chromatographed on an SiO_2 column and all of the eluates were subjected to GLC, GC/MS and

Fig. 1. A typical gas-liquid chromatogram trace of the hexane extract derived from *Tyrophagus longior*. Reproduced from total ion chromatogram by GC/MS. For analytical conditions, see text. Peak 1: α -acaridial; peak 2: tridecane; peak 3: β -acaridial; peak 4: the unknown (M^+ at m/z 164); peak 5: *n*-hexyl linolate; and peak 6: *n*-hexyl stearate.

Table 1. Alarm pheromone activity of acaridials to *Tyrophagus longior*

Dose (ng)	Isolated β -acaridial	Standard β -acaridial [#]	Synthetic α -acaridial
Control	—	—	—
10	—	—	—
50	+	+	—
100	+	+	—
1,000	+(268 ng)*	+(272 ng)*	—

+: Escaping behavior was observable to the tested filter paper. —: Inactive.

*Maximum quantity tested.

[#]Isolated from *Tyrophagus putrescentiae*. See text for details.

also to bioassay. Two fractions eluted with the 20% and the 50% ether in hexane indicated pheromone activity. The 50% eluate consisted of only one peak, which was identified as β -acaridial by GLC and GC/MS, as stated above. The yield of the compound was estimated as 536 μ g.

The other active 20% eluate contained a mixture of α - and β -acaridial, both of which were identified by GLC and GC/MS, as stated above. Bioassay of the synthetic α -acaridial revealed no activity, while the standard β -acaridial isolated from *T. putrescentiae* demonstrated the same escaping activity at 50 ng as the one obtained in the 50% eluate, as summarized in Table 1.

Tridecane was recovered as the hexane and the 1% eluate. The 5% eluate contained *n*-hexyl linolate and *n*-hexyl stearate. The 10% and the 20% eluates consisted of α -acaridial.

As a result, β -acaridial was identified as the alarm pheromone of the species belonging to the genus *Tyrophagus*. Although the compound has been widely distributed among 9 species of astigmatid mites, functioning as the sex pheromone (Leal et al., 1989c) and as an antifungal agent (Kuwahara et al., 1989b), it is the first identification of the compound functioning as an alarm pheromone, not only among the genus *Tyrophagus* but also among 31 species of astigmatid mites so far examined (Kuwahara, 1990, 1995).

Although both neryl and neryl formate are the most widely distributed active principles of the alarm pheromone among astigmatid mites composed of seven families, and a total of 16

species of Astigmata (Kuwahara, 1990, 1995; Akiyama et al., 1997), including the present species, use 12 compounds as their active principle, no general rule has been elucidated between chemical structure and these pheromones.

The pheromone of five species belonging to the genus *Tyrophagus* is species-specific (Kuwahara et al., 1989a), including the present study of *T. longior*, while those of four *Rhizoglyphus* species of the same family Acaridae use neryl formate as the common active principle, that is, the pheromone is non species-specific (Akiyama et al., 1997). The other 7 alarm pheromones are distributed among 6 families consisting of 7 genera (Kuwahara, 1995); however, no conclusive facts are available at present.

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