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Chemical Ecology of Astigmatid Mites—XLV. (2*R*,3*R*)-Epoxyneral: Sex Pheromone of the Acarid Mite *Caloglyphus* sp. (Acarina: Acaridae)[1]

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Abstract—(2*R*,3*R*)-2,3-Epoxy-3,7-dimethyl-6-octenal [(2*R*,3*R*)-epoxyneral] was identified as the female sex pheromone from an acarid mite, *Caloglyphus* sp. (Astigmata:Acaridae), whose phoretic hypopi had been collected from the Cockchafer, *Melolontha japonica* (Coleoptera: Scarabaeidae). Sexual activity of the males was induced by exposure to a 0.1–1 ng dose of synthetic (2*R*,3*R*)-epoxyneral. The enantiomer of the pheromone, (2*S*, 3*S*)-epoxyneral, was inactive and its admixture did not inhibit the activity of the natural pheromone. Copyright © 1996 Elsevier Science Ltd

Introduction

The presence of a female-producing sex pheromone for sexual excitation of males and of a male-producing one for female attraction has been confirmed based on the biological observation of the acarid mite *Acarus siro* (Acaridae).[2] Compounds responsible for the activity of each sex pheromone identified consist of 2-hydroxy-6-methylbenzaldehyde for the female sex pheromone and a mixture of male-specific and nonspecific hydrocarbons for the male sex pheromone, respectively, using the species of the same genus *Acarus immobilis*.[3] The presence of female sex pheromones has also been confirmed in the following three astigmatid mites: *Caloglyphus polyphyllae*,[4] *Aleuroglyphus ovatus*[5] and *Caloglyphus rodriguezi*,[6] whose active principles were identified as β-acaridial [2(*E*)-(4-methyl-3-pentenylidene)butanedial], 2-hydroxy-6-methylbenzaldehyde and undecane, respectively. None of the corresponding male sex pheromones have been identified so far in these species. All of the female sex pheromones described above have been observed to attract conspecific males and to induce the males to copulate.

Although the active principle of the insect sex pheromone is mostly limited to one sex as in the case of lepidopteran species (Insecta), the female sex pheromone of astigmatid mites was detected not only in females but also in males. Furthermore, even nymphs were found to possess female sex pheromones in *A. immobilis*[3] and *C. polyphyllae*.† In such cases, it may be interesting to determine whether these astigmatid mites are able to discriminate conspecific females and nymphs from males. There are two examples of optically active pheromones in astigmatid mites. One is the alarm pheromone in *Tyrophagus similis* (Acaridae),[7] in which (*S*)(+)-isopiperitenone was confirmed to be the active principle, while the (*R*)-enantiomer was inactive. The other is the aggregation pheromone of *Lardoglyphus konoi* (Lardoglyphidae), in which (1*R*,3*R*,5*R*,7*R*)-1,3,5,7-tetramethyldecyl formate has been identified as the active principle, while admixture of the other 15 isomers, which is theoretically possible, prevented the pheromone activity from developing.[8]

We report here the identification of the optically active sex pheromone, which is produced by the *Caloglyphus* sp. females and functions as a male sexual excitant. The relationships between pheromonal activities and stereochemistry were studied in detail. Distribution of the female sex pheromone among sexes and developmental stages, and possible sexual recognition between males and females were examined.

Results

When the male mites were exposed to a piece of filter paper containing the hexane extract of female mites, they came close to it and stayed around the introduced filter paper in the bioassay method (1). Furthermore, copulation behavior was observed between males. The sex pheromonal activity was detected at the rate of 0.1 female equivalent.

The GLC profile of the extract from the females and purification procedure of the sex pheromone are shown in Figure 1. Peak 1 (t_R = 8.04 min) was found to correspond to epoxyneral, as stated later. Peak 2 (t_R = 12.02 min) corresponded to pentadecene based on GC–MS,

Key words: Sex pheromone, (2*R*,3*R*)-epoxyneral, *Caloglyphus* sp. Astigmata, mite.

†Protonymph of *C. polyphyllae* was detected to contain its sex pheromone as major component (not published).

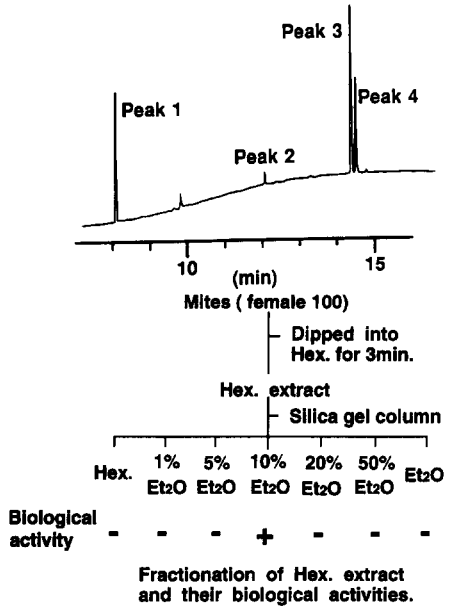

Figure 1. Typical gas liquid chromatogram of extract derived from females (100) and purification procedure for the sex pheromone of *Caloglyphus* sp.

which gave a M^+ ion at *m/z* 210 (21.3%) and the base ion at *m/z* 41 (100%) with the following diagnostic ions at *m/z* 182 (5.2%), 125 (10.5%), 97 (51.8%), 69 (77.2%), 57 (61.3%), 55 (87.9%) and 43 (90.2%). Peak 3 (t_R = 14.32 min) was associated with heptadecadiene, which implied the presence of a M^+ ion at *m/z* 236 (26.5%) and the base ion at *m/z* 67 (100%) with the following diagnostic ions at *m/z* 124 (14.7%), 123 (11.8%), 110 (32.5%), 109 (24.9%), 96 (46.7%), 95 (50.8%), 82 (58.9%), 81 (75.1%), 43 (42.0%) and 41 (73.4%). Peak 4 (t_R = 14.43 min) corresponded to heptadecene based on GC–MS, which indicated the presence of a M^+ ion at *m/z* 238 (24.0%) and the base ion at *m/z* 43 (100%) with the following diagnostic ions at *m/z* 126 (5.3%), 125 (12.6%), 112 (13.8%), 111 (32.1 %), 98 (19.1%), 97 (58.0%), 84 (35.0%), 83 (66.0%), 70 (47.5%), 69 (82.3%), 57 (70.4%), 55 (88.4%) and 41 (93.6%). Double bond positions and geometrical structure of these hydrocarbons were not elucidated.

The hexane extract from 100 females was chromatographed on a silica gel column and eluted stepwise using hexane:ether mixtures. The activity at 0.1 female equivalent was recovered by method (1) in a fraction eluted with 10% ether in hexane, which consisted of peak 1. All the other column eluates were completely inactive. GC–MS of peak 1 indicated the presence of the base ion at *m/z* 41 (100%) with the following diagnostic ions at *m/z* 153 (0.6%), 150 (1.5%), 135 (6.0%), 109 (40%) and 69 (73%), which were identical with those of (2*S*,3*S*)-epoxyneral, as previously reported.[9] Both (2*R*,3*R*)- and (2*S*,3*S*)-epoxynerals were synthesized to analyze the stereochemistry of the natural product.

(2*R*,3*R*)- and (2*S*,3*S*)-Epoxynerals (2,3-epoxy-3,7-dimethyl-6-octenal) were synthesized as follows[10]: Nerol was asymmetrically oxidized to both enantiomers of epoxynerols,[11] which further led to the formation of corresponding aldehydes. Since the optical purity of the epoxyalcohols was low (77% ee), the alcohols were reacted with 3,5-dinitrobenzoyl chloride to give 2,3-epoxy-3,7-dimethyl-6-octenyl 3,5-dinitrobenzoates. Both benzoates were repeatedly recrystallized to improve the optical purity.[12] After recrystallization, both purer esters were obtained from each mother liquid, which led to the formation of (2*R*,3*R*)-epoxyneral (97% ee) and (2*S*,3*S*)-epoxyneral (96% ee) after hydrolysis and subsequent oxidation.

By GLC on a capillary column with a chiral stationary phase, synthetic (2*R*,3*R*)- and (2*S*,3*S*)-epoxynerals each gave a separate peak at t_R = 17.50 and 17.27 min, respectively. The retention time of peak 1 was identical with that of (2*R*,3*R*)-epoxyneral, which was further confirmed by co-chromatography using the same chiral column.

Pheromone activity of synthetic epoxynerals

The dose–response relationship determined by method (2) is summarized in Figure 2 for the following three synthetic epoxynerals; (2*R*,3*R*)-epoxyneral with 97% ee, (2*S*,3*S*)-epoxyneral with 96% ee and (2*S*,3*S*)-epoxyneral with 77% ee. All the epoxynerals in each range have the same convex curves. (2*R*,3*R*)-Epoxyneral with 97% ee showed the highest activity at 0.1 and 1 ng doses (Kruskal–Wallis, both $p<0.01$), as well as (2*S*,3*S*)-epoxyneral with 77% ee at 1 and 10 ng doses (Kruskal–Wallis, $p<0.05$, 0.01) and (2*S*,3*S*)-epoxyneral with 96% ee at 10 and 100 ng doses (Kruskal–Wallis, $p<0.01$, 0.05). Optimum ranges of activities among these three epoxynerals were correlated with the content of the (2*R*,3*R*)-enantiomer and, therefore, it was concluded that the active compound was (2*R*,3*R*)-epoxyneral. No inhibitory activity was detected for the other enantiomers involved.

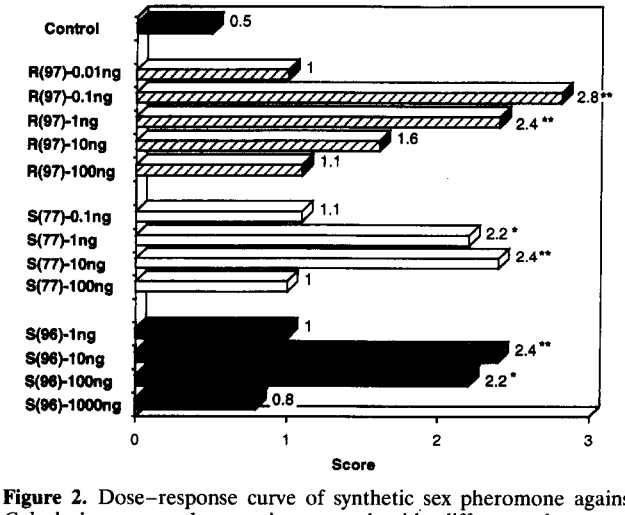

Figure 2. Dose–response curve of synthetic sex pheromone against *Caloglyphus* sp. males to tips treated with different pheromone concentrations. The results are significantly different from the control (**: $p<0.01$, *:$p<0.05$, Kruskal–Wallis test followed by Dann's multiple-comparison test).

Quantitative determination of the pheromone

The pheromone (2*R*,3*R*)-epoxyneral was identified as a major component of the hexane extract, not only from the female but also from the male and nymphal stages, as shown in Figure 3. Even the nymph possessed the pheromone as major component. The content was calculated to be 25.7±2.2 ng (mean±SEM, $n=30$) for the female, 18.0±2.0 ng (mean±SEM, $n=30$) for the male and 3.4±0.5 ng (mean±SEM, $n=10$) for the protonymph. Significant differences were observed in the content of the sex pheromone between sexes (Mann–Whitney U test, $p=0.013$, $n=30$), as shown in Figure 4.

Male extracts showed the same activity as the female extracts and no quantitative differences were observed between both sexes. Pheromonal activity of hexane extracts was compared between both sexes, using method (2). As summarized in Figure 5, no quantitative differences were detected between female and male extracts. The strongest activity was observed both at 0.1 mite equivalent. The dose–response curve of the activity showed a convex shape and doses at 0.01 and 1 mite equivalent indicated a lower activity than that of 0.1 mite equivalent.

When males or females were introduced into the assay chamber for the bioassay (3), the distribution of the time required for the initiation of mounting attempts is shown in Figure 6. No significant differences in the time required were detected between the sexes (Mann–Whitney U test, $p=0.197$, $n=25$), presumably because the female sex pheromone was present in both sexes.

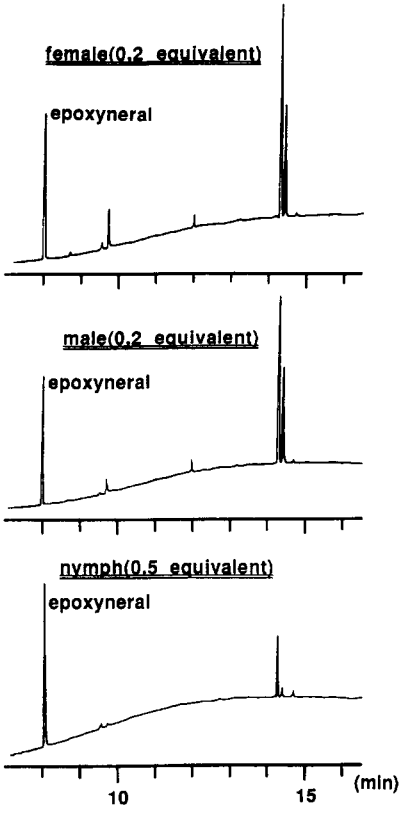

Figure 3. Typical gas liquid chromatograms of extracts from (1) 0.2 female equivalent, (2) 0.2 male equivalent and (3) 0.5 nymphal equivalent.

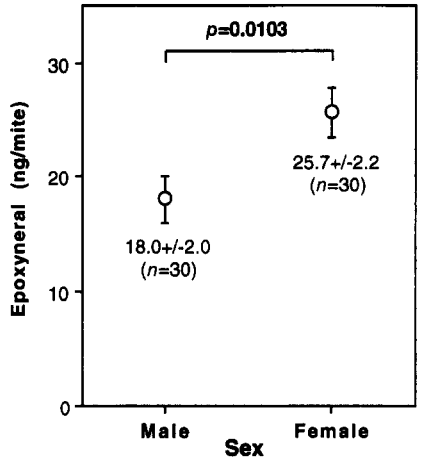

Figure 4. Comparison of the female sex pheromone contents (mean±SEM, $n=30$) of male and female. The Mann–Whitney paired comparison revealed a significantly different content of the sex pheromone between both sexes ($p=0.013$).

Discussion

(2*R*,3*R*)-Epoxyneral was detected as the sex pheromone of an unidentified *Caloglyphus* sp., whose enantiomer, (2*S*,3*S*)-epoxyneral, had been known to be a component of the opisthonotal secretion from *Tyrophagus perniciosus*.[9] Males of the present species were attracted to the pheromone source in the mini-Petri dish test, as reported for *Acarus immobilis*,[3] *C. polyphillae*[4] and *A. ovatus*.[5] The compound also stimulated males sexually to mount and gave a convex shape

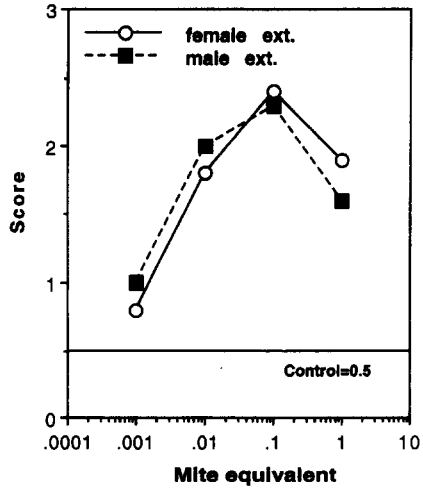

Figure 5. Response of *Caloglyphus* sp. males to tips treated with hexane extracts from males and females.

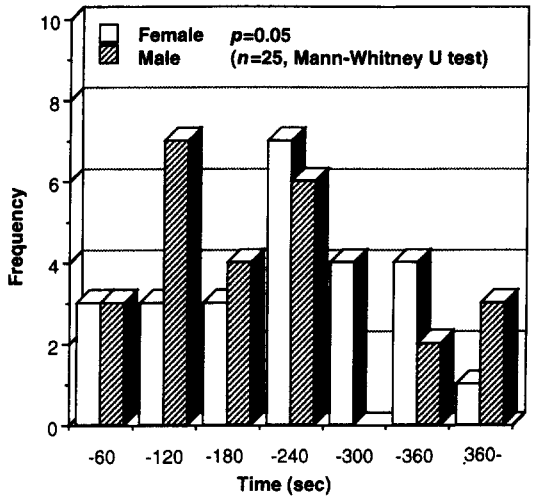

Figure 6. Distribution of frequency of time required for the initiation of mounting attempts in males or females introduced to conditioned males (Mann–Whitney U test, $p=0.197$, $n=25$).

for the dose–response curve, as reported for *C. rodriguezi*.[6]

Males responded to a small piece of filter paper to which hexane extracts derived not only from females but also from males were preliminary applied. No quantitative differences were detected in the pheromonal activity between extracts from females and males, as shown in Figure 5. Quantitative analyses of (2*R*,3*R*)-epoxyneral showed that the amount in males (18.0 ± 2.0 ng/mite) was slightly lower than that of females (25.7 ± 2.2 ng/mite). Although there were 1.4-fold (female/male) differences in the content of female sex pheromone in both sexes, the difference was not sufficient for males of this species to discriminate females from males. This assumption agreed with the fact that the time required for the initiation of mounting attempts was not significantly different between male and female introduction, as shown in Figure 6. On the other hand, it was observed that males of *C. rodriguezi* could discriminate conspecific females from males due to the 6.3-fold (female/male) difference in the sex pheromone content between sexes.[6] Comparison of the present *Caloglyphus* sp. with *C. rodriguezi*, showed that the males of the latter species had developed a more efficient strategy to detect females than the present species through the differences in the female sex pheromone content. A male of *Caloglyphus* sp. was occasionally observed to display mounting trials even in the presence of nymphs in the culture stock.

Furthermore, as far as the distribution of the female sex pheromone among developmental stages was concerned, female sex pheromones of astigmatid mites could be classified into the following two groups: Type 1, the pheromone was present in all the sexes and stages as a major component and Type 2, although the pheromone was distributed in both sexes, it was not a major component in the nymphal stages. *A. immobilis*[3], *C. polyphyllae*[†] and the present species belong to Type 1, while *A. ovatus*[5] and *C. rodriguezi*[6] to Type 2. However, the extreme situation, in which a female-specific compound would function as the sex pheromone as in the case of insect sex pheromones, has not been observed hitherto. Especially in the study of female sex pheromones in the present species and *C. rodriguezi*, it was interesting to note that in the case of *C. rodriguezi*, which could discriminate females from males due to the difference in the content of the sex pheromone, the pheromone was adult-specific, whereas in the present species, where the difference in the content of the pheromone was not sufficient to discriminate females from males, the sex pheromone was detected in the adults and protonymphs. Further studies should be carried out to categorize the female sex pheromone of the species in the genus *Caloglyphus*.

In insects relationships between stereochemistry and pheromonal activity have been divided into the following eight groups[13]. (a) Only one enantiomer is bioactive, and the antipode does not inhibit the action of the pheromone, (b) one enantiomer is bioactive and the antipode or diastereomers inhibit the action of the pheromone, (c) all the stereoisomers are bioactive, (d) even in the same genus different species use different enantiomers, (e) both enantiomers are required for bioactivity, (f) only one enantiomer is as active as the natural pheromone, but its activity can be enhanced by the addition of a less active stereoisomer, (g) one enantiomer is active on male insects, while the other is active on the female and (h) only the *meso*-isomer is active. The present study revealed that (2*R*,3*R*)-epoxyneral (97% ee) was active at 0.1–1 ng doses, while (2*S*,3*S*)-epoxyneral (96% ee) at 10–100 ng doses and (2*S*,3*S*)-epoxyneral (77% ee) at 1–10 ng doses. Taking account of the enantiomeric excess in the (2*S*,3*S*) samples, a small amount of the (2*R*,3*R*) isomer was contained in each sample. The amount of (2*R*,3*R*)-isomer in (2*S*,3*S*)-epoxyneral (77% ee) at 1–10 ng and (2*S*,3*S*)-epoxyneral (96% ee) at 10–100 ng was calculated to be 0.115–1.5 ng and 0.2–2 ng, respectively. These contents of the (2*R*,3*R*) isomer in each (2*S*,3*S*) sample agreed well with the active ranges of (2*R*,3*R*)-epoxyneral (97% ee). It was concluded that (2*R*,3*R*)-epoxyneral was the only active compound and the other enantiomers did not mask the activity of the active one, and that the pheromone belonged to group (a). The antipode, (2*S*,3*S*)-epoxyneral, is also a component of mites and its presence was confirmed in *T. perniciosus*, although the actual function has not been elucidated. The relationship of both epoxynerals in the two mites is typical of group (d). Optical activity has further been demonstrated in two other mite pheromones than these in the present study. The first is (*S*)(+)-isopiperitenone, which functions as the alarm pheromone of *T. similis* (Acaridae)[7]. (*S*)(+)-Isopiperitenone elicits the activity and the (*R*) isomer shows a weaker activity than the (*S*) enantiomer. The isomer does not appear to inhibit the activity of the pheromone and the pheromone also belongs to group (a).

[†]See footnote on p. 289.

The second is (1*R*,3*R*,5*R*,7*R*)-1,3,5,7-tetramethyldecyl formate identified as the aggregation pheromone of *L. konoi*. This compound is an example of (b). The enantiomer (1*S*,3*S*,5*S*,7*S*)-1,3,5,7-tetramethyldecyl formate does not display any pheromone activity and also does not act as the kairomone against the test mite *C. lactis*. Based on a bioassay of the experimental mixture of (*R*) and (*S*) isomer, the (*S*) isomer is known to function as an inhibitor of the pheromone activity, but not to mask the kairomone activity of the (*R*) isomer.[8] Even though further studies are required to substantiate the above-mentioned classification, three groups among the eight have been demonstrated in mite compounds.

Experimental

Mites

Unidentified species of the genus *Caloglyphus* was used, whose phoretic hypopi had been collected from the Cockchafer *M. japonica* (Coleoptera: Scarabaeidae) in Tokyo and Kyoto, Japan. This type of association between astigmatid mites and Insecta is very old and a large number of insect families act as phoretic hosts to Astigmata.[14] The strain was reared at 20 °C on dried yeast in a Petri dish (85 mm i.d., 20 mm ht), in which a sheet of moistened filter paper was placed. Two Petri dishes at a time were kept in a plastic bag with a zipper (240 × 170 × 0.04 mm) to preserve the humidity level.

Biological assays

Two methods (1) and (2) were applied using mini-Petri dishes (10 mm, i.d. 5 mm, ht). The bottom surface of the dish was covered with a small piece of damp filter paper, and small amounts of moistened dry yeast were placed on the paper. The chamber, after introduction of the test mites, was closed with a cover glass to prevent mites from escaping. Method (1) was applied to detect the active fraction in each purification step. Ten males (or females) as a group were placed in each chamber and conditioned overnight. A piece of filter paper (3 × 3 mm) impregnated with each separated fraction was placed in the center of the chamber. When males moved toward the filter paper along with mounting, the impregnated fraction was considered to be active. Method (2) was designed to quantify the sex pheromonal activity. To the same chamber as that conditioned and used for method (1) in which 10 males were placed, a small glass rod with the tip consisting of an egg-shaped ball (0.5 × 1 mm) was inserted, after a known amount of a candidate material was placed on the ball surface. Mite behavior was observed for 3 min after insertion of the glass rod. When at least one mite attempted to mount the tip within 60 s, the activity of the sample was scored as three points. Two points were given in the case of 60–120 s, and one point in the case of 120–180 s. When no males started to mount the tip within 180 s, the score was zero point. The assay was repeated 10–20 times and the total of the scores was processed using a Kruskal–Wallis test followed by a Dann's multiple-comparison test. All the bioassays were conducted at rt and under ambient humidity conditions. Hexane was used as the control treatment. Method (3) was designed to examine whether male mites could discriminate between male and female. The assay chamber was devised using a Pasteur pipette, as follows: both ends of a Pasteur pipette (7 o.d. × 140 mm, Iwaki–Glass Co. Ltd) were cut off, resulting in a funnel-like tube (40 mm in length, ca 15 mm from the funnel mouth to capillary end). Ten males were kept with moistened dry yeast in the chamber and both ends were blocked with cotton to prevent mites from escaping. After conditioning for 1 h and after almost all the mites had settled on the moistened yeast, one mite (female or male) was introduced to the chamber through the capillary ends with minimum disturbance. The time required for the initiation of mounting attempts was measured for conditioned males and introduced ones. The assay was repeated 25 times each for male or female introduction and a Mann–Whitney U test was applied for evaluating significant differences in the time between the two cases.

Extraction and purification

Following groups of mites were collected with a needle into a tube with a conical bottom (handmade, 8 mm o.d. × 30 mm ht) for quantitative studies, respectively; four females (without determining whether they were virgin or had mated), four males and five nymphs. Each group of mites was soaked for 3 min in hexane (20 μL for females and males, 10 μL for nymphs), containing tetradecane as an internal standard (10 ng /μL). Portions of 1 μL of each hexane extract, corresponding to either 0.2 female equivalent, 0.2 male equivalent or 0.5 nymph equivalent, were subjected to GLC.

Three females or males were separately dipped into 300 μL of hexane for 3 min and a 1 μL portion of the aliquot was applied for the assay method (2), which corresponded to a 0.01 female or 0.01 male equivalent. All the materials based on mite equivalent were prepared in the same way.

One hundred mites (females or males) were collected with a needle and dipped into hexane (100 μL) for 3 min. The hexane extract was separated on a conventional silica gel column (500 mg, Wako-gel C-200), by cluting each with 3 mL of hexane, ether:hexane mixture (1% ether in hexane, 5%, 10%, 20% and 50%) and ether, respectively. For all the column eluates method (1) was applied to locate the active fraction.

General chemical procedures

^{1}H NMR spectra were recorded with TMS as an internal standard at 90 MHz on a Jeol JNM-FX 90Q spectrometer. ^{13}C NMR spectra were recorded with $CDCl_3$ as an internal standard at δ 77.0, using the same spectrometer. IR spectra were recorded on a Shimadzu IR-400 spectrometer. Optical rotation was measured

on a Jasco DIP-370 polarimeter. GLC analyses were performed on a Hewlett Packard 5790A apparatus equipped with a HP-1 capillary column (Hewlett Packard). Chromatograms were processed using a Hewlett Packard Integrator model 3390A. GC–MS spectra were recorded on a Hitachi M-80, operated at 70 eV. Column chromatography was carried out on columns packed with Wakogel C-200 (Wako Pure Chemical Industry).

Enantiomeric resolution of synthetic epoxyneral was achieved on a capillary column with chiral stationary phase (CP-cyclodextrin b-2,3,6-M-19 column, 25 m × 0.25 mm, Chrompack), operated at 100 °C with split mode (50:1). Enantiomeric excess of epoxyalcohols and epoxybenzoates was determined by GLC of the epoxynerals obtained from the corresponding alcohols and benzoates.

(2*S*,3*R*)- and (2*R*,3*S*)-Epoxynerols. Titanium tetraisopropoxide (5.7 g, 20 mmol) was added to the stirred dry CH_2Cl_2 (200 mL) at −23 °C under N_2 flow. After (+)- diethyl tartrate (4.2 g, 20 mmol) was added to the preparation, the mixture was stirred for 5 min at −23 °C. Nerol (3.1 g, 20 mmol) in dry CH_2Cl_2 and 3.0 M anhydrous *tert*-butyl hydroperoxide in toluene was added to the mixture at −23 °C for 20 h. The reaction was quenched by the addition of 10% tartaric acid aq (50 mL) at −23 °C. After stirring for 30 min at −23 °C, the solution was warmed up at room temperature and stirred for 2 h. The organic layer was removed, washed with water, dried (Na_2SO_4) and evaporated on a rotary evaporator. The oil was diluted with ether (150 mL) and the solution was cooled in an ice-bath and then 1 N sodium hydroxide (60 mL) solution was added with stirring for 30 min. The ether layer was separated, washed with brine, dried (Na_2SO_4) and concentrated to an oil form. The residue was chromatographed on a silica gel column to give (2*S*,3*R*)-epoxynerol (2.7 g, 79.5% yield); $[\alpha]_D^{14}$ −14.4° (*c* 3.11, hexane); IR (film): ν 3400 (br, O—H), 1440 (s, C—H), 1350 (s, C—H), 1240 (w, C—O of epoxide), 1050 cm^{-1} (s, C—O); ^1H NMR ($CDCl_3$): δ 1.34 (3H, s), 1.62 (3H, br, s), 1.70 (3H, d, J=1.1 Hz), 1.6–2.1 (4H, m), 2.47 (1H, m), 2.96 (1H, dd, J=4.8, 6.6 Hz), 3.73 (2H, m), 5.09 (1H, m); ^{13}C NMR ($CDCl_3$): δ 17.6, 22.2, 24.2, 25.6, 33.2, 61.3, 61.5, 64.4, 123.4, 132.5; MS *m/z* 155 ($M^+ - CH_3$, 1.0%), 152 ($M^+ - H_2O$, 2.3%), 109 (80.6%), 69 (80.5%), 41 (100%). The enantiomeric excess was 77% ee

The (2*R*,3*S*) isomer was prepared in the same manner using (−)-diethyl tartrate; yield: 2.5 g (73.5%); $[\alpha]_D^{14}$ +14.5° (*c* 3.78, hexane). The IR, ^1H NMR, ^{13}C NMR and MS spectra were identical to those of (2*S*,3*R*)-epoxynerol. The enantiomeric excess was also 77% ee.

(2*S*,3*R*)- and (2*R*,3*S*)-Epoxy-3,7-dimethyl-6-octenyl 3,5-dinitrobenzoate. 3, 5-Dinitrobenzoyl chloride (2.5 g, 11 mmol) was added to a solution of (2*S*,3*R*)-epoxynerol (1.4 g, 7.6 mmol) in dry ether (30 mL) and dry pyridine (3 mL) with stirring and ice-cooling for 12 h. The mixture was quenched by the addition of ice-water and extracted with ether. The ether layer was washed with satd $CuSO_4$ aq, water and brine, dried (Na_2SO_4) and concentrated in vacuo. The preparation was repeatedly recrystallized from hexane:ether (5:1) to give crystalline (2*S*,3*R*)-epoxy-3,7-dimethyl-6-octenyl 3,5-dinitrobenzoate (562 mg, 18.3% yield) and oil (2*S*,3*R*)-epoxy-3,7-dimethyl-6-octenyl 3,5-dinitrobenzoate (1.6 g, 54% yield) from the mother liquor after chromatographic purification. Crystalline benzoate: $[\alpha]_D^{27}$ −14.0° (*c* 0.90, benzene); IR (film): ν 3080 (m, Ar—H), 1730 (s, C=O), 1540 (s, N—O), 1340 (s, N—O), 1260 (s, C—O), 1150 cm^{-1} (s, C—O); ^1H NMR ($CDCl_3$): δ 1.41 (3H, s), 1.64 (3H, br,s), 1.70 (3H, br,s), 1.6–2.3 (4H, m), 3.17 (1H, dd, J=3.7, 7.4 Hz), 4.36 (1H, dd, J=7.4, 12.1 Hz), 4.76 (1H, dd, J=3.7, 12.1 Hz), 5.13 (1H, m), 9.20 (3H, m); ^{13}C NMR ($CDCl_3$): δ 17.7, 22.0, 24.2, 25.7, 33.4, 60.5, 61.2, 65.8, 122.6, 123.0, 129.5, 132.8, 133.6, 148.9, 162.5, 198.8; MS *m/z* 364 (M^+, 1.0%), 346 ($M^+ - H_2O$, 5.2%), 239 (44.8%), 195 (100%), 149 (70.9%), 109 (77.1%). The enantiomeric excess was 26% ee. Oily benzoate: $[\alpha]_D^{30}$ −52.1° (*c* 1.14, benzene). The IR, ^1H NMR, ^{13}C NMR and MS spectra were identical with those of crystalline benzoate. The enantiomeric excess was 97% ee.

The (2*R*,3*S*) isomer was prepared from (2*R*,3*S*)-epoxynerol (77% ee) in the same manner. Crystalline benzoate: 374 mg (13% yield); $[\alpha]_D^{26}$ +13.4° (*c* 0.90, benzene). The enantiomeric excess was 24% ee; Oily benzoate: 1.4 g (50% yield); $[\alpha]_D^{28}$ +52.6° (*c* 1.06, benzene). The enantiomeric excess was 96% ee. The IR, ^1H NMR, ^{13}C NMR and MS spectra were identical with those shown above.

Hydrolysis of (2*R*,3*S*)-epoxy-3,7-dimethyl-6-octenyl 3,5-dinitrobenzoate. The (2*S*,3*R*)-epoxy-3,7-dimethyl-6-octenyl 3,5-dinitrobenzoate obtained from the mother liquor was treated with K_2CO_3 (60 mg) in MeOH (10 mL) and THF (10 mL) with stirring and ice-cooling for 30 min to give (2*S*,3*R*)-epoxynerol with a higher optical purity; yield: 172 mg (82%); $[\alpha]_D^{27}$ −18.1° (*c* 1.12, hexane). The enantiomeric excess was 97% ee. The IR, ^1H NMR, ^{13}C NMR and MS spectra were identical with those shown above.

The (2*R*,3*S*) isomer obtained from the mother liquor was prepared in the same manner; yield: 367 mg (84%); $[\alpha]_D^{27}$ +17.2° (*c* 0.97, hexane). The enantiomeric excess was 96% ee. The IR, ^1H NMR, ^{13}C NMR and MS spectra were identical with those shown above.

(2*R*,3*R*)- and (2*S*,3*S*)-Epoxyneral. After treatment of (2*S*,3*R*)-epoxynerol (97% ee, 1.2 mmol) with pyridinium dichromate (PDC, 1 g) in dry dichloromethane (10 mL) for 22 h, the residue was chromatographed on a silica gel column to give (2*R*,3*R*)-epoxyneral (125 mg, 62%) with 97% ee; $[\alpha]_D^{28}$ +127.9° (*c* 1.12, hexane); IR (film) ν 2950 cm^{-1} (m, C—H), 2900 (m, C—H), 2850 (m, C—H), 1720 (s,

C=O), 1450 (m, C—H), 1350 (m, C—H), 1240 cm^{-1} (w, C—O of epoxide); ^1H NMR ($CDCl_3$): δ 1.42 (3H, s), 1.59 (3H, br,s), 1.66 (3H, br,s), 1.7–2.3 (4H, m), 3.14 (1H, d, $J=5.3$ Hz), 5.04 (1H, m), 9.41 (1H, d, $J=5.3$ Hz); ^{13}C NMR ($CDCl_3$): δ 17.6, 22.2, 24.2, 25.6, 33.5, 64.5, 64.6, 122.5, 133.3, 198.8; MS *m/z* 153 ($M^+ - CH_3$, 0.9%), 150 ($M^+ - H_2O$, 1.5%), 109 (44%), 69 (78%), 41 (100%); Found: C, 71.10; H, 9.83. Calcd for $C_{10}H_{16}O_2$: C, 71.40; H, 9.59%. The enantiomeric excess was 97% ee.

The (2*S*,3*S*) isomer was prepared from (2*S*,3*R*)-epoxynerol (96% ee) in the same manner; yield : 123 mg (63%); $[\alpha]_D^{28} - 128.2°$ (*c* 0.89, hexane). The enantiomeric excess was 96% ee. The IR, ^1H NMR, ^{13}C NMR and MS spectra were identical with those of (2*R*,3*R*)-epoxyneral (97% ee).

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