Chemical Identification and Ethological Function of Soldier-Specific Secretion in Japanese Subterranean Termite *Reticulitermes speratus* (Rhinotermitidae)

Tuan T. Nguyen,1,† Kenji Kanaori,2 Masaru K. Hojo,1,‡ Tatsuro Kawada,1 Ryohei Yamaoka,1 and Toshiharu Akino1

1 Chemical Ecology Laboratory, Kyoto Institute of Technology, Matsugasaki, Kyoto 606-8585, Japan
2 Department of Biomolecular Engineering, Kyoto Institute of Technology, Matsugasaki, Kyoto 606-8585, Japan

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We identified the soldier-specific compounds in the Japanese subterranean termite, *Reticulitermes speratus*, to clarify their ethological roles. Silica gel column chromatography separated one major soldier-specific compound in the hexane fraction accounting for 70–80% of the total amount of the fraction, while cuticular hydrocarbons constituted the rest. We identified the compound as β-selinene by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Comparative GC analyses of the major exocrine glands detected the compound in the soldier’s frontal gland. Both soldiers and workers made aggregation to the hexane fraction, as well as to the crushed heads and head extract of the soldiers. They did not aggregate to cuticular hydrocarbons, making it likely that β-selinene was the aggregation pheromone in this species. The opportunistic predator of this termite, *Lasius japonicus*, was also attracted to the compounds. The ant workers, therefore, would use the termite aggregation pheromone as a kairomone for hunting them.

Key words: *Reticulitermes speratus*; β-selinene; *Lasius japonicus*; kairomone; aggregation pheromone

Termite colonies have different castes that include workers, soldiers and reproducitives each caste having different tasks in the nest. Termites often rely on pheromonal communication because of their blindness.1) The characteristic head morphology of the soldier termites includes mandibular and frontal glands that are necessary for mechanical and chemical defense.2–4) Soldier frontal glands are located in the head or extend over the abdomen in some genera of Rhinotermitidae.5,6) The chemical composition of the secretion from this gland has been studied in various termite species: it is a multi-component mixture which may contain different chemical compounds that include alkane, alkenes, nitroalkanes, vinyl ketones, ketoaldehydes, monoterpenes, sesquiterpenes, and organic acid compounds.6–16) Past research has shown that some individual elements of these compound groups act as irritants or effective toxicants to predators or competitors, i.e., the monoterpenes α-pinene, β-pinene, limonene, 3-carene,17–19) (E)-1-nitropentadec-1-ene and geranyl linalool.20–22)

In addition to its defensive role, the frontal gland secretion has also been reported for alarm communication in *Nasutitermes* (Termitidae), *Prorhinotermites* and *Reticulitermes* (Rhinotermitidae) termites. Such single monoterpenes as α-pinene, limonene, and carene served as alarm pheromones in *Nasutitermes*.5,23) The sesquiterpene compound of *Prorhinotermites canalifrons*, (E,E)-α-farnesene, has been mentioned as an alarm substance.23) The role of soldier-specific chemical secretion has been also studied in four European *Reticulitermes* termites (*R. santonensis*, *R. lucifugus*, *R. gracilis*, and *R. banyulensis*) which are very common in the United States and Asia. These species displayed alarm behavior when contacting individual compounds or mixtures of monoterpenes, sesquiterpenes, and diterpenes that are characterized as attractive components and stimulate such termite behavior as jittering and jerking, antennation of nestmates, mandible snapping or head-banging.26,27)

*Reticulitermes speratus* is the major termite species of Japan, China and Korea and often causes serious economic damage to older wooden buildings.28,29) There have been many studies on the influence of plant chemicals on this termite,28,30–33) but little is known about intra-specific chemical communication, except for the trail pheromone.34) *n*-Butyl *n*-butyrate and 2-methyl-1-butanol have recently been identified as the pheromone that suppresses the differentiation of new female neotenics.35) We focused this present study on the soldier secretion compounds, as *R. speratus* soldiers have characteristic head morphs that differ from those of the worker castes. We expected that the soldiers would possess caste-specific chemicals suitable for their communication tasks, as is known in the other *Reticulitermes* species. Aggregation responses were used as an indica-
tor to evaluate the attraction activity of the soldier secretion chemicals in their communication.

We also focused on the inter-specific interactions between *R. speratus* and the Japanese black garden ant, *Lasius japonicus*, because they often nest in the same rotting tree trunks. Although this ant species usually feeds on floral honey and honeydew from symbiotic aphids, it occasionally hunts small invertebrates, including termites. It was considered likely that the ants would tap the termite chemical secretions and use them to locate the termites. This hypothesis was tested in this study by evaluating the attraction of the termite chemicals to the ants.

**Materials and Methods**

Insects. *R. speratus* termites were collected in September 2008 and from April to June 2009 from wild colonies located in rotting logs at Matsugasaki in the northern suburbs of Kyoto in Japan. They were kept in plastic boxes (35 cm × 25.5 cm × 4.5 cm) with their nest wood retained as a food resource. These boxes were stored in an incubator at 27°C, and water was occasionally supplied to maintain humidity.

Two colonies of *L. japonicus*, comprising ca. 2000 workers with broods, were collected at Matsugasaki in September 2009. They were kept in a large plastic box (65 cm × 40 cm × 8.5 cm) at room temperature and supplied with an aqueous sugar solution (10% v/v) every day.

Extraction and separation. The termite chemicals were extracted to test bioactivity and identify the chemical structure. Hexane extracts of the termite head were prepared by separately immersing 100 heads of the workers and soldiers in 1 mL of hexane for 30 min. Each of these extracts was divided into two equal parts. Ten µL of the first part of each extract, or one termite head equivalent, was used in the bioassay. Each of the other half of the soldier extract was evaporated in vacuo, chromatographed on approximately 0.2 g of silica gel, and successively eluted with 2 mL each of hexane and diethyl ether. Each fraction was re-dissolved in 500 µL of hexane after evaporating the solvent, and 10 µL of each fraction was used for the bioassay.

The chemical secretions of the workers and soldiers were compared by separately immersing 10 heads of the workers and soldiers in 100 µL each of hexane for 30 min, and analyzing one-tenth individual equivalents of the respective extracts by gas chromatography (GC).

The frontal gland secretion was collected as described by Przkorski *et al.* to confirm the origin of the termite chemicals. The mandibular glands were also dissected from 10 soldiers and soaked in 100 µL hexane for 30 min. To quantify the soldier-specific chemicals, three heads and one whole body of the soldiers were separately immersed in 50 µL hexane solvent for ca. 30 min, and 1 µL of each extract (0.06 soldier head and 0.02 whole-body equivalents) was analyzed by GC. A 1-µg amount of n-pentadecane was also used to quantify the soldier-specific secretion compound by an analysis of the peak area.

The chemical structure was identified after soaking the whole bodies of 1000 soldier termites in 50 mL of hexane solvent (10 mL × 5 times) for 24 h. The hexane extract was then chromatographed on approximately 5 g of silica gel (230–400 mesh ASTM, Merck, Germany) packed in a glass column (30 cm length and 1 cm dia.), successively eluting with 50 mL each of hexane and diethyl ether. The hexane eluate, which contained a large amount of the soldier-specific compound, was kept in a deep freezer until needed for GC-MS and NMR analyses.

Bioassays.

**Investigating the intra-specific interaction.** To evaluate the attraction activity toward nestmate termites, a cylinder (4.5 cm dia. and 3.0 cm height) made from a transparent sheet was placed in the center of a plastic container (22 × 16 × 4 cm), and 3 small pieces of filter paper (2 cm dia. and 180 µm thickness, Whatman Int., England) were put around the outside of the cylinder at equal intervals. The distance between the edge of each filter paper piece and the cylinder was approximately 1.0 cm. Two of the filter paper pieces were respectively used for test 1 and test 2, while the third piece was used as a control sample. The test and control samples were crushed worker heads (test 1), crushed soldier heads (test 2) and plain paper (control) in series 1; the worker head extract (test 1), soldier head extract (test 2) and hexane (control) in series 2; the hexane fraction (test 1) and ether fraction (test 2) were separated from the extract of the soldier heads and hexane (control) in series 3. A group of 50 *R. speratus* workers and 10 soldiers were placed in the center of the cylinder. All of them had been transferred from the mother colonies and kept in small Petri dishes for at least 20 min and then in the experimental site for 10 min before the experiments to avoid any effects of translocation. The cylinder was then gently removed after the solvent had evaporated, and the number of termites present on each filter paper was counted every minute for 10 min. The experiment was repeated 10 times for series 1 and 2, and 15 times for series 3. New test and control samples, as well as groups of different individual termites were used for the replicates, and the position of the test samples was rotated in a clockwise direction. All bioassays were conducted between 10:00 and 16:00 under red light.

**Investigating the inter-specific interaction.** Experiments were conducted in a rectangular plastic box (9 × 7 × 2 cm) to evaluate the ant attraction to the termite chemicals, half of box being provided as an ant nest and covered with red plastic to keep the area inside of it dark. Samples of each series 1–3 that were tested individually were used to treat small pieces of filter paper placed at the end of the box opposite to the nest, approximately 1.5 cm away. Each trial was conducted with a group of 10 *L. japonicus* workers that had been moved from the mother colony to the box 24 h before the trial. The experiments were started when all the ants were in the nest. The number of ants that approached the test samples was counted every 10 s for 3 min. The experiment was repeated 30 times on the test samples of series 1, and 15 times on the test samples of series 2 and 3. New test and control samples as well as groups of different individual ants were used for the replicates.

**Chemical analyses.** Gas chromatography (GC) was conducted on a Shimadzu GC-14A instrument equipped with a flame-ionization detector (FID), and either a DB1-HT apolary capillary column (15 m in length × 0.25 mm ID; 0.1 µm in film thickness) or a DB-Wax polar capillary column (30 m in length × 0.25 mm ID; 0.25 µm in film thickness). Helium was used as the carrier gas, and the column head pressure was set at 100 kg/cm². Both the injection port and detector were set at 300°C. The column oven temperature was set at 60°C for 5 min, programmed to 300°C at 10°C/min, and held at the final temperature for 10 min when the apolar column was used. In contrast, the injection port and detector were set at 200°C and the column oven was set at 60°C for 5 min, programmed to 220°C at 10°C/min, and held for 10 min at the final temperature for 10 min when the polar column was used. Data was stored and analyzed by a Shimadzu C-RE6A chromatopac integrator. Retention indices were calculated for the termite chemicals by injecting with authentic hydrocarbon mixtures from nC10 to nC20, as suggested by Kovats. 35

Gas chromatography coupled with mass spectrometry (GC-MS) was conducted on a Shimadzu QP-5000 instrument linked with a Shimadzu GC 17A. A DB1-HT apolary capillary column (30 m in length × 0.25 mm ID; 0.1 µm in film thickness, J&W Scientific, USA) was used for the analyses. Helium was used as the carrier gas, with a column head pressure of 100 kPa. The column oven temperature was set at 60°C for 10 min, increased by 20°C/1 min up to 300°C, and held there for 10 min. EI-mass spectra were obtained at 70 eV. The data was analyzed by a Fujitsu computer with Class-5000 software from Shimadzu. The number of unsaturated bonds of the termite chemicals was estimated by GC-MS analyses after the hexane fraction had been partially reduced with hydrazine, according to the methods of Yamaoka et al. 50,56

NMR spectra were acquired after dissolving approximately 3.0 mg of the major compound in the hexane fraction in 400 µL of a chloroform-d solution (CDCl₃). All the NMR experiments were conducted at 27°C with a Bruker ARX-500 spectrometer (500.13 MHz for ¹H and 125.6 MHz for ¹³C). Two-dimensional DQF-COSY, TOCSY, NOESY, ¹H-¹³C HMOC, and ¹H-¹³C HMBIC experiments were performed with standard XWINNMR software (Bruker). The signal due to the residual proton of CDCl₃ and its carbon
signal were used as internal standards ($\delta = 7.26$ ppm for $^1$H and $\delta = 77.0$ ppm for $^13$C).

The purity of the NMR sample was determined to be about 80% on the basis of the intensity of the $^{13}$C=:$^{13}$C proton resonances. MS and NMR spectral data were acquired for the major compound ($\beta$-selinene). EI-MS $m/z$: 204(2), 189(27), 175(8), 161(37), 147(50), 133(32), 121(42), 107(67), 93(100), 81(92), 67(82), 41(92).

$^1$H-NMR (CDCl$_3$): 0.73 (s, 3H, H-2a), 1.27 (m, 1H, H-4a), 1.29 (m, 3H, H-5a, H-6a and H-8a), 1.45 (m, 1H, H-4b), 1.50 (m, 1H, H-5b), 1.56 (m, 1H, H-6b), 1.61 (m, 2H, H-3a and H-3b), 1.75 (s, 3H, H-8a and H-8b), 1.82 (br d, 1H, H-11), 1.87 (br d, J = 11.7 Hz, 1H, H-9a), 1.97 (m, 1H, H-7), 2.01 (m, 1H, H-2a), 2.31 (br d, J = 13.1 Hz, 1H, H-2b), 4.44 (s, 1H, H-13a), 4.70 (s, 1H, H-10a), 4.72 (s, 1H, H-13b), 4.73 (s, 1H, H-10b).

$^{13}$C-NMR (CDCl$_3$): 16.3 (C-12), 21.0 (C-11), 23.3 (C-3), 26.8 (C-8), 29.4 (C-6), 31.9 (C-4a), 36.8 (C-2), 41.2 (C-5), 41.9 (C-4), 45.9 (C-7), 49.9 (C-8a), 105.3 (C-10), 108.1 (C-13), 150.8 (C-1), 151.0 (C-9).

Statistical analyses. The average numbers of worker and soldier termites that were present on the test samples every minute were separately calculated and used for analyses in the intra-specific assay. Data were analyzed by the Friedman test and subsequent Bonferroni-corrected Wilcoxon matched-pairs test with SPSS version 16 software (SAS Institute, USA).

The inter-specific assay was conducted on the average number of worker ants that were attracted to the test samples every 10 s. Data were analyzed by the Kruskal-Wallis test and subsequent corrected Wilcoxon matched-pairs test with SPSS version 16 software (SAS Institute, USA).

Results

Intra-specific interaction

The workers were attracted by the crushed soldier heads rather than by the crushed worker heads or control sample. There were significant differences between the number of workers attracted to the crushed soldier heads and the other two samples, but there was no significant difference between those attracted to the crushed worker heads and the control. Such significant differences were also apparent when the soldiers were tested (Table 1, $p < 0.05$).

The numbers of worker and soldier termites that aggregated to the soldier head extract were significantly larger than those of the termites that aggregated to the worker head extract and the control. There was no significant difference between the numbers of worker and soldier termites that aggregated to the worker head extract and the control (Table 1, $p > 0.05$).

The hexane fraction of the soldier head extract was much more attractive to both the soldiers and workers than the ether fraction or solvent. There was no significant differences in attractant activity between the ether fraction and the solvent, although the ether fraction showed a little attractiveness to the workers (Table 1, $p > 0.05$).

Inter-specific interaction

We noted that most of the ants gathered in their nest when observing their behavior toward only the crushed worker heads or plain filter paper for 3 min. A few sometimes walked slowly and calmly to the untreated or paper treated with crushed worker heads. There was no significant difference in the number of worker-ant approaches to filter paper treated with crushed worker heads and the untreated paper (Table 2, $p > 0.05$). In contrast, the ants’ responses were much more pronounced when the crushed soldier heads were applied. A general state of alertness was observed, this starting from the detection of chemical odor by the ants, who would then show excited behavior such as rapid approach to the termite chemical source, and displays of mandibles and jerking of the body. The average number of ants attracted to the crushed soldier heads differed significantly from those to the plain filter paper and crushed worker heads (Table 2, $p < 0.05$).

Unlike the worker head extract and control solvent, the soldier head extract induced strong attraction behavior by Lasius. Statistically significant differences were confirmed for the attractiveness of the soldier head extract, worker head extract and control solvent (Table 2, $p < 0.05$).

The hexane fraction of the soldier head extract had attractiveness similar to that of the head extract, and was higher than that of either the ether fraction or control. The average number of ants attracted to the fractions differed significantly (Table 2, $p < 0.05$).

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Number of termites attracted</th>
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<tbody>
<tr>
<td></td>
<td>Workers</td>
</tr>
<tr>
<td><strong>Series 1 (N=10)</strong></td>
<td></td>
</tr>
<tr>
<td>Filter paper (control)</td>
<td>1.2 (0.8 to 1.8)</td>
</tr>
<tr>
<td>Crushed worker head (test 1)</td>
<td>1.7 (0.8 to 3.2)</td>
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<tr>
<td>Crushed soldier head (test 2)</td>
<td>8.5 (7.7 to 12.9)</td>
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<tr>
<td><strong>Series 2 (N=10)</strong></td>
<td></td>
</tr>
<tr>
<td>Hexane (control)</td>
<td>0.8 (0.2 to 1.7)</td>
</tr>
<tr>
<td>Worker head extract (test 1)</td>
<td>1.6 (0.7 to 2.9)</td>
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<tr>
<td>Soldier head extract (test 2)</td>
<td>9.9 (7.1 to 14.8)</td>
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<tr>
<td><strong>Series 3 (N=15)</strong></td>
<td></td>
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<tr>
<td>Hexane (control)</td>
<td>1.8 (0.2 to 2.4)</td>
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<tr>
<td>Hexane fraction (test 1)</td>
<td>8.4 (6.0 to 14.1)</td>
</tr>
<tr>
<td>Ether fraction (test 2)</td>
<td>2.0 (0.2 to 2.0)</td>
</tr>
</tbody>
</table>

Table 1. Median, Lower (25%) and Upper (75%) Quartiles (in brackets) of Average Number of Termites Attracted to Test Samples

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Number of ants attracted</th>
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<tbody>
<tr>
<td></td>
<td>Workers</td>
</tr>
<tr>
<td><strong>Series 1 (N=30)</strong></td>
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<tr>
<td>Filter paper (control)</td>
<td>0.4 (0.0 to 0.7)</td>
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<tr>
<td>Crushed worker head (test 1)</td>
<td>0.5 (0.0 to 1.3)</td>
</tr>
<tr>
<td>Crushed soldier head (test 2)</td>
<td>1.9 (0.8 to 2.9)</td>
</tr>
<tr>
<td><strong>Series 2 (N=15)</strong></td>
<td></td>
</tr>
<tr>
<td>Hexane (control)</td>
<td>0.8 (0.3 to 1.4)</td>
</tr>
<tr>
<td>Worker head extract (test 1)</td>
<td>0.3 (0.0 to 1.8)</td>
</tr>
<tr>
<td>Soldier head extract (test 2)</td>
<td>2.8 (2.4 to 4.2)</td>
</tr>
<tr>
<td><strong>Series 3 (N=15)</strong></td>
<td></td>
</tr>
<tr>
<td>Hexane (control)</td>
<td>1.0 (0.3 to 1.7)</td>
</tr>
<tr>
<td>Hexane fraction (test 1)</td>
<td>2.8 (2.1 to 3.7)</td>
</tr>
<tr>
<td>Ether fraction (test 2)</td>
<td>1.3 (0.6 to 2.2)</td>
</tr>
</tbody>
</table>

Table 2. Median, Lower (25%) and Upper (75%) Quartiles (in brackets) of Average Number of Ants Attracted to Test Samples

Chemical analyses

GC analyses indicated that the cephalic or whole-body extracts of soldier termites and their hexane fraction contained a large amount of the major compound which had a retention time of ca. 12.3 min (Fig. 1). This constituent was absent in the worker termites, and was found in the frontal gland of the soldier termite but not in the mandibular gland.
The amount of this main compound found in individual *R. speratus* soldiers was estimated to be 4.07 ± 0.26 μg in a head (mean ± SE, N = 10), less than that found in the entire body (9.91 ± 1.18 μg, mean ± SE, N = 20). Its respective Kovats indices were 1370 and 1774 in apolar (DB1-HT) and polar (DB-WAX) columns.

The quantitatively major compound specific to the *R. speratus* soldier termite was eluted in the hydrocarbon fraction, and the molecular weight was estimated to be 204. Hydrazine partial reduction showed two pi bonds in the molecular structure with a degree of unsaturation of four. This suggests that the molecule had two ring structures. The fragmentation patterns in the EI-MS data for this compound fit well with those of β-selinene. This compound was therefore presumed to be β-selinene (Fig. 2) and is supported by the NMR spectra of the compound, i.e., methyl signals at 0.73 ppm for H-12 and at 1.75 ppm for H-11. The upfield methyl group should be the bridgehead methyl in *trans*-fused decalin. Two terminal methane groups (H₂C=C) were observed in the range of 4.44–4.73 ppm; the signals at 4.44 and 4.72 ppm for H-13, and those at 4.70 and 4.73 ppm for H-10 were confirmed by the HMBC and NOESY cross-peaks to the methyl group of H-11 at 1.75 ppm. Compared with previous data for various sesquiterpenes with reported stereochemistry, the chemical shifts of the H-10 protons and C-7 carbon are indicators of the latter’s stereochemistry. Two tertiary carbon signals (45.9 and 49.9 ppm) in the 13C-DEPT spectra were respectively assigned to C-7 and C-8α by the two-dimensional NMR spectra. The H-7 proton (1.97 ppm) was unambiguously assigned by the connection to C-7 (45.9 ppm) in the HMOC spectrum. The smaller 1H chemical shifts of H-10 (4.70 and 4.73 ppm) and the larger 13C chemical shift of C-7 confirm that the compound was β-selinene, with an isopropenyl group that was equatorial. Moreover, the H-7 signal was very broad caused by large coupling constants, probably with the H6 and H8 axial protons, which also coincides with the axial H-7 and the equatorial isopropenyl group. The H-8α proton showed a broad doublet signal (∆ = 11.7 Hz), indicating that H-8α was also axial. All the 13C-NMR chemical shifts of β-selinene shown in this study coincide with those reported by Momin et al. 43)

**Discussion**

The sesquiterpenic compound, β-selinene, which was identified by GC, GC-MS and NMR data, is a known component of celery seeds (*Apium Graveolens L.*). It has also been mentioned as a minor sesquiterpenic compound in some *Reticulitermes* and *Nasutitermes* species.10,16,21) To the best of our knowledge, although such a termite sesquiterpene has been previously reported, almost the authors have not shown full data identifying its chemical structure due to its low or trace amount.10,16) The report of Momin et al. 44) has presented its 1H and 13C spectral data, but they did not show a precise 1H assignment nor any stereochemical evidence for some reasons. This is therefore the first report confirming the presence of β-selinene as a major component in *R. speratus* soldier and presenting a full and detailed description of its chemical structure.

The glandular source of β-selinene is the frontal gland, as it was found in both the head part and whole body of the soldier termite. It is well known that the soldier frontal gland reservoir is often located in the head capsules but also often extends into the prothorax and abdomen in some *Reticulitermes* species.27,45) And that sesquiterpenic compounds have often reported as major components in the frontal gland secretion of *Reticulitermes* species.4,12,20) Approximately 4.07 μg of β-selinene in the head part and 9.91 μg in the whole body per soldier were confirmed. Its amount in the *R. speratus* soldier is relatively high and predominant in comparison with that in European and North America *Reticulitermes*.12,16,46) The reason for this could be the different geographical zones in which termites live.

The soldier-specific compounds, including β-selinene, have been reported as chemical signals inducing attraction and alarm in European *Reticulitermes* termites.26,27) Our results indicate that they also triggered the attraction of termite workers and soldiers of *R. speratus*, and when being disturbed, they were expelled from the soldier termites, this being confirmed by using the monontrap technique. We therefore presume that the soldier caste of *R. speratus*, having a large amount of β-selinene in their heads and attracting nestmates, might be to serve as an aggregation pheromone. For example, if a soldier was attacked or killed by enemies when defending the colony, the chemicals released might be signals to inform (alarm) the nestmate workers and soldiers to aggregate together against the enemies. Another possibility is that these soldiers might use the aggregation pheromone to suppress the differentiation from workers to soldiers. Watanabe et al. have reported that the presence of *R. speratus* soldiers inhibited the formation of soldiers from workers by decreasing the JH titer in the workers.27) However, the regulatory factors for soldier caste differentiation are unknown. Okot-Kotber et al. have showed that the mixture of an *R. flavipes* soldier head extract in

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**Fig. 1.** Gas Chromatogram of Compounds in the Hexane Fraction of the Soldier Termite Extract of *R. speratus*. Major compound A accounted for 70–80% of the fraction.

**Fig. 2.** Chemical Structure of β-Selinene.
dichloromethane and synthetic JH analogs reduced soldier formation.\textsuperscript{40} Based on this, we think that the presence of \( \beta \)-selinene in \emph{R. speratus} soldiers might have a strong affect on the formation of soldiers, although this notion needs to be confirmed in future studies.

Although we expected that the termite soldier-specific compounds might have repellency against predatory ants, they in fact attracted \emph{Lasius} ant workers. This ant species often sympatrically nests with the termites in wood trunks, and we observed them to quickly come and hunt the termites when the termite nest was seriously damaged in our preliminary study. Although \emph{Lasius} ants are not specialist predators of the termite, they presumably hunt termites as one of their food sources. We presume that the ants have acquired an ability to detect the termite soldier-specific compounds that include \( \beta \)-selinene, and might use the pheromone as a kairomonal signal for predation. A recent study has revealed that the predatory ponerine ant, \emph{Pachycondyla analis}, responded to the mixtures of volatile hydrocarbons and esters from the termite worker of the \emph{Odontotermes} sp. species.\textsuperscript{49} Chemical signals are therefore highly significant in subterranean intra- and inter-specific interaction.

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\section*{References}

\begin{enumerate}
\item Eisner T, Kriston I, and Aneshansley D, \textit{Behav. Ecol. Sociobiol.}, 1, 83–125 (1976).
\item Watanabe D, Gotoh H, Miura T, and Maekawa K, \textit{J. Insect Physiol.}, in press.
\item Yusuf AA, Dissertation, University of Pretoria (2010).
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