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Determination of the absolute configuration of the male aggregation pheromone, 2-methyl-6-(4′-methylenebicyclo[3.1.0]-hexyl)hept-2-en-1-ol, of the stink bug *Erysarcoris lewisi* (Distant) as 2*Z*,6*R*,1′*S*,5′*S* by its synthesis

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Abstract—Lipase-catalyzed asymmetric acetylation of a mixture of (6*R*,1′*S*,4′*S*,5′*R*)- and (6*R*,1′*R*,4′*R*,5′*S*)-7′-norsesquisabinen-4′-ol (**3**) afforded a separable mixture of the recovered former and the acetate of the latter. The recovered alcohol was oxidized to (6*R*,1′*S*,5′*R*)-sesquisabina ketone (**2**), whose absolute configuration could be assigned by its CD comparison with (1*R*,5*S*)-sabina ketone (**4**). Conversion of (6*R*,1′*S*,5′*R*)-sesquisabina ketone (**2**) to the bioactive pheromone revealed the stereostructure of the male aggregation pheromone of the stink bug *Erysarcoris lewisi* (Distant) to be (2*Z*,6*R*,1′*S*,5′*S*)-2-methyl-6-(4′-methylenebicyclo[3.1.0]hexyl)hept-2-en-1-ol (sesquisabinen-1-ol, **1**).

Pecky rice (rice grain damaged by insects) is a serious economic problem in Japanese rice production.[1] A stink bug, *Erysarcoris lewisi* (Distant) (Heteroptera: Pentatomidae), is known as one of the major species of rice bugs that causes pecky rice in northern Japan.[2] Takita therefore examined the possibility of using its pheromone for the purpose of monitoring its population.[3,4] Takita then proposed the structure of the male-produced aggregation pheromone of *E. lewisi* as (*E*)-2-methyl-6-(4′-methylenebicyclo[3.1.0]hexyl)hept-2-en-1-ol [**1**, (*E*)-sesquisabinen-1-ol, Scheme 1].[5] Isolation and characterization of the pheromone have recently been reported in detail.[6] A crude extract containing 60,200 male equivalents (a male equivalent = volatiles obtained from a single male within a day) was used for the characterization.[6]

However, Mori's synthetic work in early 2007, in which citronellal was employed as the starting material, revealed the male pheromone of *E. lewisi* to be (2*Z*,6*R*)-2-methyl-6-(4′-methylenebicyclo[3.1.0]hexyl)hept-2-en-1-ol (**1**).[7] This Letter describes the determination of the absolute configuration of the pheromone as (2*Z*,6*R*,1′*S*,5′*S*)-**1**. The key to this result was reduction followed by lipase-catalyzed asymmetric acetylation of a diastereomeric mixture of sesquisabina ketone (6*R*,1′*RS*,5′*SR*)-**2** to give recovered and intact (6*R*,1′*S*,4′*S*,5′*R*)-**3** and acetylated (6*R*,1′*R*,4′*R*,5′*S*)-isomer. Oxidation of (6*R*,1′*S*,4′*S*,5′*R*)-**3** gave sesquisabina ketone (6*R*,1′*S*,5′*R*)-**2**, whose absolute configuration could be assigned by its CD comparison with (1*R*,5*S*)-sabina ketone (**4**).

The key diastereomeric mixture of sesquisabina ketone (6*R*,1′*RS*,5′*SR*)-**2** was prepared from (*R*)-citronellal (**5**,

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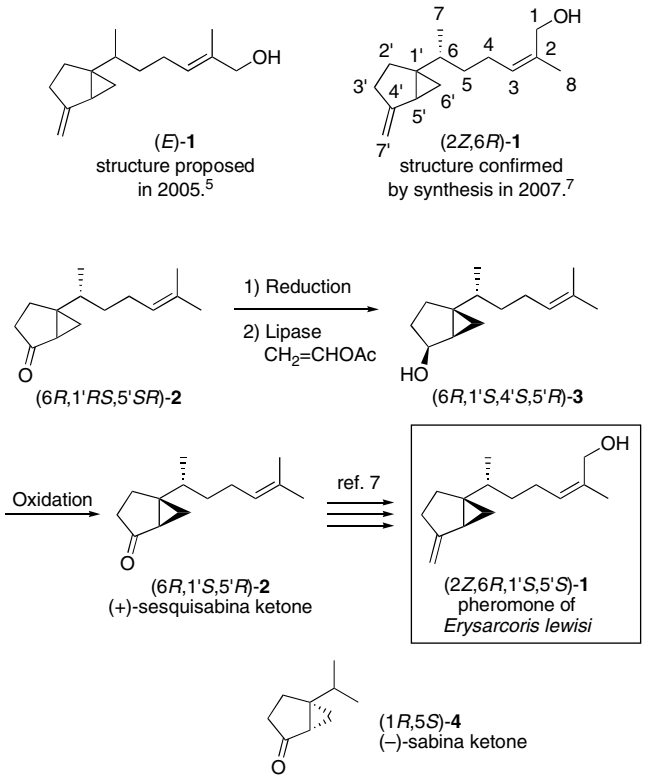
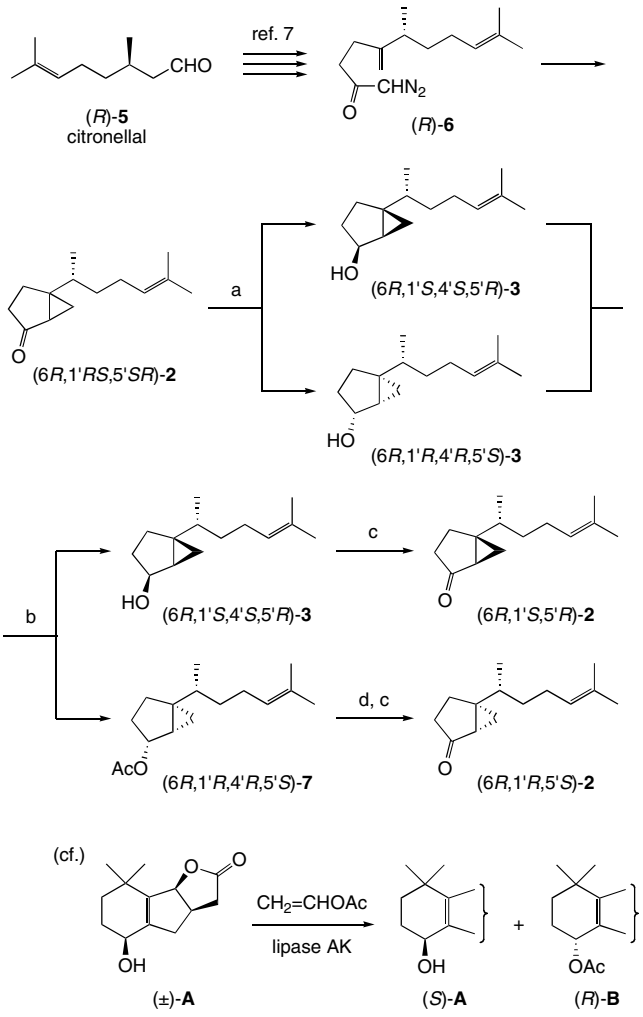


Scheme 1. Structure and synthetic plan of the pheromone of *Erysarcoris lewisi* (Distant), (2*Z*,6*R*,1′*S*,5′*S*)-**1**.



Scheme 2. Synthesis of (6*R*,1′*S*,5′*R*)- and (6*R*,1′*R*,5′*S*)-sesquisabina ketone (**2**). Reagents and conditions: (a) LiB(*sec*-Bu)$_3$H, THF, −60 °C to −20 °C, 5 h; then 3 M NaOH aq soln., 30% H_2O_2, 5 °C to rt, 2 h (94%); (b) lipase PS-D (Amano), CH_2=CHOAc, rt, 10 h, repeated three times [32% for (6*R*,1′*S*,4′*S*,5′*R*)-**3** and 11% for (6*R*,1′*R*,4′*R*,5′*S*)-**7**]; (c) TPAP, NMO, powdered MS 4A, CH_2Cl_2, rt, 1.5 h (quant.); (d) K_2CO_3, MeOH, rt (quant.).

Takasago, 97% ee) via diazoketone (*R*)-**6** as reported previously (Scheme 2).[7] Reduction of **2** with lithium tri(*sec*-butyl)borohydride (L-Selectride®) was followed by treatment with alkaline hydrogen peroxide to give a diastereomeric mixture of alcohols (6*R*,1′*S*,4′*S*,5′*R*)- and (6*R*,1′*R*,4′*R*,5′*S*)-**3** in 94% yield. The configuration of the newly generated hydroxy group of **3** was assigned as depicted in the formula on the basis of the ^{1}H NMR analysis of **3**, since the proton at C-4′ absorbed at $\delta = 4.52$ with ddd, $J_{H4',H5'} = 8.4$ Hz (dihedral angle $\Phi_{H4',H5'} = 36°$ as calculated by MM2).[8] When (±)-sabina ketone (**4**) was reduced with L-Selectride®, two alcohols were obtained, one with signals due to C*H*OH at $\delta = 4.50$ with ddd, $J_{H4',H5'} = 8.0$ Hz ($\Phi_{H4',H5'} = 33°$)[8] and the other with the corresponding signal due to C*H*OH at $\delta = 4.17$ with br d, $J_{H4',H5'} =$ ca. 0 Hz ($\Phi_{H4',H5'} = 83°$).[8] In the case of the reduction of **2**, the more sterically demanding side chain must have fixed the conformation of **2** in the course of the reduction to give *endo*-alcohols **3** selectively.

Asymmetric acetylation of the diastereomeric mixture of **3** was executed with vinyl acetate in the presence of lipase PS-D (Amano) I (lipase of *Burkholderia cepacia*). This enzyme was selected after preliminary screening with lipases PS, PS-C, PS-D, AK, and AH-S. Three repetitions of the asymmetric acetylation yielded the recovered (6*R*,1′*S*,4′*S*,5′*R*)-**3** {dr = 14:1 as revealed by its ^{1}H NMR analysis (400 MHz, $CDCl_3$) observing the intensity of the signals at $\delta = 0.25$ [due to (6*R*,1′*S*,4′*S*,5′*R*)-**3**] and $\delta = 0.34$ [due to (6*R*,1′*R*,4′*R*,5′*S*)-**3**]} in 32% yield and (6*R*,1′*R*,4′*R*,5′*S*)-**7** (dr = 18:1) in 11% yield, respectively. As to the absolute configuration of the two products of asymmetric acetylation, (6*R*,1′*S*,4′*S*,5′*R*)-configuration was tentatively assigned to the recovered alcohol **3**, while (6*R*,1′*R*,4′*R*,5′*S*)-configuration was given to acetate **7**, in analogy with the result of a similar asymmetric acetylation (±)-**A** → (*S*)-**A** + (*R*)-**B** in the case of our strigol synthesis.[9]

The recovered alcohol (6*R*,1′*S*,4′*S*,5′*R*)-**3** was then oxidized with tetra(*n*-propyl)ammonium perruthenate (TPAP) in the presence of *N*-methylmorpholine *N*-oxide (NMO) to give (6*R*,1′*S*,5′*R*)-**2**. Acetate **7** was deacetylated, and the resulting (6*R*,1′*R*,4′*R*,5′*S*)-**3** was oxidized to give (6*R*,1′*R*,5′*S*)-**2**. The tentatively assigned stereostructures of the diastereomers of **2** were confirmed at this stage by circular dichroism (CD) spectroscopy. The CD spectrum of (6*R*,1′*S*,5′*R*)-**2** in MeOH as $\Delta\varepsilon = +6.2$ (282.4 nm) and −16.3 (204.7 nm) was nearly antipodal to that [$\Delta\varepsilon = -7.9$ (282.6 nm) and +17.9

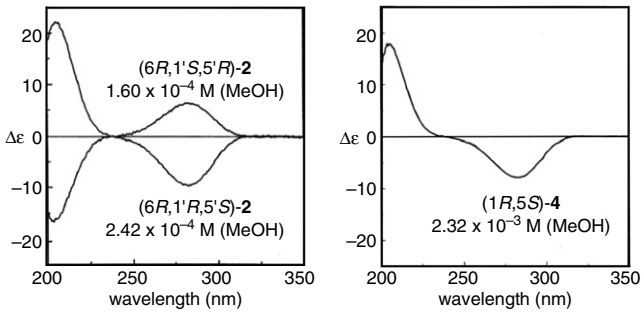
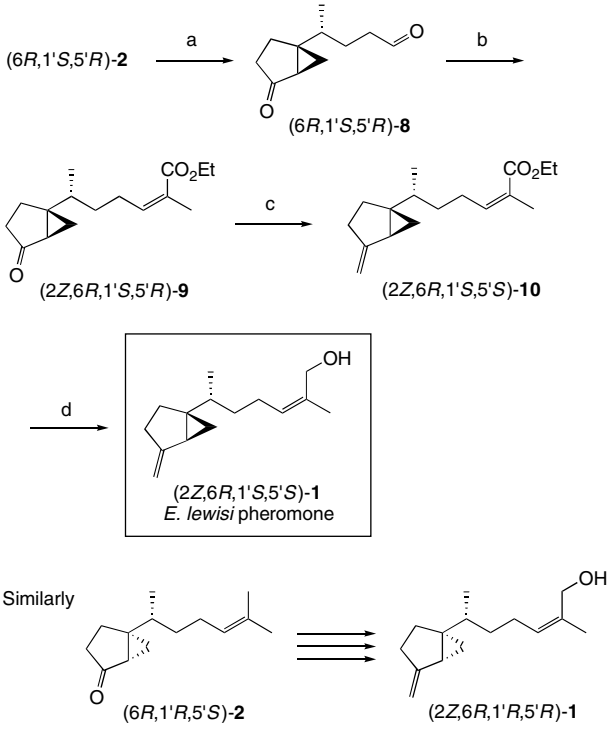


Figure 1. CD spectra of (6*R*,1′*S*,5′*R*)-**2**, (6*R*,1′*R*,5′*S*)-**2**, and (1*R*,5*S*)-**4**.

(205.5 nm)] of (1*R*,5*S*)-sabina ketone {**4**, $[\alpha]_D^{20}$ −41.2 (EtOH); 96.6% ee as estimated by GC on Chiramix}, while (6*R*,1′*R*,5′*S*)-**2** showed CD spectrum [$\Delta\varepsilon = -9.8$ (282.3 nm) and +22.3 (204.8 nm)] as almost the same as that of (1*R*,5*S*)-**4** (see Fig. 1).

The two diastereomers of ketone **2** were converted to the two diastereomers of (2*Z*,6*R*)-**1** according to the method reported previously (Scheme 3).[7] Oxidation of (6*R*,1′*S*,5′*R*)-**2** with osmium tetroxide and sodium periodate gave (6*R*,1′*S*,5′*R*)-**8** in 72% yield. Olefination of **8** with Ando's *Z*-selective Horner–Wadsworth–Emmons reagent, ethyl 2-(di-*o*-tolylphosphono)propanoate,[7,10] furnished (2*Z*,6*R*,1′*S*,5′*R*)-**9** in 85% yield. Methylenation of **9** under the conventional Wittig conditions afforded (2*Z*,6*R*,1′*S*,5′*S*)-**10** in 58% yield. Finally, treatment of ester **10** with diisobutylaluminum hydride gave (2*Z*,6*R*,1′*S*,5′*S*)-**1** (60.7 mg), $[\alpha]_D^{27}$ −37.9 (*c* 1.19, hexane), in 91% yield.[11] The overall yield of (2*Z*,6*R*,1′*S*,5′*S*)-**1** was 3.6% (15 steps) based on (*R*)-citronellal (**5**). Similarly, the diastereomeric ketone (6*R*,1′*R*,5′*S*)-**2** afforded (2*Z*,6*R*,1′*R*,5′*R*)-**1** (14.1 mg), $[\alpha]_D^{25}$ +59.8 (*c* 0.61, hexane).[12]



Scheme 3. Synthesis of the pheromone of *Erysarcoris lewisi* (Distant) (2*Z*,6*R*,1′*S*,5′*S*)-**1** and its diastereomer (2*Z*,6*R*,1′*R*,5′*R*)-**1**. Reagents and conditions: (a) OsO_4, $NaIO_4$, THF, H_2O, rt, 19 h (72%); (b) (*o*-$MeC_6H_4O)_2P(O)CHMeCO_2Et$, NaH, THF, −78 °C, 1 h, then 0 °C, 1 h (85%); (c) $(C_6H_5)_3PMeBr$, *n*-BuLi, THF, 0 °C (58%); (d) (*i*-$Bu)_2AlH$, toluene, CH_2Cl_2, −78 °C, 0.5 h, then 0 °C, 0.5 h (91%).

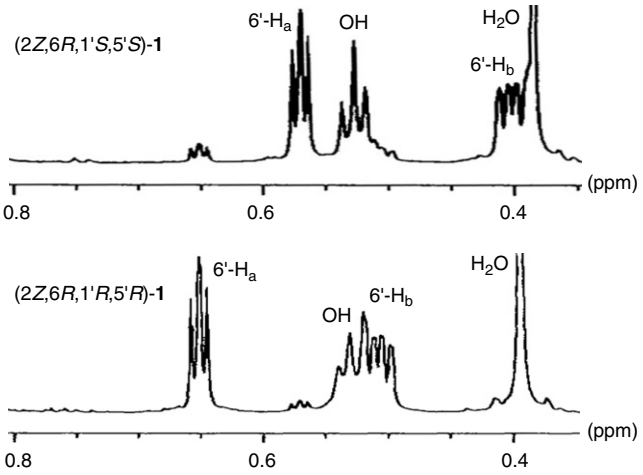


Figure 2. ^1H NMR spectra of (2*Z*,6*R*,1′*S*,5′*S*)-**1** (dr = 10.5:1) and (2*Z*,6*R*,1′*R*,5′*R*)-**1** (dr = 18:1) at 600 MHz (C_6D_6).

The ^1H NMR spectra at 600 MHz of these two diastereomers of **1** were slightly different, especially at the high field region due to cyclopropane protons, and (2*Z*,6*R*,1′*S*,5′*S*)-**1** showed the ^1H and ^{13}C NMR spectra identical to those of the natural pheromone (for ^1H NMR spectra, see Fig. 2). The identity of (2*Z*,6*R*,1′*S*,5′*S*)-**1** with the natural pheromone was also confirmed by GC analysis on CP-Chirasil-Dex CB column (25 m × 0.25 mm i.d.) at 60 °C (1 min) → 6 °C/min → 200 °C. The retention time of the natural pheromone was 22.13 min, while that of the synthetic (2*Z*,6*R*,1′*S*,5′*S*)-**1** was 22.12 min and that of (2*Z*,6*R*,1′*R*,5′*R*)-**1** was 22.47 min.

Finally, field bioassay in Yamagata definitely confirmed the attractancy of (2*Z*,6*R*,1′*S*,5′*S*)-**1** against *E. lewisi*.[13] As shown in Table 1, (2*Z*,6*R*,1′*S*,5′*S*)-**1** attracted as many stink bugs as the natural pheromone. Another experiment by Yoshimura indicates that (2*Z*,6*S*)-**1** is neither bioactive nor inhibitory.[15]

In conclusion, the stereostructure of the male-produced aggregation pheromone of the stink bug *E. lewisi* (Distant) was established as (2*Z*,6*R*,1′*S*,5′*S*)-2-methyl-6-(4′-methylenebicyclo[3.1.0]hexyl)hept-2-en-1-ol (**1**). The acetate, $[\alpha]_D^{24}$ −39.5 (*c* 0.2, $CHCl_3$), isolated from an African plant *Haplocarpha scaposa* (Harv.) and identified as the acetate of **1** by Bohlmann and Wallmeyer in 1982[16] turned out to be the acetate of the present pheromone, because the acetate prepared from (2*Z*,6*R*,1′*S*,5′*S*)-**1** showed $[\alpha]_D^{22}$ −41.8 (*c* 1.07, $CHCl_3$). The reason why the African plant produces the acetate of the pheromone of *E. lewisi* remains as a mystery.

Table 1. Number of individuals of *Erysarcoris lewisi* (Distant) attracted to the traps baited with the natural pheromone, synthetic isomers of the pheromone, and virgin males producing the pheromone

Attractant	Number of individuals (mean ± SE)[a]			
	Nymph	Adult female	Adult male	Total
Natural pheromone	11.3 ± 3.93	2.00 ± 1.53	0	13.3 ± 5.36
(2*Z*,6*R*,1′*S*,5′*S*)-**1**	12.0 ± 2.00	0.67 ± 0.33	0	12.7 ± 2.19
(2*Z*,6*R*,1′*R*,5′*R*)-**1**	2.67 ± 0.33	0	0	2.67 ± 0.33[b]
10 virgin males	13.3 ± 10.9	3.00 ± 3.00	0.33 ± 0.33	16.7 ± 14.2

[a] The traps were set for 3 days, and the experiment was repeated three times between September 3 and 14, 2007.

[b] The number of individuals attracted to (2*Z*,6*R*,1′*R*,5′*R*)-**1** was significantly less than that to (2*Z*,6*R*,1′*S*,5′*S*)-**1** (*t*-test, $P < 0.05$).[13] The data (X) were transformed to $(X + 0.5)^{1/2}$ before the statistical calculation.[14]

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11. Physical data of (2*Z*,6*R*,1′*S*,5′*S*)-**1**: n_D^{27} 1.5040; $[\alpha]_D^{27}$ −37.9 (*c* 1.19, hexane); ν_{max} (film): 3330 (s, OH), 1650 (s, C=C), 1450 (s), 1375 (m), 1010 (br s, C–O), 865 (s) cm^{-1}; δ_H (600 MHz, C_6D_6) 0.40 (1H, ddd, *J* 1.2, 4.8, 7.8), 0.53 (1H, t, *J* 5.4), 0.57 (1H, dd, *J* 3.6, 4.8), 0.85 (3H, d, *J* 6.6), 1.01 (1H, q, *J* 7.2), 1.19–1.26 (1H, m), 1.40–1.47 (2H, m), 1.56 (1H, dd, *J* 3.0, 8.4), 1.57–1.62 (1H, m), 1.75 (3H, d, *J* 1.2), 1.84–1.92 (1H, m), 1.92–2.00 (2H, m), 2.05 (1H, dd, *J* 9.0, 16), 3.93–3.98 (2H, m), 4.79 (1H, s), 5.00 (1H, s), 5.17 (1H, t, *J* 7.2); δ_C (150 MHz, C_6D_6) 16.2, 18.3, 21.3, 25.8, 26.5, 29.0, 31.7, 35.2, 36.4, 38.0, 61.4, 102.5, 128.1, 135.0, 153.8. These ^1H and ^{13}C NMR spectra were identical with those of the natural pheromone (see Ref. 6); HRMS calcd for $C_{15}H_{24}O$ (M^+) 220.1827; found, 220.1838.
12. Physical data of (2*Z*,6*R*,1′*R*,5′*R*)-**1**: n_D^{25} 1.5033; $[\alpha]_D^{25}$ +59.8 (*c* 0.61, hexane); ν_{max} (film): 3330 (s, OH), 1650 (s, C=C), 1450 (s), 1375 (m), 1010 (br s, C–O), 860 (s) cm^{-1}; δ_H (600 MHz, C_6D_6) 0.51 (1H, ddd, *J* 1.2, 6.0, 7.2), 0.53 (1H, t, *J* 5.4), 0.65 (1H, dd, *J* 3.6, 4.2), 0.87 (3H, d, *J* 6.6), 0.99 (1H, q, *J* 6.6), 1.12–1.28 (2H, m), 1.31–1.38 (1H, m), 1.43 (1H, dd, *J* 8.4, 11), 1.47 (1H, dd, *J* 3.0, 7.8), 1.65 (1H, dq, *J* 1.8, 10), 1.76 (3H, d, *J* 0.6), 1.87–1.94 (1H, m), 1.98 (1H, dt, *J* 7.8, 15.6), 2.06 (1H, dd, *J* 9.0, 16), 3.94 (2H, d, *J* 5.4), 4.79 (1H, s), 5.01 (1H, s), 5.19 (1H, t, *J* 7.2); δ_C (150 MHz, C_6D_6) 17.6, 18.4, 21.3, 26.0, 26.4, 29.2, 30.1, 35.8, 36.7, 38.1, 61.4, 102.4, 128.2, 134.9, 153.9; HRMS calcd for $C_{15}H_{24}O$ (M^+) 220.1827; found, 220.1825.
13. *Method of field bioassay*. Attraction activity was investigated by field tests. These tests were carried out in weeds at Yamagata General Agricultural Research Center. The crude natural pheromone was collected from the air in flasks containing males older than 10 days and was purified using HPLC equipped with silica gel column (Inertsil SIL-100A, 4.3 mm i.d. × 250 mm, GL Science Inc., Tokyo). Each of the isolated natural pheromone and synthetics was absorbed in cotton ropes (0.7 mm × 3 cm) and placed in screen baskets (5 cm diameter, 8 cm height). The baskets were set over water pan traps (45 cm i.d.), and their height was adjusted to be about 1 cm from the surface of the water. As controls, 10 adult males, which were over ten days old in a basket with a water supply bottle and brown rice, were used. The traps were separated by over 3.5 m. The number of insects drowned in the water, those remaining at the water's edge in the pan, and those on the basket was counted. All the insects were removed after counting. The positions of the traps were changed in each replication.
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