Chemical Ecology of Astigmatid Mites LXXVI
Identification of α-Acarial as the Female Sex Pheromone of
Schwiebea similis (Acari: Acaridae)

Karin Nishimura1, Naoki Mori1, Kimiko Okabe2 and Yasumasa Kuwahara1,*

1) Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan
2) Forestry and Forest Products Research Institute, P. O. Box 16, Norin Kenkyuu Danchi,
Tsukuba, Ibaraki 305-8687, Japan

(Received: January 14, 2004; Accepted: March 158, 2004)

無気門亜目ダニの化学生態学第LXXVI報. α-アカリジアールをSchwiebea similis
の雌性フェロモンとして同定 西村栄俊1・森 直樹1・関部貴美子2・桑原保正1
(1)京都大学大学院農学研究科, 2)独立行政法人森林総合研究所)

Schwiebea similis 雌のへキサン抽出物中に雄を性的に興奮させ, 雄同士のタッピング行動を活発化させる雌性フェロモンの存在を確認し, その同定を行った. ダニのへキサン抽出物はシリカゲルカラムで分画し, GC/MSによって活性成分をα-アカリジアール [2(E)-(4-methyl-3-pentenyl)-butenedial] と同定した. α-アカリジアールは30~300ngの投与で, 雛抽出物と同様の活性を示した. α-アカリジアールは雌1頭当たり30.4ng, 雄1頭当たり13.4ng含まれていた. 本研究は無気門亜目ダニにおいてα-アカリジアールが雌性フェロモンとして機能する2番目の例である.

This study showed the sex pheromone activity of a female hexane extract from Schwiebea similis, which caused increased tapping behavior among the test males. The active principle of the hexane extract was isolated using SiO2 column chromatography, and identified as α-acarial [2(E)-(4-methyl-3-pentenyl)-butenedial]. At doses of 30 to 100 ng, synthetic α-acarial showed the same activity as that of the female extract. Its content was determined by GC/MS as 30.4 ng in the females and 13.4 ng in the males. This is the second example to show that α-acarial functions as a female sex pheromone in astigmatid mites.

Key words: Sex pheromone, Schwiebea similis, α-Acarial, 2(E)-(4-Methyl-3-
pentenyl)-butenedial, Astigmata

* Corresponding author: zkuwa@kyotogakuen.ac.jp
Introduction

Female sex pheromones, which cause sexual arousal and trigger a tapping behavior among males, are known in 10 species of Astigmata (Kuwahara 1999, 2001; Tatami et al., 2001; Ryono et al., 2001; Hiraoka et al., 2002; Mizoguchi et al., 2003). These pheromones can be chemically divided into three groups, hydrocarbons, aromatics and monoterpenoids, however, the chemical structures and differing characteristics of these three groups with regards to taxonomic status have yet to be elucidated. In general, these sex pheromones are species-specific, however, three species, Acarus immobilis, A.leuroglyphus ovatus and Cosmoglyphus hughesii, share the same aromatics. Furthermore, as summarized by Kuwahara (2001), the female sex pheromones are found not only in females but also in males and juveniles.

A cultured line of Schwiebea similis collected from organic soil has been maintained in our laboratory. This species has also been found on shallot bulbs in Okayama prefecture, and cluster-amarilis bulbs at the Nangoku campus, Kochi University, therefore it seems to be a potential agricultural pest. S. similis is composed of 80% females and 20% males (Okabe and O’Connor, 2001), and mating pairs are constantly observable in the stock culture. When a female was temporarily transferred into a container of males using a needle and creating little disturbance, the males became sexually aroused and started a tapping behavior. Based on this observation and the information on the supposedly normal sexual behavior of this species, the presence of a sex pheromone was suggested as the cause of this arousal.

In the present paper, the presence of a female sex pheromone in S. similis was confirmed, and the active compound composing this pheromone was purified by monitoring its activity with a bioassay. Its chemical structure was also elucidated. This is the first time a sex pheromone has been identified in this species, and the first example in the genus Schwiebea.

Materials and Methods

Mites

Schwiebea similis (Acari: Acaridae) was obtained from organic soil in the botanical garden of the Science Faculty, Kyoto University, in 1994. The stock culture was maintained at 20°C under high-humidity in a disposable polyethylene culture dish (9 cm in diameter x 18 mm in height), in which a sheet of filter paper was used as the foothold. The culture was fed dried yeast and kept in a polyethylene bag with a zipper (Seinichi, Unipack; 240 x 170 x 0.04 mm) to maintain humidity.

Bioassay

Sex pheromone activity was evaluated as previously reported (Mori et al., 1996) with minor modifications. Ten males were placed in a culture dish (7 mm in diameter x 5 mm in height) with a cover glass (18 x 18 mm), the bottom surface of which was preliminarily covered with moistened filter paper, at room temperature and ambient humidity. A small lump of moistened yeast was placed on the paper as food. After conditioning for 30 min, a living female or piece of filter paper (3 x 3 mm) impregnated with a candidate material at each indicated dose was introduced into the assay chamber, and the subsequent behavior of the males was observed under a binocular microscope for 3 min. A male mite or a hexane extract from a male mite was used as the control. The tapping behavior frequencies of the males were recorded 5-8 times for each group of males. The group was awarded 3 points when more than one male started to tap during the 0-1 min observation period, 2 points if observed...
during the 1-2 min period and, 1 point if observed during the 2-3 min period. Total scores were calculated and evaluated using the Mann-Whitney U test or Kruskal-Wallis test followed by the Dunn's multiple-comparison test to determine any significant differences.

**Extraction and purification**

The following extraction and purification procedures were used to isolate the sex pheromone: mites of all developmental stages (0.28 g) were separated from the stock culture using a saline flotation method (Matsumoto, 1965) and dipped into hexane (2 ml) for 3 min. The hexane extract was then collected by filtration and without being concentrated, applied to an SiO2 column (300 mg, Wako-gel C-200) and eluted successively with 2 ml each of the following hexane and Et2O mixtures: (100:0), (99:1), (97:3), (95:5), (90:10), (80:20), (50:50) and (0:100). All fractions were then subjected to gas chromatograph/mass spectrometer (GC/MS) and bioassay analyses, respectively, to locate the active fraction.

**GC/MS and GC analyses**

GC/MS analysis was carried out with a gas chromatograph/mass spectrometer (Hewlett Packard HP-5890) operated at 70 eV in a splitless mode, using an HP-5 MS capillary column (0.25 mm x 30 m; film thickness, 0.25 μm). Helium at 1.23 ml/min was used as the carrier gas and the oven temperature was programmed from 60°C (2 min hold) to 290°C at a rate of 10°C/min. GC was performed with a Hewlett Packard HP-5890 equipped with a flame ionization detector (FID) and using an HP-5 capillary column (0.25 mm x 30 m; film thickness, 0.25 μm) under the same conditions as those used for GC/MS analysis.

Ten males or 10 females were collected from the stock culture using a needle and placed in a conical bottomed tube (8 mm in diameter x 30 mm in height, handmade). Hexane (4 μl) was then added to the tube using a microsyringe (10 μl, Hamilton Co. Ltd. U. S. A.), and removed after 3 min. The collected extract was subjected to GC or GC/MS analysis.

**Quantitative determination of the sex pheromone**

α-Acaridial content was estimated using single ion (monitored at m/z 166) mass chromatograms with GC/MS analysis, because α-acaridial appeared as an unresolved peak with neryl formate under the present GC column conditions. The chromatographic responses of α-acaridial (200 and 100 ng) were co-related to those of the hexane extracts from five mites (5 replications for each sex), and its content was calculated for each extract.

**Preparation of acaridials**

α-., β- and γ-Acaridials were prepared according to previously reported methods (Suzuki et al., 1992; Shimizu et al., 2003; Sakata and Kuwahara, 2001, respectively).

**Results**

**Biological activity**

When introduced into the male chamber, females provoked more frequent tapping behavior than if a male was introduced (Fig. 1). The tapping frequency scores for female and male introduction were 14.1±0.8 (n = 8) and 9.2±0.9 (n = 6), respectively. Significantly different results (p<0.01, n = 5) were obtained using filter papers preliminary impregnated with a mite equivalent of the hexane extract prepared from females or males (Fig. 1). The score of the female extract (15.2±1.6) was two times larger than that of the male extract (7.5±1.5). No peculiar behavior was observed among females upon introduction of a male or exposure to a male extract (data not shown).

**Identification of mite compounds**

Both the male and female extracts were composed
Fig. 1 Sex pheromone activity of male and female introductions (A), and male and female extracts (B). For assay method see the text. The alphabetical letters above the bars indicate statistical differences at \( p < 0.01 \) using the Mann-Whitney U test.

Table 1  GC-mass spectrum of compounds in the hexane extract from *Schwiebea simillis*

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Compound identified</th>
<th>Retention time (min)</th>
<th>Diagnostic ions m/z (relative intensity %)</th>
<th>Identified Method, and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Neral</td>
<td>9.448</td>
<td>152 (M+, 1), 94 (18), 84 (17), 69 (68), 41 (100)</td>
<td>GLC, GC/MS; Kuwahara et al. (1991)</td>
</tr>
<tr>
<td>B</td>
<td>α-Acaridial</td>
<td>10.047</td>
<td>166 (M+, 11), 133 (29), 123 (30), 69 (79), 41 (100)</td>
<td>GLC, GC/MS; Leal et al. (1989a)</td>
</tr>
<tr>
<td></td>
<td>Neryl formate</td>
<td>10.009</td>
<td>136 (M+ -46, 20), 121 (11), 93 (29), 69 (100), 41 (84)</td>
<td>GLC, GC/MS; Kuwahara et al. (1975, 1980)</td>
</tr>
<tr>
<td>C</td>
<td>3-Hydroxybenzene-1,2-dicaraldehyde</td>
<td>10.164</td>
<td>150 (M+, 98), 121 (100), 93 (35), 65 (26)</td>
<td>GLC, GC/MS; Sakata and Kuwahara (2001)</td>
</tr>
<tr>
<td>D</td>
<td>Tridecane</td>
<td>10.229</td>
<td>184 (M+, 16), 85 (40), 71 (63), 57 (100), 43 (64)</td>
<td>GLC, GC/MS</td>
</tr>
<tr>
<td>E</td>
<td>β-Acaridial</td>
<td>10.984</td>
<td>166 (M+, 25), 133 (65), 95 (64), 67 (85), 41 (100)</td>
<td>GLC, GC/MS; Leal et al. (1989b)</td>
</tr>
<tr>
<td>F</td>
<td>Unknown</td>
<td>11.949</td>
<td>166 (M+, 17), 148 (36), 137 (98), 119 (47), 109 (45), 98 (56), 91 (34), 79 (35), 69 (100), 53 (30), 41 (86)</td>
<td>GLC, GC/MS</td>
</tr>
</tbody>
</table>

of six peaks (A, B, C, D, E and F) (Fig. 2). Each identified compound is shown in Table 1. With peak A (\( t_R: 9.448 \) min) M+ occurred at \( m/z\) 152 (1%) and the base ion at \( m/z\) 41 (100%) with the following diagnostic ions: 94 (18%), 84 (17%) and 69 (63%). The mass spectrum with GC \( t_R \) was identical to that obtained from the standard neral [3,7-dimethyl-(Z)-2,6-octadienal] and to that
\( \alpha \)-Acridial: Female Sex Pheromone of \textit{Schwiea similis}

![chromatogram](image)

**Fig. 2** Typical gas liquid chromatograms of ten female (1) and male (2) extracts. A: neral, B: mixture of neryl formate and \( \alpha \)-acridial, C: \( \gamma \)-acridial, D: tridecane, E: \( \beta \)-acridial, F: structure not determined. \* : n-butyl p-hydroxybenzoate, \** : squalene, and \( \dagger \) : cholesterol derived from the culture medium.

Previously reported (Kuwahara et al., 1980).

Peak B \((t_R : 10.048\ \text{min})\) was composed of a mixture of two peaks \((t_R : 10.009\ \text{min} \text{ and } 10.048\ \text{min})\), and was resolved by single ion mass chromatography using \(m/z\ 136\) and \(m/z\ 166\) (Fig. 3). The \(t_R\) 10.009 min compounds gave \(M^+\) at \(m/z\ 136\) (20\%\) and the base ion peak at \(m/z\ 69\) (100\%) with the following diagnostic ions: 121 (11\%), 93 (29\%) and 41 (84\%). The mass spectrum with GC \(t_R\) was identical to that obtained from the standard neryl formate [3,7-dimethyl-(\(Z\))-2,6-octadienyl formate] and to that previously reported (Kuwahara et al., 1975, 1979). The \(t_R\) 10.047 min compound gave \(M^+\) at \(m/z\ 166\) (11\%) and the base ion at \(m/z\ 41\) (100\%) with the following diagnostic ions: \(m/z\ 133\) (29\%), 123 (30\%) and 69 (79\%). Its spectrum was identical to that of \(\alpha\)-acridial [2(\(E\))-(4-methyl-3-pentenyl)-butenedi-]

Peak C \((t_R : 10.164\ \text{min})\) gave \(M^+\) at \(m/z\ 150\) (98\%) and the base ion at \(m/z\ 121\), along with \(m/z\ 93\) (35\%) and 65 (26\%). The spectrum was identical to that of 3-hydroxybenzene-1,2-dicarbalddehyde (tentatively named as \(\gamma\)-acridial) as previously reported (Sakata and Kuwahara, 2001).

Peak D \((t_R : 10.229\ \text{min})\) indicated \(M^+\) at \(m/z\ 184\) (16\%) and the base ion at \(m/z\ 57\) (100\%) with the following diagnostic ions: 85 (41\%), 71 (63\%) and 43 (64\%). It was identified as tridecane from the GC \(t_R\) and GC/MS results, using tridecane as the standard.

Peak E \((t_R : 10.984\ \text{min})\) gave \(M^+\) at \(m/z\ 166\) (25\%) and the base ion at 41 (100\%) with the following diagnostic ions: 133 (65\%), 95 (64\%) and 67 (85\%), which along with the GC retention time were identical to those of \(\beta\)-acridial [2(\(E\))-(4-
methyl-3-pentenylidene)-butanedial] as previously reported (Leal et al., 1989b).

It was not possible to determine the structure of peak F ($t_R: 11.949$ min), in which $M^+$ occurred at $m/z$ 166 (17%) and the base ion peak at 69 with the following fragments: 148 (36%), 137 (98%), 119 (47%), 109 (45%), 98 (56%), 91 (34%), 79 (35%), 53 (30%) and 41 (86%).

In the present analysis (Fig. 2), the highest peak in the female extracts was B (a mixture of neryl formate and $\alpha$-acaridial), followed by C ($\gamma$-acaridial), D (tridecane), E ($\beta$-acaridial), A (neral), and F. The latter two compounds appeared in reverse order in the male extracts. The peak height ratios between sexes were all biased to the female extract, except for peak F, as follows: peak A, 1.13; peak B, 1.57; peak C, 1.51; peak D, 1.26; peak E, 1.23; and peak F, 0.41. In peak B (Fig. 3), the ratios of neryl formate and $\alpha$-acaridial were 0.89 and 1.78, respectively. As a result, peak F and neryl formate were found to be biased to males, while all others were biased to females.

Identification of the pheromone

The hexane extract from the mites (0.28 g) was chromatographed using a SiO$_2$ column. Sex pheromone activity was recovered in two fractions (90:10 and 80:20 eluates) by bioassay (Fig. 4), both of which indicated statistically significant bioassay results ($p<0.05$) compared to the control. According to GC/MS analysis, the former fraction consisted of $\alpha$-acaridial only (a component of peak B) while the latter contained a mixture of $\alpha$- and $\beta$-acaridials (peak E). The inactive fractions consisted of the following compounds: tridecane (peak D) in the hexane fraction, neryl formate (a component of peak B) in the hexane-ether (97:3) fraction, and neral (peak A) in the (95:5) fraction. Possibly because of the minute amounts contained in the other hexane-ether fractions (99:1, 50:50 and 0:100), no detectable components were detected using GC analysis, including peaks C ($\gamma$-acaridial), that is not recovered in any fractions separated by an SiO$_2$ column as reported (Sakata and Kuwahara, 2001), and F.

The tapping scores of the two inactive fractions (the hexane eluate and the (97:3) fraction), and the control were $6.6 \pm 1.2$, $7.4 \pm 1.5$, and $5.5 \pm 1.0$, respectively, whereas those of the two active fractions (the 90:10 and 80:20 eluates) were $14.1 \pm 1.3$ and $13.4 \pm 1.4$, respectively (Fig. 4). The latter

![Fig. 3 Analyses of the peak B from females (1) and males (2) using single ion monitoring. a: Enlarged GC response of peak B, b: monitored by m/z 166 (M$^+$ ion of $\alpha$-acaridial), and c: monitored by m/z 136 (M$^+$ ion of neryl formate - HCOOH).](image-url)
scores were twice as large as that of the control. An identical sex pheromone activity was demonstrated using doses of 30 to 100 ng of synthetic $\alpha$-acaridial (Fig. 5), while $\beta$-acaridial produced no activity at the same doses (data not shown).

**Dose response**

![Graph showing dose response](image)

*Fig. 4* Pheromone activity of the SiO$_2$ column eluates. Statistical significance compared to the control at $p<0.05$ using the Kruskal-Wallis test followed by the Dunn's multiple-comparison tests is indicated as in *Fig. 1*.

At doses of 30 to 100 ng, $\alpha$-acaridial displayed the highest tapping scores and subsequent mounting behavior (significant at $p<0.01$ and $p<0.05$, respectively, compared to the control using the Kruskal-Wallis test followed by Dunn's multiple-comparison test). Both lower and higher doses gave low and sta-

![Graph showing dose response](image)

*Fig. 5* Sex pheromone activity of $\alpha$-acaridial. Statistical significance at $p<0.05$ level was calculated and indicated as in *Fig. 4*. 

—113—
tistically insignificant scores. The dose-response relationship was composed of a convex curve (Fig. 5).

**Pheromone content**

The hexane extract obtained from five females contained 151.3 ± 22.7 ng of α-acaridial, which corresponded to 30.4 ng per female, while the content of the male extract was determined as 40.2 ± 22.8 ng, 13.4 ng per male.

**Discussion**

Female mites and female extracts sexually aroused the test males, however, the test females did not indicate any particular behavior upon exposure to a male or male extract. These observations suggest the presence of a female sex pheromone. The active component of this pheromone was determined as α-acaridial, the active dose of which (30 to 100 ng) was almost identical to that found in the female mites. Such concentrations have also been observed in other mite sex pheromones (Leal et al., 1989c). This is the 11th identified female sex pheromone in Astigmata (Kuwahara, 1999, 2001; Tatami et al., 2001; Ryono et al., 2001; Hiraoka et al., 2002; Mizoguchi et al., 2003). There was no evidence indicative of a male sex pheromone, as with other acarid mites except two species belonging to the genus *Acarus* (Kuwahara, 2001).

α-Acaridial has been isolated from *Tyrophagus pteriosos* (Leal et al., 1989a), and is distributed in 20 of the 52 species examined from the following genera: *Tyrophagus, Histiodaster, Rhizoglyphus*, and *Sancassania* (= *Caloglyphus*) (Kuwahara, 1999). In these species, it is one of the components of the opisthonotal gland and shows potent antifungal activity against four species of fungi tested (Kuwahara et al., 1989). This study is the second to demonstrate the pheromone activity of α-acaridial; the first was determined in the bulb mite *Rhizoglyphus robini* (Mizoguchi et al., 2003). Patch tests also showed this compound to be a potent sensitizer of atopic dermatitis (Sakurai et al., 1997).

Female astigmatid mite sex pheromones are characterized by their puzzling distribution pattern. They are found at different species-specific ratios in males as well as females. The female/male ratio of the present species was calculated as 2.3. Similar values were noticed in the following 10 species whose pheromones have also been identified: *Sancassania* (= *Caloglyphus*) sp. MJ (2R, 3R-epoxy-neral; female/male ratio, 1.4/1; Mori et al., 1996), *S. polyphyllae* (β-acaridial; 3.4/1; Leal et al., 1989c), *S. rodriguezi* (undecane; 6.3/1; Mori et al., 1995), *Sancassania* (= *Caloglyphus*) HP (rose-furan; 8.4:1; Mori et al., 1998), *Acarus immobile* [2-hydroxy-6-methylbenzaldehyde (2,6-HMB); 2.86/3.78; Sato et al., 1993], *Auleuroglyphus ovatus* (2,6-HMB; 12.7/15.4; Kuwahara et al., 1992), *Cosmoglyphus Hughesii* (2,6-HMB; 2.1/1; Ryono et al., 2001). *Dermapthogoides farinae* (2,6-HMB; 8.4/1; Tatami et al., 2001), *R. robini* (α-acaridial; 2.4/1; Mizoguchi et al., 2003), and *Histiodaster* sp. (neral; 5.2/1; Hiraoka et al., 2002).

To rationalize this unusual distribution of what appear to be sex pheromones, an evolutionary trend from the initial state in which the sex pheromone is distributed in males and females at all stages (for example, in *S. polyphyllae* and the unidentified *Sancassania* sp. "MJ") to the derived state in which distribution becomes biased towards the females (for example, in *S. rodriguezi* and unidentified *Sancassania* sp. "HP"), has been postulated for four *Sancassania* species (Mori and Kuwahara, 2000; Kuwahara et al., 1998).

There is also another possible explanation for this puzzling distribution of sex pheromone components based on recent observations of *S. polyphyllae*. When a group of mites at all developmental stages
are transferred to a new culture medium and (or) place, they initially aggregate. The active principle mediating this temporary aggregation phenomenon has been identified as \( \beta \)-acaridial (Shimizu et al., 2001), which is also a sex pheromone component of this species. With \( S. \) elongata, at high doses neral functions as an alarm pheromone (Kuwahara et al., 2001), whereas at lower doses it elicits aggregate responses (Nishimura et al., 2002).

These observations suggest that dose and context are critically important in the interpretation of mite pheromonal signals. In the context of disturbance or the colonization of new habitats, it would be beneficial for both sexes and nymphs to produce chemical signals associated with alarm or aggregation. In the context of reproduction in an undisturbed and colonized habitat, the same chemical signal released by females might trigger mating behavior in males. Further clarification of these alternatives is required using examples from other mite species.

References


Nishimura et al.


α-Acarial: Female Sex Pheromone of Schwieba similis


Tatami, K., N. Mori, R. Nishida and Y. Kuwahara (2001)  