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Research Article

Deuterated analogues of 4,8-dimethyldecanal, the aggregation pheromone of *Tribolium castaneum*: synthesis and pheromonal activity

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Summary

To elucidate the deuterium isotope effect (DIE) in pheromonal activity and to investigate the biosynthetic pathway of 4,8-dimethyldecanal (4,8-DMD; **1**), the aggregation pheromone of the red flour beetle (*Tribolium castaneum*), deuterated analogues of 4,8-DMDs (**2**, **3**, **4**, and **5**), were synthesized and their pheromonal activities were tested using a two-hole pitfall olfactometer. Although no apparent DIE was observed in their pheromonal activities, 4,8-DMD-*1*-d_1 (**2**) was less attractive than other analogues, which suggested that the bond distance between the formyl group of **1** and its receptor was critical in pheromone recognition by *T. castaneum*.

Key Words: *Tribolium castaneum*; aggregation pheromone; 4,8-dimethyldecanal; deuterated 4,8-dimethyldecanals; deuterium isotope effect

Introduction

Deuterated compounds have been utilized for investigation of binding interactions or structure–odour relationships between molecules of biological interest and their receptors, the so-called deuterium isotope effect (DIE), and also used as mass-labeled precursors for biosynthetic studies of insect pheromones.[1–3] Some of the deuterated semiochemicals affected insect behavior, and those phenomena were interpreted by the spatial and/or binding affinity between molecules and their receptor surfaces due to the short bond length of C–D (1.1071 Å) versus that of C–H (1.1122 Å).[1,4,5]

4,8-Dimethyldecanal (4,8-DMD, **1**; Figure 1.) is the male-produced aggregation pheromone of the red flour beetle, *Tribolium castaneum*

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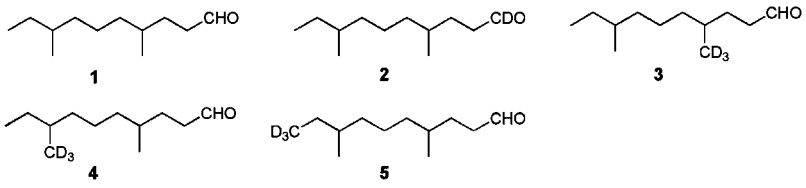


Figure 1. The aggregation pheromone (1) of *T. castaneum* and its deuterated analogues (2, 3, 4, and 5)

(Coleoptera; Tenebrionidae), identified by one of the authors.[6] *T. castaneum* is known as a serious stored-product pest distributed throughout the world. The natural pheromone is a (4*R*,8*R*)-optical isomer, and the racemic 4,8-DMD shows one hundredth of the attraction of the natural pheromone.[7] It is thought that 4,8-DMD has a four-group-binding site to its receptor or a pheromone-binding protein, that is, –CHO, 4-CH_3, 8-CH_3 and the terminal CH_3 group in the molecule, and these groups appear to be responsible for pheromone recognition by the insect. Therefore, deuterated analogues of 4,8-DMDs (**2**, **3**, **4**, and **5**; Figure 1) were synthesized to elucidate their DIEs. Our final objective is to elucidate the biosynthetic pathway of the pheromone, which is unclear to date. Deuterium labeled putative precursors and deuterated 4,8-DMDs are expected to be useful for this purpose.

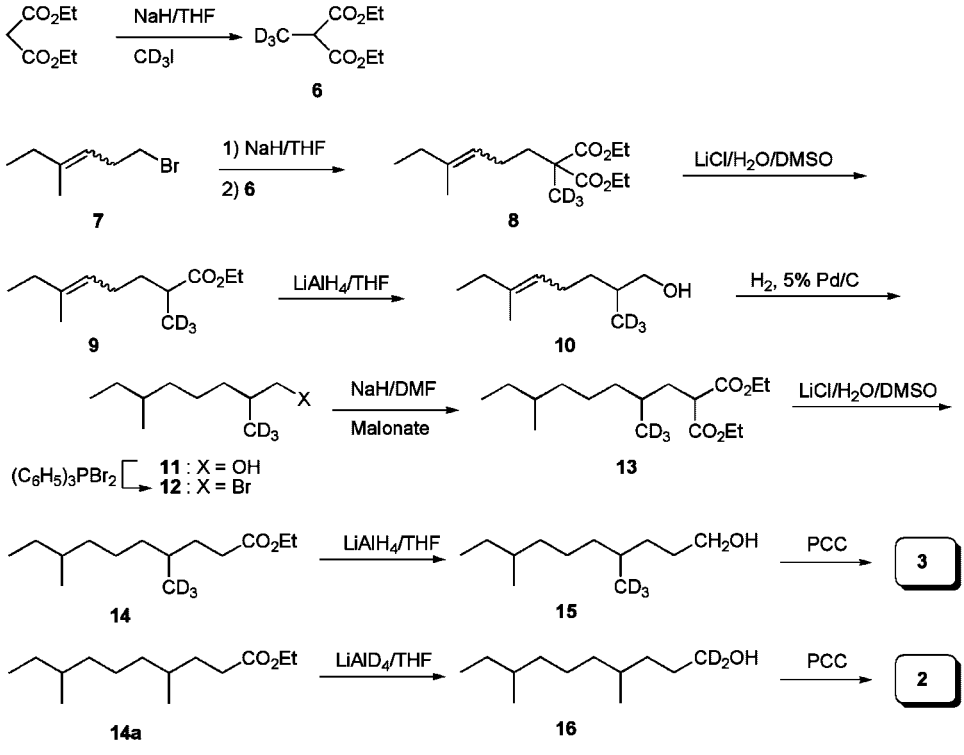
Here we report the detailed synthesis of the deuterated 4,8-DMDs and their DIEs in the pheromonal activities of *T. castaneum*.

Results and discussion

Synthesis

Two synthetic routes (A, B) were employed as described below. The racemic 4,8-DMDs (**1a** and **1b**), prepared by different procedures, are expected to show equal levels of activity.

Synthesis of 1a, 2, and 3 (Route A). Deuterium at the C-1 position of **2** and CD_3 at the C-4 position of **3** were introduced using $LiAlD_4$ and CD_3I, respectively, as outlined in Scheme 1. The key reaction is alkylation of a malonate followed by decarboxylation (malonic ester synthesis). Malonate **6**, prepared with CD_3I and diethyl malonate, was alkylated with **7** followed by decarboxylation to give the deuterated ester **9**, which was converted into **10** by $LiAlH_4$ reduction. Hydrogenation and subsequent bromination yielded the saturated bromide **12**. This compound was submitted to malonic ester synthesis again. The desired aldehyde **3** was obtained by PCC oxidation of **15**, prepared as described above *via* ester **14**. Synthesis of aldehyde **2** was accomplished by the same procedure using ester **14a**, prepared in the same



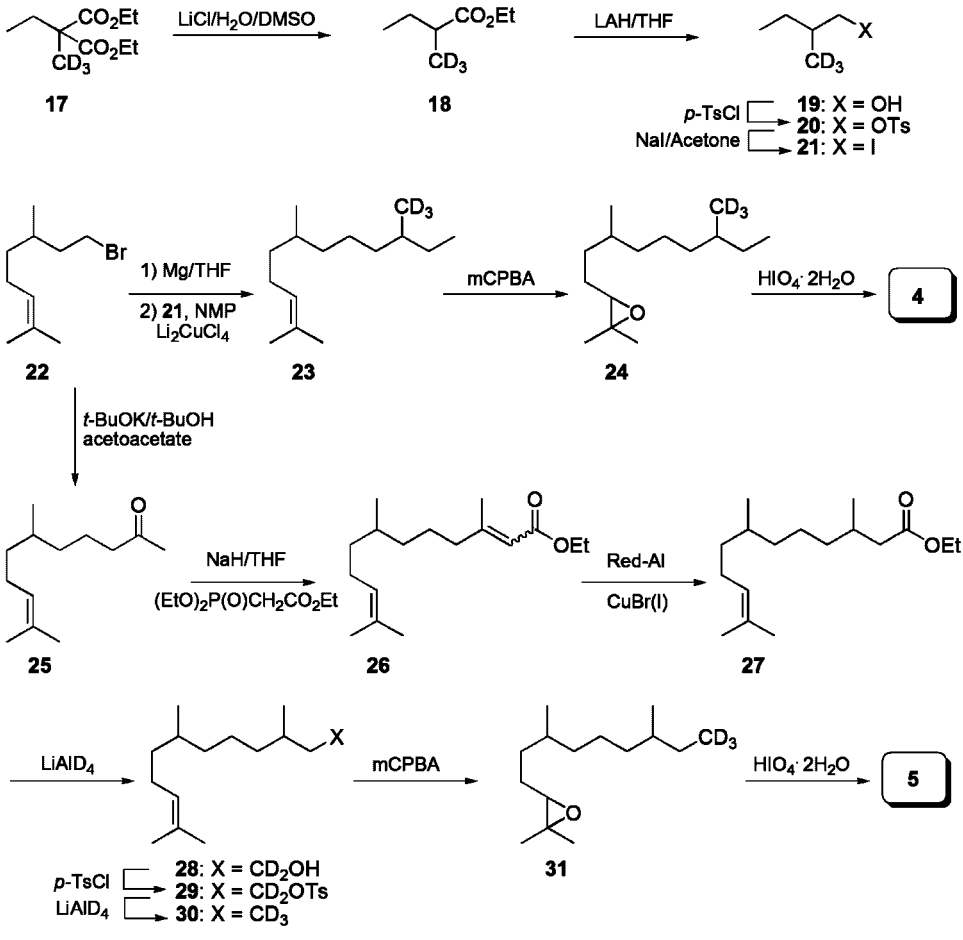
Scheme 1. Synthesis of deuterated 4,8-DMDs (2, 3; Route A)

manner as for **14**, using diethyl methylmalonate instead of **6** and $LiAlD_4$, while the unlabeled aldehyde **1a** was synthesized using **14a** and $LiAlH_4$.

Synthesis of 1b, 4, and 5 (Route B). CD_3 at the C-8 position of **4** and CD_3 at the C-10 position of **5** were introduced using CD_3I and $LiAlD_4$, respectively (Scheme 2). The main reactions are the Grignard coupling reaction in the presence of $LiCuCl_4$ and oxidative cleavage of a double bond to an aldehyde. The Grignard coupling of citronellyl bromide **22** and iodide **21**, prepared by malonic ester synthesis, gave alkene **23**, which was submitted to oxidative cleavage to yield the target aldehyde **4**.[8–10] The α,β-unsaturated ester **26** was obtained by the acetoacetic ester synthesis of **22** followed by the Wittig–Horner reaction. The desired aldehyde **5** was produced by oxidative cleavage of alkene **30** prepared by selective hydrogenation of **26** with Red-Al^{TM} and CuBr(I).

Bioassay

The bioassay results of 4,8-DMD and its deuterated analogues on *T. castaneum* are summarized in Table 1. All compounds (**1a**, **2**, and **3**) prepared



Scheme 2. Synthesis of deuterated 4,8-DMDs (4, 5; Route B)

Table 1. Attraction of 4,8-DMD and its analogues on *T. castaneum*

	Attractiveness (%, mean ± SD)[a]					
	Compounds (Route A)			Compounds (Route B)		
Dose (ng/disk)	**1a**	**2**	**3**	**1b**	**4**	**5**
1	−1.3 ± 22.0	−1.7 ± 18.5	−4.2 ± 30.2	9.2 ± 14.4	−7.1 ± 30.6	−4.2 ± 12.8
10	2.9 ± 20.1	15.0 ± 21.5	8.8 ± 12.3	23.8 ± 22.8*	15.0 ± 13.9*	33.3 ± 14.6**
100	38.8 ± 22.4**	20.0 ± 21.2**	30.0 ± 29.3**	43.3 ± 11.8**	28.3 ± 20.4**	30.8 ± 19.2**
1000	46.7 ± 22.5**	27.9 ± 13.0*	42.9 ± 16.9*	30.0 ± 15.0**	29.2 ± 22.2**	38.3 ± 14.9**

[a] Thirty 4-day-old beetles were used per replicate (8 replicates). *,**: Significant at $p<0.05$ and $p<0.01$, respectively, by *t*-test.

in Route A were active to the extent of more than 100 ng, while all compounds (**1b**, **4**, and **5**) prepared in Route B showed an attraction of more than 10 ng. Although the racemic 4,8-DMDs (**1a** and **1b**) prepared by different synthetic

routes (Routes A and B) were expected to show equal levels of activity, **1b** was ten times more active than **1a** (the lowest active limit: **1a** – 100 ng; **1b** – 10 ng). The pheromonal activity of 4,8-DMD was affected by the composition of optical isomers involved.[7] Since compounds **9** and **22** were optically inactive, the ratio of optical isomers at the C-4 position of 4,8-DMDs in each route would be same. Therefore, the different activity between the compounds prepared by Routes A and B would be the result of the different ratio of optical isomers at the C-8 position of 4,8-DMDs, which was supported by the fact that the 8:2 mixture of the (4*R*,8*R*)- and (4*R*,8*S*)-isomers showed maximum activity, although the natural pheromone was identified as the (4*R*,8*R*)-isomer.[7]

No apparent DIE was observed in all deuterated 4,8-DMDs, whereas **2** showed relatively less attraction than **1a** at doses of 100 and 1000 ng.

Pheromonal activity, in general, decreased or disappeared when the functional group and/or carbon chain length of a pheromone was converted. Compound **2** has a chain length equal to the original compound **1a** and maintains the characteristics of the functional group. However the binding distance to its receptor would change due to the shorter bond length of C–D versus that of C–H. Therefore, the lower attraction of **2** suggests that the bond distance between the CHO group of 4,8-DMD and its receptor was critical in pheromone recognition by *T. castaneum*.

Experimental

General analytical procedure

GC analyses were performed on a HP 5890 Series II gas chromatograph equipped with FID and a 007-5MS column [25 m × 0.25 mm (I.D) × 0.25 μm, Quardrex]. Mass spectra were obtained either on a HP MSD 5972 Series coupled with a HP 5890 Series II Plus equipped with a HP-5MS column [30 m × 0.25 mm (I.D) × 0.25 μm, J&W Scientific] or a JEOL MS Router MS-600 coupled with a HP 6890N equipped with a HP-1MS column [30 m × 0.25 mm (I.D) × 0.25 μm, J&W Scientific]. NMR spectra were recorded in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard using a Bruker Avance 500 spectrometer at 500 MHz for ^{1}H spectra and at 125 MHz for ^{13}C spectra. IR spectra were run on a JASCO IRA-1 (Japan Spectroscopic Co., Ltd.) spectrometer.

Reagents and solvents

Deuterium-labeled reagents (CD_3I and $LiAlD_4$) were purchased from Aldrich. Tetrahydrofuran (THF) was dried and distilled from sodium benzophenone ketyl prior to use. Diethyl ether was dried over sodium wire and used without

purification. Acetone was dried over K_2CO_3 and distilled prior to use. Column chromatography was performed using Wakogel® (C-200, Wako, Japan).

Synthesis

*Diethyl trideuteriomethylmalonate (**6**).* To a solution of NaH (2.21 g, 60.0 mmol; 65%) in THF (60 ml), diethyl malonate (8.00 g, 50.0 mmol) in THF (20 ml) was added at 0°C under N_2. After stirring for 30 min at room temperature, iodomethane-d_3 (3.73 ml, 60.0 mmol) in THF (20 ml) was added at 0°C. The solution was refluxed for 2 h, and then ice-cold water (200 ml) was added. The mixture was extracted with ether (3×60 ml). The combined extracts were washed with saturated Na_2SO_3, H_2O, brine, then dried and concentrated *in vacuo*. Distillation of the residue gave 8.22 g of **6**, containing ca. 30% diethyl ditrideuteriomethylmalonate as a by-product. This material was employed in the next step without further purification. bp: 95–96°C/21 mmHg. ^{1}H NMR: 4.20 (4H, q, $J = 7.1$), 3.41 (1H, s), 1.27 (6H, t, $J = 7.14$). ^{13}C NMR: 170.6 (C × 2), 61.7 (CH_2 × 2), 46.4 (CH), 14.4 (CH_3 × 2). GC-MS: 177 (M^+, 8.3), 150 (21.1), 132 (100), 105 (31.1), 77 (74.0), 60 (31.2), 59 (35.2). IR: 2950 (s), 2220 (w), 1730 (s), 1360 (m), 1230 (s), 1120 (s), 1020 (s).

*1-Bromo-4-methyl-3-hexene (**7**).* Compound **7** was prepared in the usual manner.[13] Yield 53.2% (3*E*:3*Z* = 3:1, calculated on GC). bp: 85–86°C/35 mmHg. ^{1}H NMR: 5.13 (1H, tq, $J = 7.1$,1.4), 3.34 (2H, t, $J = 7.4$), 2.57 (2H, dq, $J = 7.3$, 0.7), 2.01 (2H, q, $J = 7.4$), 1.63 (3H, s), 1.00 (3H, t, $J = 7.4$). ^{13}C NMR: 140.8 (C), 119.8 (CH), 33.4 (CH_2), 32.7 (CH_2), 32.1 (CH_2), 16.6 (CH_3), 13.0 (CH_3). GC-MS (A): 178 (M^+, 10.2), 176 (11.0), 97 (40.3), 83 (17.7), 55 (100). IR: 2950 (s), 1650 (w).

*Diethyl (4-methyl-3-hexenyl)(trideuteriomethyl)malonate (**8**).* To a suspension of NaH (0.98 g, 26.55 mmol) in THF (10 ml), **6** (5.76 g, 22.13 mmol, 70% pure) in THF (15 ml) was added at 0°C under N_2, and the solution was stirred for a further 30 min at room temperature and then a solution of **7** (4.70 g, 26.55 mmol) in THF (15 ml) was added at 0°C. After the mixture was refluxed for 12 h, ice-cold water (70 ml) was added. The mixture was extracted with ether (3×60 ml). The combined extracts were washed with saturated Na_2SO_3, H_2O, brine, then dried and concentrated to give 9.52 g of crude **8**. This material was employed in the next step without further purification. GC-MS: 228 (M^+-OMe, 2.2), 177 (100), 131 (73.2), 103 (30.0), 81 (12.2).

*Ethyl 2-trideuteriomethyl-6-methyl-5-octenoate (**9**).* According to the modified procedure in the literature, **9** was prepared.[14] To a solution of crude **8** (9.52 g, 34.87 mmol) and LiCl (2.96 g, 69.74 mmol) in DMSO (70 ml), H_2O (4.5 ml) was added, and then the mixture was refluxed for 4 h by stirring. After being cooled

to room temperature, water (200 ml) was added to the mixture. The solution was extracted with ether (3 × 60 ml) and the organic phases were washed with H_2O, brine, then dried and concentrated *in vacuo*. Distillation of the residue gave 3.45 g of **9** (17.20 mmol, 77.6% based on **7**). bp: 109–111°C/15 mmHg. ^{1}H NMR: 5.08 (1H, tq, $J_=$7.1, 1.3), 4.13 (2H, q, J=7.1), 2.41 (1H, t, J=7.0), 2.03-1.96 (4H, m), 1.70 (1H, dq, J=6.7, 0.9) 1.59 (3H, s), 1.43 (1H, dq, J=6.3, 1.6), 1.26 (3H, t, J=7.1), 0.98 (3H, t, J=7.4). ^{13}C NMR: 177.3 (C), 138.0 (C), 122.5 (CH), 60.5 (CH_2), 39.3 (CH), 34.2 (CH_2), 32.7 (CH_2), 25.6 (CH_2), 16.2 (CH_3), 14.7 (CH_3), 13.1 (CH_3). GC-MS: 201 (M^+, 1.5), 156 (11.6), 105 (100), 97 (25.4), 96 (29.1), 77 (96.8). IR: 2900 (s), 2210 (m), 2130 (m), 2050 (w), 1730 (s), 1150 (s), 1010 (s), 840 (m).

*2-Trideuteriomethyl-6-methyloctanol (***11***).* Ester **9** was converted to the corresponding alcohol **10** with $LiAlH_4$ according to the usual procedure (Yield 66.3%). Compound **10** (1.81 g, 11.38 mmol) was submitted for catalytic hydrogenation to give crude **11**, which was purified by column chromatography (silica 20 g, hexane:ether = 1:1). Yield 67.1%. ^{1}H NMR: 3.51 (1H, ddd, J=10.5, 5.8, 2.0), 3.41 (1H, dd, J=10.5, 6.6), 1.59 (1H, m), 1.47 (1H, br. s), 1.40-1.23 (6H, m), 1.18-1.01 (3H, m), 0.86 (3H, t, J=7.2), 0.85 (3H, dd, J=6.5, 1.1). ^{13}C NMR: 68.8 (CH_2), 37.3 (CH_2), 36.0 (CH), 34.8 (CH), 33.8 (CH_2), 30.0 (CH_2), 24.8 (CH_2), 19.6 (CH_3), 11.8 (CH_3). GC-MS: 143 (M^+-H_2O, 0.4), 114 (64.4), 88 (20.1), 83 (18.2), 74 (30.5), 72 (63.0), 71 (61.1), 70 (100), 57 (78.6), 43 (72.5). IR: 3300 (br, s), 2900 (s), 2300 (w), 2200 (m), 1020 (s).

*1-Bromo-2-trideuteriomethyl-6-methyloctane (***12***).* To a solution of triphenylphosphine (2.20 g, 8.40 mmol) in CH_2Cl_2 (20 ml), bromine (0.43 ml, 8.40 mmol) was added dropwise at 0°C and the mixture was stirred for 30 min with cooling. To this solution, **11** (1.23 g, 7.64 mmol) and triethylamine (1.60 ml, 11.46 mmol) in CH_2Cl_2 (10 ml) was added. After stirring for 2 h at 0°C, the mixture was filtered and the solvent was evaporated *in vacuo*. The residue was extracted with petroleum ether (100 ml) and filtered off. The filter cake was rinsed with petroleum ether (2 × 50 ml). The combined filtrates were concentrated. After this procedure was done twice, the residue was chromatographed over silica (20 g, hexane) to give 1.30 g of **12** (5.80 mmol, 76.0%). ^{1}H NMR: 3.40 (1H, ddd, J=9.8, 4.9, 1.9), 3.32 (1H, ddd, J=9.8, 6.2, 1.1), 1.77 (1H, m), 1.46-1.04 (9H, m), 0.86 (3H, t, J=7.2), 0.85 (3H, dd, J=6.1, 0.8). ^{13}C NMR: 42.0 (CH_2), 37.0 (CH_2), 35.5 (CH_2), 35.4 (CH), 34.7 (CH), 29.9 (CH_2), 24.7 (CH_2), 19.6 (CH_3), 11.8 (CH_3). GC-MS: 196 (M^+-Et, 1.6), 194 (M^+-Et, 1.6), 168 (60.4), 166 (62.4), 114 (36.1), 72 (55.3), 57 (100.0), 56 (45.6), 55 (36.1). IR: 2910 (s), 2200 (m), 1040 (m).

Ethyl 4-trideuteriomethyl-8-methyldecanoate (**14**). Crude malonate **13** (1.82 g), prepared by a method similar to that used for **8** with DMF, was decarboxylated as described previously to give 0.71 g of **14** (53.0% yield in 2 steps). ^{1}H NMR: 4.12 (2H, q, $J = 7.1$), 2.38-2.26 (2H, m), 1.70-1.63 (1H, m), 1.48-1.38 (2H, m), 1.37-1.20 (6H, m), 1.26 (3H, t, $J = 7.1$), 1.16-1.02 (3H, m), 0.85 (3H, t, $J = 7.3$), 0.84 (3H, dd, $J = 6.1$, 0.7). ^{13}C NMR: 174.6 (C), 60.6 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 34.8 (CH), 32.6 (CH), 32.6 (CH_2), 32.3 (CH_2), 29.9 (CH_2), 24.8 (CH_2), 19.6 (CH_3), 14.6 (CH_3), 11.8 (CH_3). GC-MS: 231 (M^+, 0.5), 174 (11.0), 171 (10.0), 143 (4.3), 101 (100), 73 (26.2). IR: 2900 (s), 2090 (m), 1730 (s), 1350 (m), 1240 (m), 1150 (s).

4-Trideuteriomethyl-8-methyldecanal (**3**). Alcohol **15**, obtained from the corresponding ester **14** by $LiAlH_4$ reduction, was submitted for PCC oxidation. To a suspension of PCC (0.72 g, 3.33 mmol) in CH_2Cl_2 (10 ml), **15** (0.42 g, 2.22 mmol) in CH_2Cl_2 (10 ml) was added. After the mixture was stirred for 1 h at room temperature, the supernatant was decanted, and the black gum was washed with dry ether (3×50 ml). The combined organic layers were passed through a short pad of florisil column and concentrated. The residue was chromatographed over silica (10 g, hexane:ether = 95:5) and distilled to yield 0.10 g of **3** (24.8%). Compound **3** was 91.5% pure and it was used for bioassay without further purification. ^{1}H NMR: 9.78 (1H, t, $J = 1.9$), 2.45-2.40 (2H, m), 1.70-1.62 (1H, m), 1.48-1.40 (2H, m), 1.37-1.20 (6H, m), 1.17-1.02 (3H, m), 0.86 (3H, t, $J = 7.1$), 0.84 (3H, dd, $J = 6.5$, 0.8). ^{13}C NMR: 203.5 (CH), 42.1 (CH_2), 37.3 (CH_2), 37.2 (CH_2), 34.8 (CH), 32.5 (CH), 29.8 (CH_2), 29.3 (CH_2), 24.8 (CH_2), 19.6 (CH_3), 11.7 (CH_3). GC-MS: 187 (M^+, 0.4), 143 (46.5), 140 (24.9), 114 (53.5), 98 (50.2), 84 (76.5), 70 (100), 57 (88.6). IR: 2920 (s), 2710 (m), 2190 (m), 1710 (s).

4,8-Dimethyldecanol-1,1-d_2 (**16**). Ethyl 4,8-dimethyldecanoate **14a** (0.78 g, 3.42 mmol), prepared by the same procedure as used for compound **14** (starting from diethyl methylmalonate), was reduced with $LiAlD_4$ to give 0.42 g of **16** (2.28 mmol, 65.3%), which was purified by column chromatography (silica 10 g, hexane:ether = 1:1). ^{1}H NMR: 1.63-1.48 (2H, m), 1.44-1.22 (8H, m), 1.40 (1H, br. s), 1.20-1.03 (4H, m), 0.87 (3H, dd, $J = 6.4$, 0.6), 0.86 (3H, t, $J = 7.1$), 0.84 (3H, d, $J = 6.1$). ^{13}C NMR: 37.7 (CH_2), 37.3 (CH_2), 34.8 (CH), 33.3 (CH_2), 33.0 (CH), 30.5 (CH_2), 30.0 (CH_2), 24.9 (CH_2), 20.0 (CH_3), 19.6 (CH_3), 11.8 (CH_3). GC-MS: 170 (M^+-H_2O, 0.6), 140 (15.3), 111 (17.0), 99 (11.9), 85 (30.4), 71 (100), 70 (70.0), 57 (62.1). IR:3300 (br, s), 2170, 2070 (m), 1115 (m), 960 (m).

4,8-Dimethyldecanal-1-d_1 (**2**). Alcohol **16** (0.42 g, 2.22 mmol) was oxidized with PCC (0.72 g, 3.33 mmol) by stirring for 2 h, yielding 0.18 g of **2** (42.4%). Purification by column chromatography (silica 10 g, hexane:ether = 95:5) and

bulb-to-bulb distillation gave 98.8% purity of **2** and it was used for bioassay. ^{1}H NMR: 9.78 (trace, 0.01H, t, $J=1.9$), 2.45-2.40 (2H, m), 1.70-1.62 (1H, m), 1.48-1.40 (2H, m), 1.37-1.20 (6H, m), 1.17-1.02 (3H, m), 0.88 (3H, d, $J=6.1$), 0.86 (3H, t, $J=7.2$), 0.84 (3H, dd, $J=6.5$, 0.8). ^{13}C NMR: 41.9 (CH_2), 37.4 (CH_2), 37.2 (CH_2), 34.8 (CH), 32.8 (CH), 29.8 (CH_2), 29.3 (CH_2), 24.8 (CH_2), 19.7 (CH_3), 19.6 (CH_3), 11.8 (CH_3). GC-MS: 185 (M^+, 0.4), 140 (26.6), 138 (19.3), 111 (40.5), 96 (43.3), 83 (48.7), 70 (100), 57 (69.2). IR: 2910 (s), 2010 (s), 1700 (s).

*4,8-Dimethyldecanal (***1a***)*. This compound was synthesized according to Scheme 1 from nondeuterated diethyl methylmalonate as the starting material. Yield 0.12 g (24.4% based on 4,8-dimethyldecanol). Further purification gave 95.3% purity of **1a** and it was used for bioassay. ^{1}H NMR: 9.78 (1H, t, $J=1.9$), 2.45-2.40 (2H, m), 1.70-1.62 (1H, m), 1.48-1.40 (2H, m), 1.37-1.20 (6H, m), 1.17-1.02 (3H, m), 0.88 (3H, d, $J=6.1$), 0.86 (3H, t, $J=7.1$), 0.84 (3H, dd, $J=6.5$, 0.8). ^{13}C NMR: 203.5 (CH), 42.1 (CH_2), 37.4 (CH_2), 37.2 (CH_2), 34.8 (CH), 32.7 (CH), 29.9 (CH_2), 29.3 (CH_2), 24.7 (CH_2), 19.7 (CH_3), 19.6 (CH_3), 11.7 (CH_3). GC-MS: 184 (M^+, 0.2), 140 (38.5), 137 (18.0), 111 (40.7), 95 (50.7), 85 (59.5), 81 (48.9), 70 (100), 69 (72.4), 57 (96.8). IR: 2920 (s), 2700 (m), 1710 (s).

*Diethyl (ethyl)(trideuteriomethyl)malonate (***17***)*. This compound was prepared in the same manner as that used for **6** from NaH (1.77 g, 48.00 mmol), diethyl ethylmalonate (7.52 g, 40.00 mmol), and iodomethane-d_3 (3.00 ml, 48.00 mmol) as the starting materials. Yield 85.7%. bp: 120–121°C/45 mmHg. ^{1}H NMR: 4.18 (4H, q, $J=7.0$), 1.91 (2H, q, $J=7.5$), 1.25 (6H, t, $J=7.5$). 0.87 (3H, t, $J=7.5$). ^{13}C NMR: 172.8 (C × 2), 61.4 (CH_2 × 2), 54.2 (C), 28.8 (CH_2), 14.4 (CH_3 × 2), 9.0 (CH_3). GC-MS: 205 (M^+, 0.2), 177 (100), 160 (45.8), 132 (66.1), 118 (57.1), 90 (91.8), 76 (44.0). IR: 2990 (s), 2230 (m), 1720 (s), 1230 (s), 1130 (s), 1100 (s), 1015 (s).

*2-Trideuteriomethylbutan-1-ol (***19***)*. Decarboxylation of **17** (7.03 g), as described previously, gave ester **18** (2.33 g, 51.1%), followed by reduction with $LiAlH_4$ to afford the corresponding alcohol **19** (1.13 g, 70.9%). bp: 74°C/76 mmHg. ^{1}H NMR: 3.51 (1H, dd, $J=10.5$, 5.9), 3.41 (1H, dd, $J=10.5$, 6.5), 1.82 (1H, br. s), 1.52 (1H, m), 1.49-1.42 (1H, m), 1.14 (1H, dquin, $J=13.5$, 7.4), 0.91 (3H, t, $J=7.4$). ^{13}C NMR: 68.3 (CH_2), 37.5 (CH), 26.1 (CH_2), 11.7 (CH_3). GC-MS: 91 (M^+, 0.2), 73 (49.4), 60 (100), 59 (90.0). IR: 3300 (s), 2900 (s), 2200 (m), 2050 (m), 1240 (m), 1040 (s).

*1-Iodo-2-trideuteriomethylbutane (***21***)*. To a solution of NaI (3.50 g, 23.34 mmol) in dry acetone (40 ml), tosylate **20** (2.86 g), derived from **19**, was added. The solution was stirred and refluxed for 5 h. After cooling to

room temperature, water (100 ml) was added. The solution was extracted with pentane (3×70 ml). The organic phases were washed with saturated Na_2SO_3, brine and dried. After the solvent was evaporated, the residue was distilled to afford 1.61 g of **21** (8.01 mmol, 64.5% in 2 steps). bp: 62°C/70 mmHg. ^{1}H NMR: 3.23 (1H, dd, $J = 9.6$, 4.8), 3.17 (1H, dd, $J = 9.6$, 5.8), 1.44-1.35 (2H, m), 1.29-1.24 (1H, m), 0.89 (3H, t, $J = 7.3$). ^{13}C NMR: 36.6 (CH), 29.5 (CH_2), 17.8 (CH_3), 11.7 (CH_3). GC-MS: 201 (M^+, 12.4), 127 (16.3), 74 (100). IR: 2930 (s), 2220 (m), 2050 (m), 1270 (m), 1250 (m), 1180 (s), 1050 (s).

*Citronellyl bromide (***22***)*. Racemic citronellol (15.60 g, 100.00 mmol) was brominated in the same way as **12** to give 18.23 g of **22** (83.24 mmol, 83.2%). bp: 103–105°C/12 mmHg. ^{1}H NMR: 5.09 (1H, tq, $J = 7.1$, 1.4), 3.48-3.37 (2H, m), 2.05-1.84 (3H, m), 1.71-1.57 (2H, m), 1.68 (3H, d, $J = 1.3$), 1.61 (3H, s), 1.38-1.32 (1H, m), 1.21-1.14 (1H, m), 0.90 (3H, d, $J = 6.5$). ^{13}C NMR: 131.9 (C), 125.0 (CH), 40.4 (CH_2), 37.0 (CH_2), 32.4 (CH_2), 31.8 (CH), 26.1 (CH_3), 25.8 (CH_2), 19.3 (CH_3), 18.1 (CH_3). GC-MS: 220 (M^+, 8.4), 218 (M^+, 8.0), 83 (56.2), 69 (100). IR: 2910 (s), 1250 (w), 1100 (w), 870 (w), 810 (w).

*2,6-Dimethyl-10-trideuteriomethyl-2-dodecene (***23***)*. To a solution of citronellylmagnesium bromide prepared from Mg 0.77 g (0.032 g atom) and **22** (7.02 g , 32.04 mmol) in THF (40 ml), a solution of **21** (1.61 g, 8.01 mmol), *N*-methyl pyrrolidinone (12.30 ml, 128.16 mmol) and Li_2CuCl_4 (4.0 ml, 0.40 mmol; 0.1 M) in THF (20 ml) was added at 0°C under N_2. The mixture was stirred for 1.5 h at 0°C and 1 h at room temperature. The excess Grignard reagent was destroyed by dropwise addition of 2N HCl (50 ml). The precipitate formed was removed by filtration through defatted cotton and the filtrate was extracted with hexane (3×60 ml). The organic phase was washed successively with 2N HCl, saturated Na_2SO_3, H_2O, and brine, then dried, and concentrated *in vacuo*. The residue was distilled to give 0.46 g of **23** (2.16 mmol, 27.0% based on **21**). bp: 102–104°C/4 mmHg. ^{1}H NMR: 5.10 (1H, tq, $J = 7.1$, 1.4), 2.00-1.92 (2H, m), 1.68 (3H, d, $J = 1.1$), 1.60 (3H, s), 1.42-1.35 (1H, m), 1.34-1.17 (7H, m), 1.18-1.02 (4H, m), 0.86 (3H, d, $J = 6.6$), 0.85 (3H, t, $J = 7.3$). ^{13}C NMR: 131.3 (C), 125.5 (CH), 37.7 (CH_2), 37.6 (CH_2), 37.3 (CH_2), 34.6 (CH), 32.8 (CH), 29.8 (CH_2), 26.0 (CH_3), 25.7 (CH_2), 24.8 (CH_2), 20.0 (CH_3), 18.0 (CH_3), 11.8 (CH_3). GC-MS: 213 (M^+, 20.4), 111 (14.7), 73 (26.6), 69 (100). IR: 2920 (s), 2200 (m), 2050 (m), 1440 (s), 1370 (s), 1050 (m).

*2,3-Epoxy-2,6-dimethyl-10-trideuteriomethyldodecane (***24***)*. Compound **24** was prepared according to the procedure of Suzuki.[10] Yield 84.9%. GC-MS: 229 (M^+, 0.4), 86 (31.0), 85 (37.5), 73 (46.5), 59 (100). IR: 2930 (s), 2200 (m), 2030 (m), 1240 (w), 1110 (m), 1040 (w).

*8-Trideuteriomethyl-4-methyldecanal (**4**).* Epoxide **24** was cleaved with HIO_4 to give the expected aldehyde **4**. Yield 31.3%. Purification of a small portion of **4** by column chromatograph yielded 95.2% purity of **4** and it was used for bioassay. ^{1}H NMR: 9.78 (1H, t, $J = 1.8$), 2.45-2.40 (2H, m), 1.70-1.62 (1H, m), 1.46-1.41 (2H, m), 1.36-1.20 (6H, m), 1.14-1.04 (3H, m), 0.88 (3H, d, $J = 6.3$), 0.85 (3H, t, $J = 7.3$). ^{13}C NMR: 203.1 (CH), 41.7 (CH_2), 37.0 (CH_2), 36.8 (CH_2), 34.2 (CH), 32.9 (CH), 29.4 (CH_2), 28.9 (CH_2), 24.4 (CH_2), 19.4 (CH_3), 11.4 (CH_3). GC-MS: 187 (M^+, 0.5), 143 (61.0), 140 (28.1), 114 (54.0), 95 (55.9), 85 (68.7), 73 (100), 60 (77.6), 57 (90.0). IR: 2910 (s), 2700 (s), 2190 (m), 2050 (m), 1730 (s).

*Citronellylacetone (**25**).* A solution of *t*-BuOK (10.95 g, 97.80 mmol) in *t*-BuOH (70 ml) was heated and refluxed to dissolve *t*-BuOK and then cooled to 50°C. After ethyl acetoacetate (11.65 g, 89.65 mmol) was added, the mixture was stirred for 30 min, and **22** (17.84 g, 81.50 mmol) was added to the solution. The mixture was stirred and refluxed for 24 h. The reacted solution was cooled, filtered and concentrated. The residue was poured into a stirred 2N NaOH (300 ml) solution and heated for 3 h at 50–55°C. After cooling to room temperature, the alkaline solution was acidified with acetic acid (40 ml). The mixture was extracted with hexane (3×100 ml). The organic phases were washed with saturated $NaHCO_3$, dried and concentrated *in vacuo*. The residue was distilled to give 10.63 g of **25** (54.23 mmol, 66.5%). bp: 108–111°C/4 mmHg. ^{1}H NMR: 5.09 (1H, t, $J = 7.1$), 2.40 (2H, t, $J = 7.6$), 2.13 (3H, s), 1.96 (2H, m), 1.68 (3H, s), 1.60 (3H, s), 1.55 (2H, m), 1.41 (1H, m), 1.29 (2H, m), 1.13 (2H, m), 0.87 (3H, d, $J = 6.6$). ^{13}C NMR: 209.7 (C), 131.5 (C), 125.2 (CH), 44.5 (CH_2), 37.3 (CH_2), 36.8 (CH_2), 32.6 (CH), 30.2 (CH_3), 26.1 (CH_3), 25.9 (CH_2), 21.8 (CH_2), 19.9 (CH_3), 18.0 (CH_3). GC-MS: 196 (M^+, 4.8), 138 (6.4), 69 (78.8), 43 (100). IR: 2910 (s), 1710 (s), 1150 (m).

*Ethyl 3,7,11-trimethyl-2,10-dodecadienoate (**26**).* Compound **26** was prepared by the usual Wittig–Horner reaction procedure. Yield 79.9%. ^{1}H NMR: 5.66 (1H, q-like, $J = 1.1$), 5.09 (1H, t-like, $J = 7.1$), 4.14 (2H, q, $J = 7.1$), 2.15 (3H, d, $J = 1.2$), 2.11 (2H, t-like, $J = 7.7$), 1.95 (2H, m), 1.68 (3H, d, $J = 0.9$), 1.60 (3H, s), 1.48 (2H, m), 1.40 (1H, m), 1.33-1.25 (2H, m), 1.26 (3H, t, $J = 7.1$), 1.12 (2H, m), 0.87 (3H, d, $J = 6.6$). ^{13}C NMR: 167.3 (C), 160.7 (C), 131.5 (C), 125.3 (CH), 115.8 (CH), 59.8 (CH_2), 41.6 (CH_2), 37.4 (CH_2), 36.8 (CH_2), 32.6 (CH), 26.1 (CH_3), 25.9 (CH_2), 25.2 (CH_2), 19.9 (CH_3), 19.1 (CH_3), 18.0 (CH_3), 14.7 (CH_3). GC-MS: 266 (M^+, 10.4), 221 (18.2), 128 (50.4), 109 (98.0), 69 (100). IR: 2910 (s), 1705 (s), 1640 (m), 1210 (s), 1130 (s).

*Ethyl 3,7,11-trimethyl-1-dodecenoate (**27**).* Selective hydrogenation of **26** was carried out using a modified procedure described in the literature.[11,12] To a suspension of CuBr (I) (12.13 g, 84.87 mmol) in dry THF (50 ml), Red-Al^{TM}

(19.78 ml, 65.28 mmol; 65%) was added dropwise at −10°C under N_2 and the solution was stirred for a further 30 min. A solution of **26** (2.17 g, 8.16 mmol) in dry THF (15 ml) was added to the mixture and stirred for 1 h at −10°C and for 2 h at room temperature. After the reacted solution was cooled to 0°C, ice-cold water was added carefully. The black gum which formed was washed with ether (4 × 50 ml). The organic phase was filtered on a short pad of SiO_2, washed with 2N HCl, saturated $NaHCO_3$, brine, then dried and concentrated *in vacuo*. The residue was chromatographed over silica (20 g, hexane:ether = 9:1) to give 1.57 g of **27** (5.86 mmol, 71.8%). ^{1}H NMR: 5.10 (1H, t-like, J = 7.1), 4.13 (2H, q, J = 7.1), 2.28 (1H, ddd, J = 14.0, 6.0, 2.0), 2.09 (1H, ddd, J = 14.0, 8.0, 2.0), 2.03-1.90 (3H, m), 1.68 (3H, s), 1.60 (3H, s), 1.39 (1H, m), 1.36-1.02 (8H, m), 1.26 (3H, t, J = 7.1), 0.95 (3H, d, J = 6.7), 0.86 (3H, d, J = 6.5). ^{13}C NMR: 173.8 (C), 131.4 (C), 125.3 (CH), 60.4 (CH_2), 42.3 (CH_2), 37.5 (CH_2), 37.5 (CH_2), 37.44 (CH_2), 32.8 (CH), 30.8 (CH), 26.1 (CH_3), 26.0 (CH_2), 24.7 (CH_2), 20.1 (CH_3), 19.9 (CH_3), 18.0 (CH_3), 14.7 (CH_3). GC-MS: 268 (M^+, 2.8), 222 (7.7), 180 (5.2), 125 (27.5), 69 (100). IR: 2910 (s), 1735 (s), 1170 (s), 1010 (m), 900 (m).

*3,7,11-Trimethyl-10-dodecen-1-ol-1,1-d_2 (***28***)*. Reduction of **27** (1.57 g, 5.86 mmol) with $LiAlD_4$ (0.49 g, 11.72 mmol) in the usual way gave 1.09 g of **28** (4.78 mmol, 81.6%). This was purified by column chromatography (silica 15 g, hexane:ether = 7:3). ^{1}H NMR: 5.10 (1H, t-like, J = 7.1), 3.67 (trace; 0.04H, m), 1.95 (2H, m), 1.68 (3H, s), 1.64-1.54 (2H, m), 1.60 (3H, s), 1.44 (1H, br. s), 1.42-1.00 (10H, m), 0.89 (3H, d, J = 6.6), 0.86 (3H, d, J = 6.6). ^{13}C NMR: 131.4 (C), 125.5 (CH), 40.2 (CH_2), 37.8 (CH_2), 37.6 (CH_2), 37.5 (CH_2), 32.8 (CH), 29.9 (CH), 26.1 (CH_3), 26.0 (CH_2), 24.7 (CH_2), 20.1 (CH_3), 20.0 (CH_3), 18.0 (CH_3). GC-MS (B): 228 (M^+, 11.7), 210 (5.6), 125 (53.3), 109 (26.4), 83 (46.3), 69 (100). IR: 3350 (br, s), 2160 (m), 2080 (m), 950 (m).

*2,6,10-Trimethyl-2-dodecene-12,12,12-d_3 (***30***)*. Tosylate **29** (2.35 g), derived from **28**, was reduced with $LiAlD_4$ (1.00 g, 23.90 mmol) in the usual way. After the workup, the residue was chromatographed over silica (10 g, Hexane) yielding 0.63 g of **30** (2.96 mmol, 61.9% in 2 steps). ^{1}H NMR: 5.10 (1H, t-like, J = 7.1), 2.03-1.90 (2H, m), 1.68 (3H, d, J = 1.1), 1.60 (3H, s), 1.41 (1H, m), 1.35-1.20 (7H, m), 1.18-1.00 (4H, m), 0.86 (3H, d, J = 6.6), 0.84 (3H, d, J = 6.1). ^{13}C NMR: 131.3 (C), 125.5 (CH), 37.7 (CH_2), 37.5 (CH_2), 37.3 (CH_2), 34.8 (CH), 32.8 (CH), 29.6 (CH_2), 26.1 (CH_3), 26.0 (CH_2), 24.8 (CH_2), 20.0 (CH_3), 19.6 (CH_3), 18.0 (CH_3). GC-MS: 213 (M^+, 47.6), 111 (35.6), 69 (100). IR: 2980 (s), 2200 (w), 1450 (m).

*2,3-Epoxy-2,6,10-trimethyldodecane-12,12,12-d_3 (***31***)*. Compound **31** was prepared by the same procedure described for **24**. Yield 88.5%. ^{1}H NMR: 2.70

(1H, t, $J=6.3$), 1.62-1.02 (14H, m), 1.31 (3H, s), 1.27 (3H, s), 0.88 (3H, d, $J=6.6$), 0.84 (3H, d, $J=6.3$). ^{13}C NMR: 65.2 (CH), 58.6 (C), 37.6 (CH_2), 37.3 (CH_2), 34.8 (CH), 33.9 (CH_2), 33.1 (CH), 29.6 (CH_2), 26.8 (CH_2), 25.3 (CH_3), 24.8 (CH_2), 20.0 (CH_3), 19.6 (CH_3), 19.0 (CH_3). GC-MS: 229 (M^+, 5.7), 83 (72.5), 73 (87.1), 59 (100). IR: 2950 (s), 2200 (m), 2020 (w), 1100 (m), 720 (m).

*4,8-Dimethyldecanal-10,10,10-d_3 (**5**).* Cleavage of **31** with HIO_4, according to the procedure used for **4**, gave the desired aldehyde **5**. Yield 65.3%. A small portion was purified by column chromatography yielding 97.7% purity of **5** and it was used for bioassay. ^{1}H NMR: 9.78 (1H, t, $J=1.9$), 2.45-2.40 (2H, m), 1.70-1.62 (1H, m), 1.48-1.40 (2H, m), 1.37-1.20 (6H, m), 1.17-1.02 (3H, m), 0.88 (3H, d, $J=6.1$), 0.84 (3H, d, $J=6.5$). ^{13}C NMR: 203.4 (CH), 42.1 (CH_2), 37.4 (CH_2), 37.2 (CH_2), 34.7 (CH), 32.7 (CH), 29.7 (CH_2), 29.3 (CH_2), 24.7 (CH_2), 19.8 (CH_3), 19.6 (CH_3). GC-MS: 187 (M^+, 0.7), 143 (64.0), 137 (26.2), 111 (61.1), 95 (67.0), 85 (65.0), 81 (68.9), 73 (100), 60 (56.8). IR: 2910 (s), 2700 (s), 2200 (m), 2050 (m), 1720 (s).

*4,8-Dimethyldecanal (**1b**).* 4,8-Dimethyldecanal (**1b**) was prepared from **27** in the same way as described for **5** (95.2% pure), using $LiAlH_4$ instead of $LiAlD_4$, and it was used for bioassay. The spectra were identical to those of **1a**.

Behavioral tests

Insects

A laboratory colony of *T. castaneum* was used in all experiments. The beetles were reared on a mixture of whole wheat flour and 5% brewer's yeast at 27 ± 1°C, ca. 70% RH on a 16 h:8 h L/D cycle. New adult progenies were used for bioassay 4 days after emergence.

Bioassay

Pheromonal activity of the synthetic compounds was estimated with a two-hole pitfall olfactometer, which consisted of a four-compartment plastic Petri dish (ϕ 9 cm; Vermak, Canada) and a surface-roughened walking arena with two holes (ϕ 6 mm) located directly opposite from each other, 1 cm from the edge. A filter paper disk (ϕ 21 mm; Kiriyama, Japan) treated with the compounds in hexane (20 µl) and a control disk treated with hexane (20 µl) alone were placed in opposite chambers of the Petri dish after evaporation of the solvent, and then the walking arena was loaded on. Thirty adult mixed-sex beetles were released on the walking arena in each replicate. After the disk was covered, the whole device was covered with a black box. The number of beetles in each chamber were recorded after 15 min, and attraction (%) was calculated from an equation $[100 \times (T-C)/30]$, where T and C are the numbers of beetles

in the treated and control chambers, respectively. Each test was repeated eight times with new beetles. Dose-response studies of the compounds were assessed at doses ranging from 1 to 1000 ng. The data were statistically analyzed using a *t*-test.

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