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A Male-produced Aggregation Pheromone Blend Consisting of Alkanediols, Terpenoids, and an Aromatic Alcohol from the Cerambycid Beetle *Megacyllene caryae*

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Abstract Bioassays conducted with a Y-tube olfactometer provided evidence that both sexes of the cerambycid beetle *Megacyllene caryae* (Gahan) were attracted to odor produced by males. Odor collected from male *M. caryae* contained eight male-specific compounds: a 10:1 blend of (2*S*,3*R*)- and (2*R*,3*S*)-2,3-hexanediols (representing 3.2±1.3% of the total male-specific compounds), (*S*)-(−)-limonene (3.1±1.7%), 2-phenylethanol (8.0±2.4%), (−)-α-terpineol (10.0±2.8%), nerol (2.1±1.5%), neral (63.3±7.3%), and geranial (8.8±2.4%). Initial field bioassays determined that none of these compounds was attractive as a single component. Further field trials that used a subtractive bioassay strategy determined that both sexes were attracted to the complete blend of synthetic components, but the elimination of any one component resulted in a decline in trap captures. Blends that were missing (2*S*,3*R*)-2,3-hexanediol, (2*R*,3*S*)-2,3-hexanediol, or citral (a 1:1 mixture of neral and geranial) attracted no more beetles than did controls. A pheromone blend of this complexity, composed of alkanediols, terpenoids, and aromatic alcohols, is unprecedented for cerambycid species.

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Introduction

Male-produced aggregation pheromones have been identified for nine species in four tribes of the cerambycid beetle subfamily Cerambycinae (Lacey et al. 2004, 2007b; Hanks et al. 2007). In another three species, male-produced pheromones attract only females (reviewed by Lacey et al. 2004). The pheromones of all these species are comprised of one to three compounds that share a similar structural motif, consisting of molecules that are 6, 8, or 10 carbons in length, with hydroxyl or carbonyl groups at C_2 and C_3 (reviewed by Lacey et al. 2004, 2007b). There are two exceptions to this trend: the male-produced aggregation pheromone of the cerambycine *Phymatodes lecontei* Linsley, (*R*)-2-methylbutan-1-ol (Hanks et al. 2007), and a component of the sex pheromone of *Hylotrupes bajulus* (L.), 1-butanol (Reddy et al. 2005). Volatile pheromones of species in the subfamily Cerambycinae apparently are produced by glands in the prothorax and secreted through pores lying within depressions in the cuticle (Ray et al. 2006; Hanks et al. 2007; Lacey et al. 2007b). Pheromone release by males of several species has been associated with a characteristic body posture, termed the "pushup stance" (Lacey et al. 2004, 2007a, b): Males fully extend their front legs, elevating the head and thorax, and remain motionless for extended periods.

The cerambycine species *Megacyllene caryae* (Gahan) is endemic to North America. The larvae develop in woody tissues of stressed, moribund, and dead trees of a variety of

hardwood species (Linsley 1964). Adults are active in early spring, and collection records from the area of our study, east-central Illinois, range from March 29 to June 7 (Illinois Natural History Survey, Champaign, IL). The adult beetles reportedly feed on flowers of trees in the genus *Crataegus* (Rosaceae; Dusham 1921), but dissections of field-collected adults of both sexes revealed that their guts contained pollen from trees in the genus *Quercus* (Fagaceae; unpublished data). In the laboratory, beetles can live for more than 30 d if provided sugar water (Lacey, personal observation). Adults are active from ~11:00 to 18:00 hours. Both sexes aggregate on larval hosts in numbers that may exceed 40 individuals. They mate soon after emergence, and females begin ovipositing immediately. Generation time is usually 1 yr.

M. caryae exhibits several characteristics that have been associated with production of volatile pheromones in other cerambycine species. For example, adults aggregate on larval hosts (Lacey, personal observation), males have sex-specific gland pores on their prothoraces (Ray et al. 2006), and males on larval hosts commonly assume the pushup stance (Lacey, personal observation). We report here the identification and testing of sex-specific volatile pheromone components produced by adult male *M. caryae*.

Methods and Materials

Source of Insects Adult *M. caryae* used in olfactometer bioassays and for pheromone collections were reared from logs of honey locust, *Gleditsia triacanthos* L. (Fabaceae), that were naturally colonized by beetles from April to May 2003 on the campus of University of Illinois at Urbana–Champaign (UIUC). Periodically, logs were moved to a 3×2×1-m rearing cage of window screen in a laboratory room (ambient conditions: fluorescent lighting, ~12:12 L/D, 20°C, 50% relative humidity) during January and March 2004. Most adults emerged within 14 d of moving logs into the laboratory. Freshly emerged adults were caged individually in 0.1-m^3 cylindrical cages of aluminum window screen with plastic Petri dishes at top and bottom, under ambient laboratory conditions. They were provided 10% sucrose solution dispensed from 8-ml vials plugged with cotton dental rolls (Patterson Dental Supply, South Edina, MN). Adults used in experiments appeared healthy and active.

Testing for Attractant Pheromones Bioassays for attractants produced by adult *M. caryae* were carried out with a horizontal glass Y-tube olfactometer (6 cm diameter, main tube 26 cm long, arm length 22 cm, 70° angle between arms). Bioassays were conducted outdoors in partial shade because beetles either were sedentary or appeared agitated when placed in the olfactometer under laboratory conditions. We conducted bioassays between 11:00 and 16:00 hours for 4 d in April 2004 (skies clear, air temperatures 17–24°C). A 2-l plastic chamber containing a cylinder of aluminum screen as a perch was attached to each arm of the Y-tube. One chamber contained six male beetles and the other six females. Ambient air was pulled through the olfactometer with a vacuum cleaner connected to a variable power supply (air speed ~1.0 m sec^{-1}). For each trial, a beetle was released at the base of the Y-tube and allowed 10 min to respond to an odor source by crossing a line 18 cm down one arm. Beetles that did not respond within 10 min were recorded as "no response." Chambers were alternated between arms of the Y-tube every three trials to control for positional bias. Chambers and the olfactometer were washed and rinsed with acetone each day. We bioassayed 20 individuals of each sex and compared numbers of beetles responding to treatments with a χ^2 goodness-of-fit test corrected for continuity (Sokal and Rohlf 1995).

Identification of Potential Pheromone Components Volatile compounds were collected from adult *M. caryae* by placing five females and males in separate glass vacuum traps (~0.3 l, custom manufactured by the glass shop, School of Chemistry, UIUC) that were lined with an aluminum screen to provide perches. A glass tube (6 cm×4 mm inner diameter [i.d.]) containing 100 mg of 80/100 mesh SuperQ® (Alltech Associates, Deerfield, IL) held between plugs of silanized glass wool was attached to one nipple of each chamber with an 8-cm-long section of Teflon® tubing, and charcoal-purified air was pulled through the apparatus with a water aspirator (0.7 l min^{-1}). Males and females were aerated simultaneously on a laboratory windowsill from 11:00 to 16:00 hours in April and May 2004. We selected this period because it was the only time during the day that males displayed the pushup stance. Collectors were eluted with three 0.5-ml aliquots of CH_2Cl_2 and the resulting extracts analyzed at the University of California Riverside on a Hewlett-Packard® (HP) 5973 mass selective detector interfaced to an HP 6890 gas chromatograph, fitted with a DB5-MS column (30 m×0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA), temperature-programmed from 40 (held for 1 min) to 250°C at 10°C min^{-1}. Injector temperature was 250°C, and injections were made in the splitless mode. Absolute configuration of the insect-produced compounds was determined by analysis of the extract on a Cyclodex-B gas chromatography (GC) column (30 m×0.25 mm i.d., 0.25 μm film thickness, J&W Scientific) with the GC programmed from 50 (held for 1 min) to 200°C at 5°C min^{-1}; injector and detector temperatures were 100 and 200°C, respectively. Identifications of peaks were confirmed by coinjections of extracts with authentic standards.

Synthesis of Pheromone Components The stereoisomers of 2-hydroxy-3-hexanone and 2,3-hexanediol (see "Results") were synthesized as described below and shown in Fig. 1. Racemic 2-hydroxy-3-hexanone and racemic (2*R**,3*S**)- and (2*R**,3*R**)-2,3-hexanediols were available from previous studies (Lacey et al. 2004, 2007b; Hanks et al. 2007). All other compounds were obtained from Sigma-Aldrich (St Louis, MO), including (*S*)-(−)-limonene (96%), 2-phenylethanol (99%), (−)-alpha-terpineol (90%), nerol (≥90%), and citral (95%; see "Results"). Because neral and geranial (see "Results") were not readily available as pure isomers, citral (a 1:1 mixture of neral and geranial; Sigma-Aldrich) was used in the bioassays.

Tetrahydrofuran was distilled from sodium/benzophenone ketyl under argon. ^{1}H- and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Varian INOVA-400 (400 and 100.5 MHz, respectively) spectrometer as $CDCl_3$ solutions. Chemical shifts were expressed in parts per million relative to $CDCl_3$ (7.26 and 77.23 ppm for ^{1}H- and ^{13}C-NMR, respectively). Mass spectra were obtained with an HP 5890 GC interfaced to an HP 5970 mass selective detector, in electron impact mode (70 eV), with helium as the carrier gas. The GC was equipped with a DB5-MS column (25 m×0.20 mm i.d.×0.33 μm film; J&W Scientific). Solutions were dried over anhydrous Na_2SO_4 and concentrated under partial vacuum by rotary evaporation unless otherwise stated. Crude products were purified by flash or vacuum flash chromatography with silica gel (230–400 mesh, EM Science, Gibbstown, NJ). Reactions with air- or water-sensitive reagents were carried out in oven-dried glassware under argon. GC with a chiral stationary phase column was performed with an HP 5890 GC fitted with a Cyclodex B column (30 m×0.25 mm i.d.× 0.25 μm, J&W Scientific), programmed from 50 (held for 1 min) to 200°C at 5°C min^{-1}, with the injector at 100°C and helium as carrier gas (20 psi).

Ethyl (S)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate [(S)-2] 3,4-Dihydro-2H-pyran (53.4 g, 635 mmol) was added dropwise to a stirred solution of ethyl (*S*)-(−)-lactate (*S*)-1 (50.0 g, 423 mmol; Aldrich Chem., Milwaukee, WI)

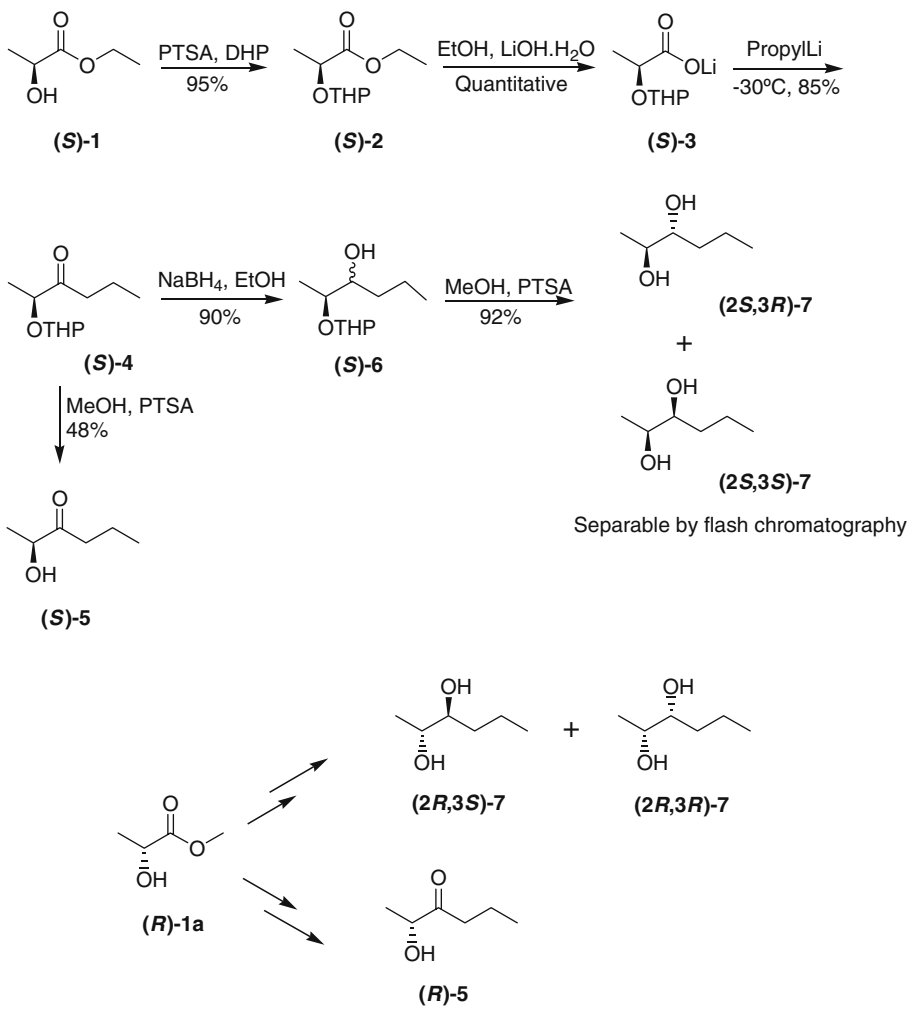

Fig. 1 Syntheses of all four 2,3-hexanediol stereoisomers and the two 2-hydroxy-3-hexanone enantiomers from ethyl (*S*)-lactate and methyl (*R*)-lactate

and 100 mg *p*-toluenesulfonic acid in 120 ml CH_2Cl_2 at 0°C under argon. The mixture was warmed to room temperature and stirred until all the starting material had been consumed (~5 hr). The mixture was then diluted with diethyl ether (60 ml) and washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. The crude product was Kugelrohr distilled (64–68°C, 0.25 mmHg), yielding 81.4 g of protected alcohol (*S*)-2 (95%) as a mixture of diastereoisomers (2:1—measured by GC). ^{1}H-NMR (major stereoisomer): δ 1.26 (t, *J*=7.2 Hz, 3H), 1.44 (d, *J*=7.0 Hz, 3H), 1.48–1.90 (m, 6H), 3.47–3.55 (m, 1H), 3.80–3.88 (m, 1H), 4.11–4.25 (m, 3H, two quadruplets overlapped with signals of other stereoisomer), 4.66–4.72 (m, 1H, dd overlapped with signals of other stereoisomer). ^{1}H-NMR (minor stereoisomer): δ 1.27 (t, *J*=7.2 Hz, 3H), 1.38 (d, *J*=6.8 Hz, 3H), 1.48–1.90 (m, 6H), 3.40–3.47 (m, 1H), 3.88–3.95 (m, 1H), 4.11–4.25 (m, 2H - quadruplet overlapped with signals of other stereoisomer), 4.40 (q, *J*= 7.0 Hz, 1H), 4.66–4.72 (m, 1H, dd overlapped with signals of other stereoisomer); mass spectrometry (MS; *m/z*: relative intensity): 144 (1), 130 (3), 129 (4), 101 (19), 85 (100), 73 (11), 67 (12), 57 (16), 55 (17), 45 (25), 43 (24).

Methyl (R)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate [(R)-2a] In the same manner as described above, methyl (*R*)-(+)-lactate (*R*)-1a (20.0 g, 192 mmol; Aldrich Chem., 96% enantiomeric excess [ee]) was converted to a mixture (2:1—measured by GC) of methyl (*R*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate diastereoisomers (*R*)-2a (34.24 g) in 95% yield after Kugelrohr distillation (62–66°C,1.3 mmHg). ^{1}H-NMR (major stereoisomer): δ 1.44 (d, *J*=7.0 Hz, 3H), 1.46–1.90 (m, 6H), 3.40–3.54 (m, 1H), 3.72 (s, 3H), 3.76–3.93 (m, 1H), 4.41 (d, *J*=7.0 Hz, 1H), 4.65–4.71 (m, 1H) (some signals overlapped with signals of the other stereoisomer). ^{1}H-NMR (minor stereoisomer): δ 1.38 (d, *J*=6.8 Hz, 3H), 1.46–1.90 (m, 6H), 3.40–3.54 (m, 1H), 3.72 (s, 3H), 3.76–3.93 (m, 1H), 4.19 (d, *J*=6.8 Hz, 1H), 4.65 – 4.71 (m, 1H) (some signals overlapped with signals of the other stereoisomer); MS (relative intensity) *m/z*: 130 (2), 129 (6), 116 (2), 101 (28), 85 (100), 73 (7), 67 (15), 57 (20), 55 (26), 45 (25), 43 (30).

Lithium (S)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate [(S)-3] Ethyl (*S*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate (*S*)-2 (80.00 g, 396 mmol) was added to a stirred suspension of $LiOH.H_2O$ (16.60 g, 396 mmol) in ethanol (200 ml) under argon at 0°C. The reaction was stirred for 30 min at 0°C, warmed to room temperature, and stirred for 3 hr. The mixture was concentrated under vacuum, and hexane (50 ml) was added to the concentrate, followed by concentration under vacuum to remove traces of ethanol and water as azeotropes. This procedure was repeated four times, and the residue was pumped under vacuum (0.1 mmHg) for 10 hr. ^{1}H-NMR showed that ethanol was still present in the salt, and so the crude lithium salt was suspended in benzene, the benzene distilled off at atmospheric pressure, and the residue again pumped under vacuum for 10 hr, affording lithium (*S*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate (*S*)-3 as a light yellow solid in quantitative (99.7%) yield. ^{1}H-NMR (major stereoisomer): δ 1.39 (d, *J*=6.8 Hz, 3H), 1.42–1.92 (m, 6H), 3.40–3.55 (m, 1H), 3.82–3.92 (m, 1H), 4.18 (q, *J*=6.8 Hz, 1H), 4.70 (t, *J* 3.5 Hz, 1H). ^{1}H NMR (minor stereoisomer): δ 1.34 (d, *J*=6.8 Hz, 3H), 1.42–1.92 (m, 6H), 3.40–3.55 (m, 1H), 4.00–4.05 (m, 1H), 4.12 (q, *J*=7.0 Hz, 1H), 4.54 (dd, *J*=2.3 and 7.0 Hz, 1H). Some NMR signals from the two diastereomers overlapped.

Lithium (R)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate [(R)-3] Methyl (*R*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate (*R*)-2a (34.20 g, 182 mmol) was converted to lithium (*R*)-2-(2-tetrahydropyranyloxy)-propanoate (*R*)-3 (32.70 g) as described above, in quantitative yield. ^{1}H-NMR and mass spectra matched those of the corresponding (*S*)-enantiomer mixture.

(S)-2-[(Tetrahydro-2H-pyran-2-yl)oxy]-3-hexanone [(S)-4] Lithium wire (1% sodium content; 1.39 g, 200 mmol, previously rinsed with hexane) was cut into small pieces directly into a three-necked flask charged with anhydrous diethyl ether (85 ml) under argon. A few drops of a 1-bromopropane (12.3 g, 100 mmol) solution in 15 ml ether were added, and the mixture was stirred at ambient temperature until the reaction started. The mixture was then cooled to −20°C, and the remaining solution of bromopropane was added dropwise over 1.5 hr. When the addition was complete, the mixture was allowed to warm to room temperature and stirred for an additional hour before use.

The resulting propyllithium solution (~1.0 M in ethyl ether, 80 ml) was added dropwise to a suspension of lithium (*S*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate (*S*)-3 (9.63 g, 53.5 mmol) in Et_2O (100 ml) under argon at −30°C. The reaction was allowed to warm to room temperature, stirred overnight, poured into crushed ice, and extracted with Et_2O (3×100 ml). The combined organic layers were washed with saturated aqueous NH_4Cl and brine, dried, and concentrated. The residue was purified by vacuum flash chromatography on silica gel (hexane: EtOAc, 9:1) affording (*S*)-2-[(tetrahydro-2H-pyran-2-yl) oxy]-3-hexanone (*S*)-4 (9.14 g, 85%). ^{1}H-NMR (major isomer): δ 0.90 (t, *J*=7.4 Hz, 3H), 1.34 (d, *J*=7.0 Hz, 3H), 1.45–1.95 (m, 8H), 2.40 (dt, *J*=7.2 and 17.3 Hz, 1H), 2.50 (dt, *J*=7.3 and 17.3 Hz, 1H), 3.40–3.53 (m, 1H), 2.77–3.90 (m, 1H), 4.27 (q, *J*=7.0 Hz, 1H), 4.54 (dd, *J*=3.1 and 4.5 Hz, 1H). Some NMR signals overlapped with the

signals from the minor stereoisomer. MS (major stereoisomer): 156 (1), 129 (3), 101 (1), 99 (1), 85 (100), 71 (9), 67 (13), 57 (14), 55 (9), 43 (30), 41 (22). ^{1}H-NMR (minor isomer): δ 0.91 (t, *J*=7.4 Hz, 3H), 1.25 (d, *J*=6.8 Hz, 3H), 1.45–1.95 (m, 8H), 2.56 (dt, *J*=7.2 and 17.8 Hz, 1H), 2.62 (dt, *J*=7.4 and 17.9 Hz, 1H), 3.40–3.53 (m, 1H), 2.77–3.90 (m, 1H), 4.07 (q, *J*=6.8 Hz, 1H), 4.60 (dd, *J*=2.8 and 5.3 Hz, 1H). MS (minor stereoisomer): 156 (1), 129 (3), 101 (1), 99 (1), 85 (100), 71 (7), 67 (12), 57 (15), 55 (10), 43 (32), 41 (22).

(R)-2-[(Tetrahydro-2H-pyran-2-yl)oxy]-3-hexanone [(R)-4] Lithium (*R*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-propanoate (*R*)-3 (10.52 g, 58.4 mmol) and *n*-propyllithium (0.73 M in ethyl ether, 120 ml) were reacted as described above, giving (*R*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-3-hexanone (*R*)-4 (7.51g, 64%). NMR and mass spectra were in accord with those of the (*S*)-2-diastereomers.

(S)-2-Hydroxy-3-hexanone [(S)-5] Pyridinium-*p*-toluene sulfonate (100 mg) was added to a stirred solution of (*S*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-3-hexanone (*S*)-4 (9.1 g, 45.5 mmol) in methanol (100 ml) at 0°C under argon. The reaction was allowed to warm to ambient temperature and stirred overnight. The methanol was removed by fractional distillation under reduced pressure. Water was added to the residue, and the mixture was extracted with Et_2O (4× 30 ml). The organic phase was washed with saturated $NaHCO_3$ and brine and dried, and the solvent was removed by distillation through a Vigreux column. The residue was purified by vacuum flash chromatography (silica gel, eluting with pentane/Et_2O 9:1), removing the solvent by distillation through a Vigreux column, followed by Kugelrohr distillation (34°C, 1.60 mmHg), giving 2.53 g (48%) of (*S*)-2-hydroxy-3-hexanone (*S*)-5. The moderate yield was primarily a result of losses during purification because of the volatility of the compound, rather than to any problem with the chemistry. ^{1}H-NMR: δ 0.92 (t, *J*=7.4Hz, 3H), 1.36 (d, *J*=7.2 Hz, 3H), 1.58–1.72 (m, 2H), 2.39 (dt, *J*=17.0 and 7.3 Hz, 1H), 2.48 (dt, *J*=17.0 and 7.2 Hz, 1H), 3.57 (d, *J*= 4.5 Hz, 1H), 4.22 (dq, *J*=4.5 and 7.2 Hz, 1H). ^{13}C-NMR: δ 13.96, 17.29, 20.03, 39.60, 72.80, 212.80. MS: 116 (M^+, 1), 87 (1), 83 (1), 74 (12), 73 (36), 72 (19), 71 (64), 55 (59), 45 (87), 43 (100), 41 (19).

(R)-2-Hydroxy-3-hexanone [(R)-5] (*R*)-2-Hydroxy-3-hexanone was obtained in 49.6% yield (2.10 g) from (*R*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-3-hexanone (*R*)-4 after purification. NMR and mass spectra were identical to those of (*S*)-2-hydroxy-3-hexanone.

(S)-2-[(Tetrahydro-2H-pyran-2-yl)oxy]-3-hexanol [(S)-6] A solution of (*S*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-3-hexanone (*S*)-4 (10.00 g, 49.9 mmol) in ethanol (25 ml) was added dropwise to a stirred suspension of $NaBH_4$ (1.89 g, 50 mmol) in 25 ml ethanol at 0°C, and the mixture was stirred overnight. The mixture was concentrated, 100 ml brine added to the residue, and the product was extracted with ethyl acetate (5×50 ml). The combined organic phases were dried and concentrated. Purification by vacuum flash chromatography (hexane/EtOAc—9:1) afforded 9.08 g (90%) of product (*S*)-6. MS (*m/z*, rel. intensity): 158 (2), 157 (2), 129 (2), 101 (10), 85 (100), 67 (12), 57 (23), 56 (12), 55 (22), 45 (20), 43 (28), 41 (29). Because there were four stereoisomers in the product mixture, ^{1}H- and ^{13}C-NMR spectra were complex and not useful.

(R)-2-[(Tetrahydro-2H-pyran-2-yl)oxy]-3-hexanol [(R)-6] Compound (*R*)-6 was obtained in similar fashion with a 93% yield from (*R*)-2-[(tetrahydro-2H-pyran-2-yl) oxy]-3-hexanone (*R*)-4. Mass and NMR spectra were in accord with the (*S*)-6 mixture.

(2S,3S)-2,3-Hexanediol ((2S,3S)-7) and (2S,3R)-2,3-hexanediol [(2S,3R)-7] A solution of (*S*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-3-hexanol (*S*)-6 (9.05 g, 45 mmol) and 100 mg *p*-toluenesulfonic acid in methanol (50 ml) was stirred overnight. After concentration, the residue was diluted with ethyl acetate (100 ml) and washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. Purification by vacuum flash chromatography (hexane/EtOAc—4:1) followed by Kugelrohr distillation (90–98°C, 4.5 mmHg) gave a mixture of (2*S*,3*S*)-2,3-hexanediol (2*S*,3*S*)-7 and (2*S*,3*R*)-2,3-hexanediol (2*S*,3*R*)-7 (48:52, respectively). The diastereoisomeric diols (6.18 g) were separated by flash chromatography in 2-g batches (hexane/acetone—5:1; column 32 cm long×5 cm diameter) followed by recrystallization at 4°C (50 ml hexane/g of diol) affording 2.55 g of pure (2*S*,3*S*)-2,3-hexanediol (diastereomeric excess [de]>98%) and 2.89 g of (2*S*,3*R*)-2,3-hexanediol (de>96%). (2*S*,3*R*)-7: ^{1}H-NMR: δ 0.96 (t, *J*=6.8 Hz, 3H), 1.15 (d, *J*=6.4 Hz, 3H), 1.30–1.46 (m, 3H), 1.46–1.60 (m, 1H), 1.99 (br s, 2H), 3.62–3.67 (m, 1H), 3.76–3.84 (m, 1H). ^{13}C NMR: δ 14.29, 16.84, 19.39, 34.13, 70.67, 74.86. MS: 103 (1), 85 (1), 75 (10), 73 (61), 72 (34), 57 (18), 55 (100), 45 (36), 43 (35), 41 (11). (2*S*,3*S*)-7: ^{1}H NMR: δ 0.94 (t, *J*=7.2 Hz, 3H), 1.19 (d, *J*=6.4 Hz, 3H),1.34–1.58 (m, 4H), 2.43 (br s, 2H), 3.31–3.37 (m, 1H), 3.59 quint, *J*=6.2 Hz, 1H). ^{13}C NMR: δ 14.26, 18.96, 19.70, 35.69, 71.13, 76.16. MS: *m/z*: 103 (1), 85 (1), 75 (16), 73 (59), 72 (35), 57 (21), 55 (100), 45 (38), 43 (37), 41 (12).

(2R,3S)-2,3-Hexanediol ((2R,3S)-7) and (2R,3R)-2,3hexanediol [(2R,3R)-7] In analogous fashion, a mixture of (2*R*,3*S*)-2,3-hexanediol (2*R*,3*S*)-7 and (2*R*,3*R*)-2,3-hexanediol (2*R*,3*R*)-7 (51:49, respectively) was obtained

in 92% yield from (*R*)-2-[(tetrahydro-2H-pyran-2-yl)oxy]-3-hexanol (*R*)-6. After purification, 2.51 g of (2*R*,3*S*)-2,3-hexanediol (de>97%) and 2.41 g (2*R*,3*R*)-2,3-hexanediol (de>98%) were obtained. NMR and mass spectra matched those of the (2*S*,3*R*)- and (2*S*,3*S*)-enantiomers, respectively.

Field Bioassays of Synthetic Pheromone Aeration extracts from male *M. caryae* contained eight male-specific compounds (see "Results"). Initially, we predicted that the hexanediols would have the greatest activity, based on pheromones that have been identified for closely related beetle species (see "Introduction"). Therefore, field bioassays of the individual components (including citral in place of neral and geranial) were first conducted before mixtures of compounds were tested. Bioassays were conducted at Allerton Park (Piatt, IL), a 600-ha mixed hardwood forest that harbors a population of *M. caryae*, between 22 and 31 May 2004 (skies clear, air temperatures ~19–28°C, average wind speed 8–20 kph). Sticky card traps ("mouse glue trap" cards, 12.6×22 cm, baited, Victor® Pest Control Products, Lititz, PA) were stapled to 1.5-m-tall wooden stakes, with the middle of the card ~1 m above the ground. Traps were positioned 10 m apart in a straight line approximately perpendicular to the prevailing wind direction. Traps were baited with a cotton dental wick that was loaded with 5 mg of the compound in 0.1 ml of methylene chloride and attached to the middle of the card. There were seven treatments that were randomly assigned to traps, as follows: racemic (2*R**,3*S**)-2,3-hexanediol, (*S*)-(−)-limonene, 2-phenylethanol, (−)-α-terpineol, nerol, citral, and a solvent control. Release rates of these lures were unknown, but similar lures loaded with 2,3-hexanediols remained attractive to *N. a. acuminatus* for at least 18 hr (Lacey et al. 2004). The study was replicated five times. For each replication, traps were set up at 11:00 hours, captured beetles were removed from traps the following morning, and the numbers and sex of beetles responding to treatments were recorded.

The 2004 bioassay was conducted late in the activity period for *M. caryae*, when populations were declining, and trap catches were low (see "Results"). Therefore, the bioassay was repeated on 8–20 May 2005 at the same site (skies clear, air temperatures ~16–27°C, wind speed 8–20 kph). By that time, we had discovered that adult beetles could extract themselves from sticky cards (Lacey et al. 2007b) and, therefore, adopted cross-vane flight-intercept panel traps (black, 1.2×0.30 m, Intercept™, model PT, APTIV, Portland, OR) that had proven efficient in capturing other species of cerambycid beetles (Lacey et al. 2007b). Traps were positioned as described above, and test chemicals were applied neat to cotton dental wicks (except [2*R**,3*S**]-2,3-hexanediols, which were dissolved in 0.025 ml of hexanes) in uncapped 1-dram vials that were hung in the open central slot of each trap (Lacey et al. 2007b). The study was replicated five times as described above. Too few beetles were captured in both the 2004 and 2005 studies to warrant statistical analysis (see "Results").

Field bioassays of blends of synthetic compounds were conducted in an abandoned plantation of mixed tree species ~1 km south of the campus of the UIUC in April to May 2006 (skies clear, air temperatures ~19–28°C, wind speed ~8–20 kph). A subtractive scheme was used to compare activity of the complete blend with seven treatments that each lacked a different component, as well as a solvent control. We used the same panel traps and lures as described above. The lures were baited with pheromone components, each diluted in 0.025 ml hexane, in ratios that approximated the mean relative abundances in extracts from aerations of males (see "Results") with two exceptions: (1) The relative proportions of hexanediols were increased because other cerambycine species had responded to relatively high quantities of similar compounds (see Lacey et al. 2004, 2007b) and (2) the total proportion of citral was decreased to compromise between the high proportion of geranial and lower proportion of neral that are produced by males (see "Results"). Lure quantities were: (2*S*,3*R*)-2,3-hexanediol (2.5 mg), (2*R*,3*S*)-2,3-hexanediol (0.5 mg), (*S*)-(−)-limonene (1.5 mg), 2-phenylethanol (5 mg), (−)-α-terpineol (5 mg), nerol (2 mg), citral (12 mg), and the control (0.175 ml hexane). Traps were positioned 8 m apart in transects approximately perpendicular to the direction of the prevailing wind. On each date, traps were baited at 10:00 hours, and beetles were collected at ~18:00 hours. Traps were cleaned with glass cleaner (Windex®, S. C. Johnson & Sons, Racine, WI) and lures replaced each day. The bioassay was replicated 14 times. Overall differences between treatments in numbers of beetles captured were tested by one-way analysis of variance (ANOVA), and differences between pairs of treatment means were subsequently tested with the least significant difference test (Analytical Software 2000). Three replicates that captured fewer than four beetles were excluded from the data analysis.

Results

Testing for Attractant Pheromones In olfactometer bioassays, 100% of female and 90% of male *M. caryae* responded by walking upwind. Of the 20 females that were tested, 19 were attracted to odor emitted by males and only one responded to odor of females. Males showed a similar response, with 16 responding to odor of males and only two responding to odor from females ($\chi^2_{1,\ 18}=21.8$, $P<0.001$).

Identification of Potential Pheromone Components GC-MS analysis of volatiles produced by male *M. caryae* revealed seven peaks that were absent in analogous samples of females. The first compound to elute (Fig. 2) was tentatively identified as 2,3-hexanediol, with a mass spectral base peak at *m/z* 55 (100) and characteristic mass fragments at *m/z* 75 (10), 73 (61), 72 (34), 45 (36), and 43 (35). The identification was confirmed by matching the mass spectrum and the retention time with those of an authentic standard of (2*R**,3*S**)-2,3-hexanediol; this diastereomer is completely resolved from the (2*R**,3*R**)-2,3-diastereomer on a DB5-MS column (Lacey et al. 2004). The enantiomeric composition of the insect-produced compound was determined to be a ~10:1 mix of (2*S*,3*R*)- and (2*R*,3*S*)-2,3-hexanediols by analysis on the Cyclodex-B column, with baseline resolution of the enantiomers. These diols represented 3.2±1.3% (*N*=7 aeration extracts) of the total male-specific compounds. The remaining compounds, in order of elution (Fig. 2), were (*S*)-(−)-limonene (3.1±1.7%), 2-phenylethanol (8.0±2.4%), (−)-α-terpineol (10.0±2.8%), nerol (2.1±1.5%), neral (63.3±7.3%), and geranial (8.8±2.4%). These compounds were tentatively identified by mass spectral fragmentation patterns, and the identifications were confirmed by retention time and mass spectral matches with authentic standards. The absolute configurations of those compounds that were chiral were determined by analyses and coinjections with appropriate standards on the Cyclodex-B GC column. Relative abundances of compounds varied between samples, with neral, 2-phenylethanol, (−)-α-terpineol, and geranial always being most abundant, whereas the hexanediols, (*S*)-limonene, and nerol were not detectable in some samples. We have found that beetles of other cerambycine species also produce pheromone sporadically under the conditions of aeration in closed chambers (unpublished data).

Syntheses of Pheromone Components Chiral 2,3-hexanediols were synthesized by modification and extension of the strategy developed by Hall et al. (2006) to produce chiral 2-hydroxy-3-decanones (Fig. 1). Thus, the alcohol function

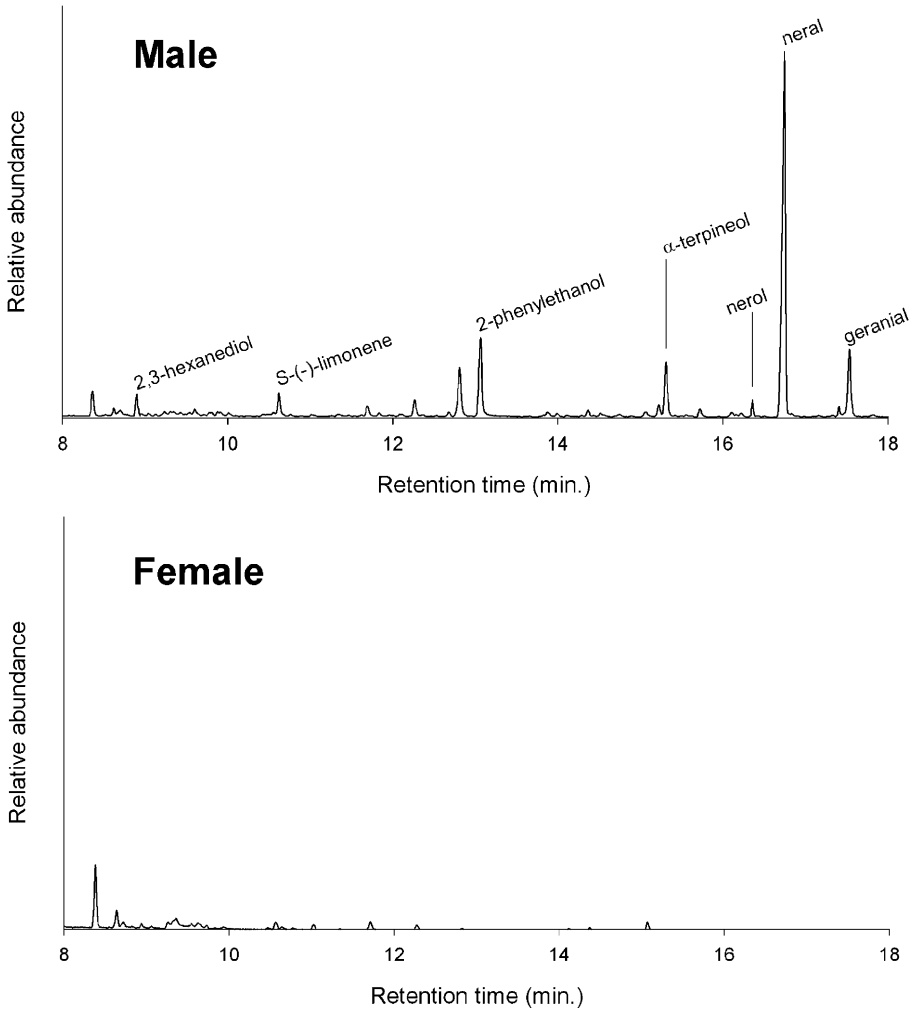

Fig. 2 Representative total ion chromatograms of extracts of headspace volatiles produced by male (*top*) and female (*bottom*) *Megacyllene caryae*

of ethyl (*S*)-(−)-lactate (*S*)-1 was protected as the tetrahydropyranyl (THP) ether (*S*)-2, followed by hydrolysis of the ester group to give the lithium salt of the carboxylic acid (*S*)-3 in quantitative yield. Alkylation of the salt with propyllithium in ether then yielded the THP-protected 2-hydroxy-3-hexanone (*S*)-4. (2*S*,3*R*)- and (2*S*,3*S*)-2,3-hexanediols were produced by reduction of THP-protected 2-hydroxy-3-hexanone (*S*)-4 with sodium borohydride in ethanol, giving the monoprotected diols mixture (*S*)-6. Acid-catalyzed removal of the THP group gave a diastereoisomeric mixture of (2*S*,3*R*)-2,3-hexanediol [(2*S*,3*R*)-7) and (2*S*,3*S*)-2,3-hexanediol ((2*S*,3*S*)-7]. The diastereomers were separated by flash chromatography on silica gel followed by recrystallization from hexanes, giving the two diols in high purity (>96% de). Analogous results were obtained for the syntheses of (2*R*,3*R*)-2,3-hexanediol [(2*R*,3*R*)-7) and (2*R*,3*S*)-2,3-hexanediol ((2*R*,3*S*)-7] starting from THP-protected hydroxyketone (*R*)-4.

Alternatively, removal of the THP group from compound 4 with acid catalysis in methanol gave (*R*)- and (*S*)-2-hydroxy-3-hexanones, (*R*)- and (*S*)-5, both of which are known pheromone components for cerambycid beetles (see Hanks et al. 2007). GC analysis on a Cyclodex-B column confirmed that no isomerization or racemization (see Lacey et al. 2007b) had occurred during the synthesis.

Field Bioassays of Synthetic Pheromone In field bioassays that tested the activity of individual compounds, sticky traps captured a total of two *M. caryae* in 2004, and panel traps captured a total of five in 2005. We concluded from this experiment that probably neither the 2,3-hexanediols nor any of the other compounds in the volatile blend produced by males were attractive to adult *M. caryae* as single components.

In bioassays that tested blends of components in 2006, traps captured 147 adult *M. caryae* (76 females and 71 males; sex ratio not significantly different from 1:1, $\chi^2_{1,\ 147}=0.17$, $P>0.05$). Treatments differed significantly in numbers of *M. caryae* that were captured (Fig. 3; sexes combined, overall ANOVA $F_{10,\ 147}=9.56$, $P<0.001$). The greatest numbers of beetles were captured in traps baited with the complete blend of pheromone components, whereas trap catches were reduced by ~50% in treatments that were missing (*S*)-(−)-limonene, 2-phenylethanol, (−)-α-terpineol, or nerol and reduced to levels not significantly different from controls in treatments missing (2*S*,3*R*)-2,3-hexanediol, (2*R*,3*S*)-2,3-hexanediol, or citral (Fig. 3).

Discussion

Attraction of both sexes of *M. caryae* to odor produced by live males in olfactometer bioassays provided the first evidence that males produce an aggregation pheromone. That this was an aggregation pheromone and not a sex pheromone was confirmed during field bioassays with synthetic pheromone, in which similar numbers of males and females were attracted to baited traps.

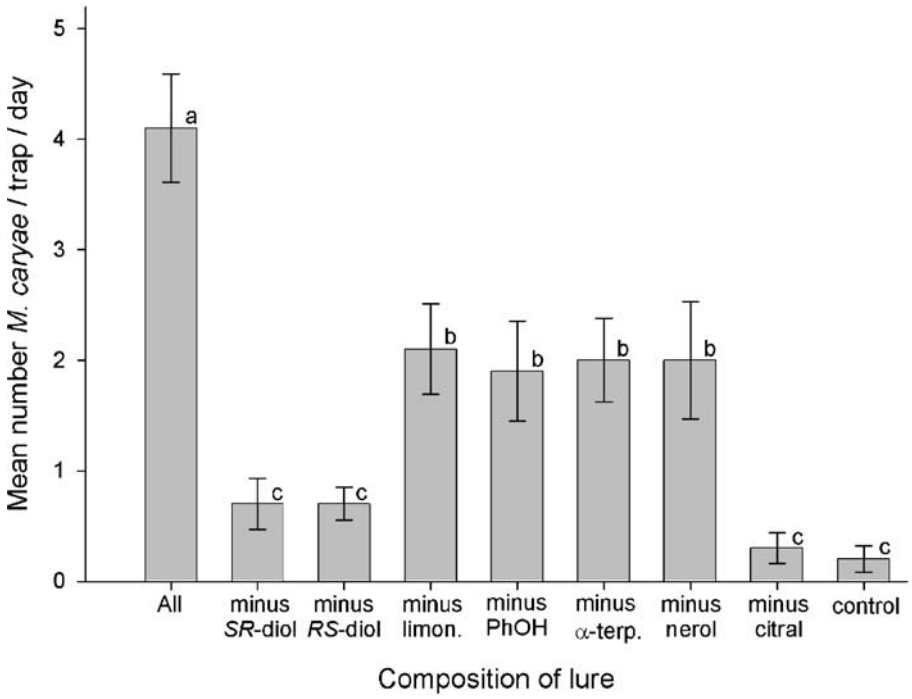

Fig. 3 Relationship between mean (±SEM) number of adult *Megacyllene caryae* caught in cross-vane panel traps (sexes combined) and composition of the lure. Treatments included the complete blend of synthetic components (All), and the complete blend minus (2*S*,3*R*)-2,3-hexanediol, (2*R*,3*S*)-2,3-hexanediol, (*S*)-(−)-limonene, 2-phenylethanol (*phoh*), (−)-α-terpineol, nerol, and citral (a 1:1 blend of neral and geranial), and a solvent control. *Means with different letters* are significantly different (LSD tests; $P<0.05$)

Although (2*S*,3*R*)- and (2*R*,3*S*)-2,3-hexanediol constituted only about 3 and 0.3%, respectively, of the total blend of volatile compounds produced by males, absence of either in synthetic blends resulted in a significant loss of activity. This finding indicated that adult *M. caryae* are sensitive to both components, unlike other clytine species that produce blends of enantiomers but respond strongly to the dominant component alone (see Hanks et al. 2007). Among the remaining components, one or both isomers of citral (neral or geranial) were equally as important to attraction as were the 2,3-hexanediols. Whereas the remaining components ([*S*]-[−]-limonene, 2-phenylethanol, [−]-α-terpineol, and nerol) increased attraction significantly, they were not necessary for attraction because beetles showed a partial response to blends lacking these compounds. The increased response of adult *M. caryae* to the complete blend of synthetic compounds confirmed that all of these compounds have a role in the natural pheromone blend.

The structure of the hexanediols produced by male *M. caryae* is consistent with the diol/hydroxyketone structural motif of pheromones reported for another 12 species in the subfamily Cerambycinae (see "Introduction"). To our knowledge, this motif is unique to cerambycine beetles. Whereas exceptions to the motif have been identified from two cerambycine species (see "Introduction"), the complex blend of compounds produced by male *M. caryae*, in addition to the components that are consistent with the structural motif, is unprecedented among cerambycines that have been studied to date.

All of the terpenoids and 2-phenylethanol produced by male *M. caryae* have been identified as pheromones of species in a broad range of insect and arachnid orders, serving a variety of behavioral functions (see Mayer and McLaughlin 1991). These compounds are also common in essential oils of plants (Budavari 1996), suggesting that beetles might acquire them from their host plants. However, it seems more likely that beetles produce the compounds *de novo* because adult male *M. caryae* from which volatile compounds were collected had been reared in the laboratory and so had not been exposed to host plants of adults.

Previous research suggests that the compounds produced by male *M. caryae* may have semiochemical functions in other species of cerambycids. For example, in electroantennograph studies of potential host plant volatiles, antennae of both sexes of *Xylotrechus pyrrhoderus* Bates and *Arhopalus tristis* (F.) responded to citral and α-terpineol, respectively (Iwabuchi et al. 1985; Suckling et al. 2001). Both α-terpineol and limonene were repellent to adult *Semanotus japonicus* Lacordaire in choice bioassays (Yatagai et al. 2002). Male *Xylotrechus quadripes* Chevrolat produce 2-phenylethanol, although it does not appear to be a pheromone component (Hall et al. 2006). A similar compound, 1-phenylethanol, is produced from metasternal glands of a congener of *M. caryae*, *M. robiniae*, and has been suggested to serve in defense against natural enemies (Wheeler et al. 1988). The fact that the terpenoids of *M. caryae* are produced by only one sex suggests that they are unlikely to have a defensive function.

The preparation of (2*S*,3*R*)- and (2*S*,3*S*)-2,3-hexanediols (and their enantiomers) in high diastereomeric and enantiomeric purity took advantage of the fact that during nonselective syntheses of mixtures of all four diol stereoisomers by the reduction of 2,3-hexanedione (Hanks et al. 2007), we observed that the diastereomeric 2,3-hexanediols were readily separable in gram quantities by flash chromatography. Thus, we reasoned that nonselective reduction of the ketone function of a chiral, THP-protected 2-hydroxy-3-hexanone precursor, in which the configuration at the carbon bearing the protected alcohol was fixed and known, should produce a mixture of the two diastereomers, each in high enantiomeric purity. Reduction of THP-protected (*S*)-2-hydroxy-3-hexanone (*S*)-4 gave an approximately 1:1 ratio of the two expected alcohol products. Removal of the THP-protecting group and separation of the resulting diol diastereomers by flash chromatography gave the (2*S*,3*R*)- and (2*S*,3*S*)-stereoisomers in quantities of several grams. Analogous reaction of the THP-protected (*R*)-2-hydroxy-3-hexanone (*R*)-4 gave the other two stereoisomers. Thus, this synthetic strategy provided ready access to all four 2,3-hexanediols in high stereoisomeric purity from readily available lactate ester synthons. In contrast, our previous syntheses of 2,3-hexanediol stereoisomers using the Sharpless asymmetric dihydroxylation protocols (Kolb et al. 1994) had produced diols with only 80–90% ee (Lacey et al. 2004). The synthetic strategy described above also has advantages in comparison to previously published syntheses of the 2,3-hexanediol stereoisomers, in which each stereoisomer was synthesized individually from a different chiral precursor (Schröder et al. 1994). It should also be noted that exactly the same strategy can be applied to the syntheses of the homologous 2,3-octanediols and 2,3-decanediols, compounds that also may be pheromone components of cerambycid beetles (e.g., Hall et al. 2006). This straightforward method of producing multigram quantities of all four stereoisomers of 2,3-alkanediols of any desired chain length should prove beneficial in the unraveling of pheromone blends for other cerambycid species.

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