Pheromone study on acarid mites III Citral: isolation and identification from four species of acarid mites, and its possible role

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Abstract: The alarm pheromone was isolated from *Carpoglyphus lactis* through the bioassay against the mold mite, *Tyrophagus putrescentiae*, and the compound was identified to be citral, (E and Z)-3,7-dimethylocta-2,6-dienal. The occurrence of citral in three other related species, *Aleuroglyphus ovatus*, *Lardoglyphus konoi* and *Dermatophagoides farinae* were also chromatographically demonstrated.

INTRODUCTION

Neryl formate, (Z)-3,7-dimethylocta-2,6-dien-1-ol formate, was isolated from the mold mite, *Tyrophagus putrescentiae* (Schrank) and identified as an alarm pheromone (Kuwahara *et al.*, 1975, 1979). The presence of the alarm pheromone has hitherto not reported in mites. It is well known that alarm pheromones, especially of social insects, are the least specific of pheromones and the same active compounds are shared in several taxonomically related species (Blum, 1969). In order to investigate the species-specificity of the mite's alarm pheromone, the crude pheromone extracts of the following five species

of mites were collected; *Tyrophagus putrescentiae*, *Aleuroglyphus ovatus*, *Lardoglyphus konoi* and *Dermatophagoides farinae*, and bioassayed against *T. putrescentiae*. Some alarm pheromone activities were detected in hexane extract of each species. In the present work the isolation and identification of the active principle(s) from the four species of mites were made and its possible role as the alarm pheromone was discussed.

MATERIALS AND METHODS

Mite rearing: *A. ovatus*, fed on dry yeast (Matsumoto, 1965) and *T. putrescentiae* reared as reported (Kuwahara *et al.*, 1975) and *C. lactis* fed on the mixture of dry yeast and sugar (1:1, by weight) were kept at 25°C, 75% RH in rearing vials, which were confined each in a desiccator with satd. NaCl solution (Matsumoto, 1965). Meanwhile *L. konoi* kept at 87% RH in a desiccator with satd. KCl solution (Matsumoto, 1968), and *D. farinae* at 64% RH with satd. NH_4NO_3 solution (Waki and Matsumoto, 1973) were both fed on the mixture of dry fish powder and dry yeast (1:1, by weight).

All species of mites employed in the pre-

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sent study were maintained under above-mentioned condition successively for years at the Department of Parasitology, The Tokyo Women's Medical College. These mites were separated periodically from culture medium by the saturated saline flotation method (Matsumoto, 1970) and stored at -80°C before use.

Bioassay method: All bioassay were conducted as reported previously using *T. putrescentiae* (Kuwahara *et al.*, 1979), unless otherwise mentioned.

Instrumentation: Gas-liquid chromatography was carried out by Yanako Model 550F, using three kinds of stainless steel columns; 1) 0.3 cm in diameter, 75 cm in length, packed with 15% PEG-20M on Chromosorb W, 60–80 mesh, at 100°C , 30 ml/min He flow rate, 2) 150 cm in length, packed with 5% DEGS on Chromosorb W, 60–80 mesh, at 100°C , 17 ml/min, and 3) 75 cm in length, packed with Thermol Q for terpenoid analysis (Shimadzu Seisakusho Ltd., Kyoto), 80–100 mesh, at 70°C , 30 ml/min.

Mass spectrum was obtained by the combined gas chromatograph-mass spectrometer (Hitachi K-53-RMU-4) on glass column (3 mm id $\times$ 2 m in length) packed with 5% PEG-20M at 20 eV and 120°C .

RESULTS AND DISCUSSION

With hexane all species of mites (5–7 ml by volume each) were soaked for several days and extracted separately. Small portions of aliquot of each extract was subjected to gas-liquid chromatograph (PEG-20M at 100°C),

for surveying the presence of neryl formate, and also to bioassay.

Bioassay results indicated that extracts of four species of mites, *T. putrescentiae*, *C. lactis*, *D. farinae* and *L. konoii* showed the alarm pheromone activity against *T. putrescentiae*. Extracts of *A. ovatus* was found to be inactive at the tested concentration. Nevertheless the peak corresponding to neryl formate (tentatively named as peak 1) was detected in all species of mites as shown in Fig. 1. The height of peak 1 was measured in the chromatogram of each species (Table 1). It was noted that the bioassay results were not always parallel to the height of peak 1, which was accompanied with the

Fig. 1 Gas-liquid chromatograms of the crude extracts from all acarid mites employed (15% PEG-20 M, 75 cm, 100°C).

Table 1 GLC peak response of neryl formate from the crude extracts of mites and their alarm pheromone activity

	Extracted Mite	volume Mite and hexane	Peak height* corresponding to neryl formate	Alarm pheromone activity against <i>Tyrophagus</i>
<i>Tyrophagus putrescentiae</i>	7.1 ml	7.8 ml	0.4 cm	+
<i>Aleuroglyphus ovatus</i>	5.9	6.6	0.6	—**
<i>Lardoglyphus konoii</i>	6.3	6.9	2.3	+
<i>Carpoglyphus lactis</i>	5.0	6.0	10.6	+
<i>Dermatophagoides farinae</i>	5.8	6.8	4.0	+

* The same quantity of aliquot of the each extracts was subjected to GLC using a 15% PEG-20 M column at 100°C .

** When the concentrated extract was assayed, positive response was observed.

other peak (tentatively named as peak 2) except the case of *T. putrescentiae*. The peak 1 was noted to be smaller than the peak 2.

Each extract of mites was chromatographed on the silicic acid column (5 g, 1.2 cm in diameter, 11 cm in height), eluted with 50 ml each of pentane and a mixture of pentane and ether (20:1, by volume). After evaporation of the solvents, the resulted residue of both fractions from each species were filled up to 1 ml with hexane, and analyzed by GLC at 100°C using a PEG-20M column. All peaks originally present in each extract were recovered separately in both fractions. It was noted that the fractions eluted with the mixture of pentane and ether consisted of two peaks (peak 1 and peak 2) in all species of mites except *T. putrescentiae*, and that the alarm pheromone activity was exclusively found in this fraction of all species except *A. ovatus*. From the quantity of peak 1 and the bioassay result of *A. ovatus* it is unlikely that peak 1 consist of neryl formate. In fact, further detailed analyses using two other GLC systems (5% DEGS and Thermol Q) revealed that these two peaks were identical to citral, [(Z and E)-3,7-dimethylocta-2,6-dienal, or the mixture of neral and geranial], and neryl formate was different in their GLC retention times as shown in Table 2.

The active fraction of *C. lactis* was further purified with a silicic acid (20 g) column by eluting with benzene. Thus-purified and isolated active compounds were still a mix-

Table 2 Identification of non-hydrocarbon volatiles from four species of acarid mites except *T. putrescentiae*

	15% PEG-20 M	Thermol Q	5 % DEGS
Unknown peak 1	14.4 min	8.3 min	6.8 min
Unknown peak 2	18.8	10.8	8.5
Neral*	14.4	8.4	6.8
Geranial*	18.8	10.8	8.5
Neryl formate	14.4	11.8	5.8

* The mixture of neral and geranial is called as "citral."

Fig. 2 Mass spectra of the isolated alarm pheromones from *C. lactis* against *T. putrescentiae*.

ture of the two peaks (yield; about 100 µg in total, neral 40 % and geranial 60 %, active at 100 ppm).

Mass spectra of these compounds were measured. Peak 1 and 2 gave the same molecular ion at m/e 152 and the same base ion peak at m/e 41 as shown in Fig. 2. The fragmentation pattern of peak 1 was identical to that of neral, and of the other peak 2 to that of geranial (Masada, 1975).

The commercially available citral (neral 35 % and geranial 65 %) were bioassayed to give the alarm pheromone activity at 100 ppm in hexane. This pheromonal activity was around one hundredth of neryl formate. From this result, it was understandable that extracts of *L. konoi*, *C. lactis* and *D. farinae* showed some alarm pheromone activity to *T. putrescentiae*. Presence of citral in *A. ovatus* was also supported from the following bioassay. When the bioassay was carried out with a filter paper impregnated with the concentrated extracts of *A. ovatus*, the filter paper distinctly showed the activity.

From the above-mentioned results, it was concluded that citral was contained in the following four species of mites, *A. ovatus*, *L. konoi*, *C. lactis* and *D. farinae*. No trace of neryl formate was found in these species, although their taxonomical status was very

close to *T. putrescentiae*. It might be interesting to know the evolutionary aspects of the alarm pheromone of mites in relation to their taxonomy.

In the preliminary experiment, both of *C. lactis* and *L. kono*i demonstrated the strong alarm pheromone activity to citral and also to the squashed bodies of themselves or the filter papers impregnated with hexane extracts of themselves. This evidence supported that citral played a role of an alarm pheromone on at least these two species of mites. Possible role of citral on the other two species was not clear. However, from these facts that citral is anti-fungal substance (Cole *et al.*, 1975, Okamoto *et al.*, 1978), and that fungi never grow in a healthy culture of mites, the citral is supposed to function as one of the defence substances against fungi.

Detailed study on the role of citral present in mites and the biological activity against these species will be discussed elsewhere.

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摘 要

コナダニ類のフェロモン研究 III

4 種のコナダニ類からのシトラールの単離と同定およびその役割

ケナガコナダニ *Tyrophagus putrescentiae* の警報フェロモンは蟻酸ネリルである。一般的に警報フェロモンは種特異的でないとされている。そこで近縁の4種のコナダニ類(サトウダニ, *Carpoglyphus lactis*, コオノホシカダニ, *Lardoglyphus kono*i, ムギコナダニ *Aleuroglyphus ovatus*, コナヒョウヒダニ, *Dermatophagoides farinae*) 粗抽出物のケナガコナダニに対する活性を調べた。4種の粗抽出物(ムギコナダニは濃縮すると)は活性を示した。ガスクロマトグラフ法分析(GLC)の結果、4種の活性部は、ケナガコナダニの場合と異なり、2成分から成っていた。最も量の多いサトウダニ抽出物を精製し、活性物質(100 µg)を単離した。質量分析および GLC の結果から、シトラール(ネラール 40%, ケラニール 60% の混合物)と同定した。ケナガコナダニ以外のダニには蟻酸ネリルは存在しない。予備的な生物試験でサトウダニとコオノホシカダニはシトラールに強い活性を示し、これらのダニではシトラールを警報フェロモンと推定した。