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Syntheses and Determination of Absolute Configurations and Biological Activities of the Enantiomers of the Longtailed Mealybug Pheromone

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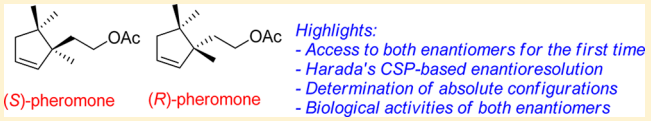
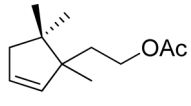
Supporting Information

ABSTRACT: Preparation and assignment of absolute configurations to both enantiomers of the sex pheromone of the longtailed mealybug, an irregular monoterpenoid with extraordinary biological activity, has been completed. Comparison of the biological activities of both enantiomers and the racemate in field trials showed that the (*S*)-(+)-enantiomer was highly attractive to male mealybugs, strongly suggesting that female longtailed mealybugs produce this enantiomer. The (*R*)-(−)-enantiomer was benign, being neither attractive nor inhibitory.



The longtailed mealybug *Pseudococcus longispinus* (Order Hemiptera: Family Pseudococcidae), is one of a group of small sucking insects that are widely distributed pests of agricultural crops and ornamental plants. Damage is caused by direct feeding, by the growth of sooty mold and other fungi on the honeydew excreted by the insects, and increasingly, through the transmission of plant pathogens.[1] Females of sexually reproducing mealybug species have been shown to produce powerful sex pheromones to attract males for mating, and some of these pheromones have been identified and commercialized for use in pest management.[2] In addition to their practical value, the structures of mealybug pheromones are intrinsically interesting because of their irregular terpenoid skeletons (Figure 1).[3]



sex pheromone (**1**)

Figure 1. Gross structure of the sex pheromone of the longtailed mealybug.

The pheromone of the longtailed mealybug was identified after collection of headspace odors produced by thousands of live unmated females over many weeks. Isolation by liquid and preparative gas chromatography produced a few micrograms of the pure material, sufficient for identification of the basic structure as 2-(1,5,5-trimethyl-2-cyclopent-2-en-yl)ethyl acetate **1**.[4] Several syntheses of the racemate have been developed, using different strategies.[4,5] The synthetic pheromone proved to be extremely biologically active; lures loaded with 25 μg of the racemate remained active for several months under field conditions.[6]

However, to date it has not been possible to determine the absolute configuration of the insect-produced compound because the pheromone, its corresponding alcohol, and several analogues were not resolved by GC on chiral stationary phases.[4] We report here the syntheses of both enantiomers of the pheromone, the determination of their absolute configurations, and the results of field trials testing their biological activity.

The most recently developed synthesis of the racemic pheromone progressed through the key intermediate **2**, in which the adjacent quaternary carbons were generated by a Claisen rearrangement of a readily accessible allylic alcohol precursor, followed by ring-closing metathesis of **2** to close the cyclopentene ring with the endo double bond in the correct position.[5c] Although the alcohol function in **2** was separated from the stereogenic center by two carbon atoms, we reasoned that the enantiomers still might be separable as diastereomeric derivatives of the alcohol. Furthermore, at least one of the two resulting diastereomers must be crystalline so that the relative and absolute configurations could be unambiguously determined from an X-ray crystal structure determination. After careful examination of the literature, Harada's camphorsultam phthalic (CSP) acid appeared to be a suitable candidate for the optically active auxiliary.[7] Thus, alcohol **2**, available in gram quantities from the synthesis of the racemate,[5c] was readily esterified (DMAP, DCC) with CSP acid to produce the mixture of diastereomers **3a** and **3b**. Although the diaster-

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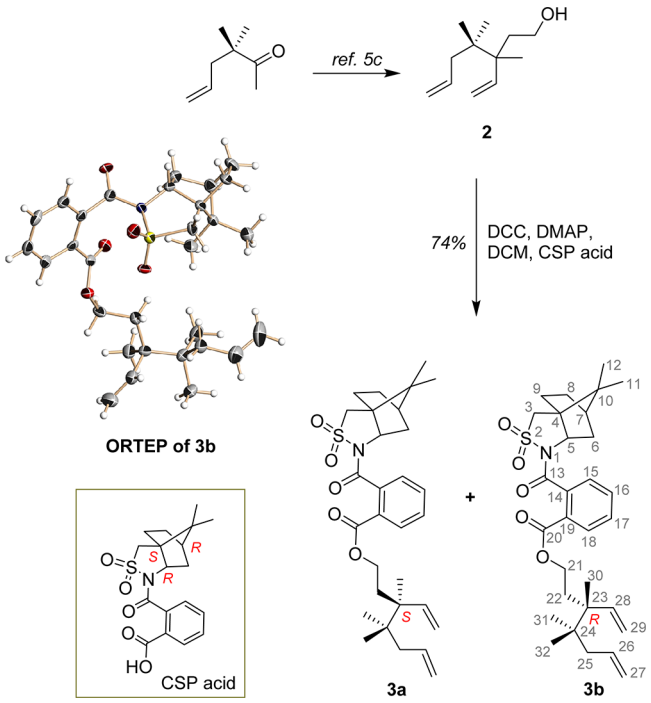
eomers were not separable on achiral stationary phases, they were resolved to baseline on an enantioselective column, eluting with *n*-hexane:EtOH:MeOH (90:10:2). NMR data of compounds **3a** and **3b** were very similar (See Table S1 in the Supporting Information).

After recrystallization from petroleum ether, the relative and absolute configurations of **3b** were determined through X-ray crystal structure analysis, revealing that the chiral center in the synthetic intermediate had the (*R*)-configuration (Scheme 1).

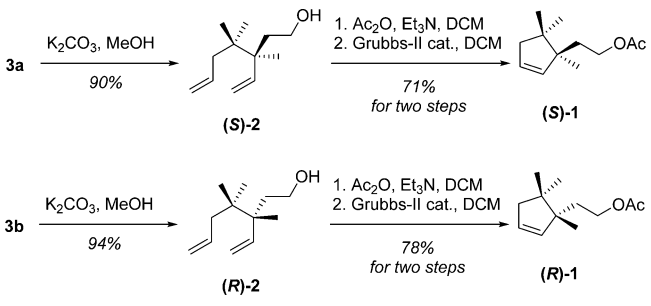
Scheme 1



Diastereomer **3a** was hydrolyzed with K_2CO_3 in MeOH to obtain alcohol (*S*)-**2**, which was subsequently transformed to the target pheromone (*S*)-(+)-**1** by acetylation followed by ring-closing metathesis as previously described (Scheme 2).[5c] As expected, the spectral data of (*S*)-(+)-**1** were in agreement with those of the racemate. The other diasteromer **3b** was converted to (*R*)-(−)-**1** in similar fashion.

With both enantiomers of the pheromone in hand, the biological activities of each enantiomer and the racemate were tested in field trials. The results showed that the (*S*)-(+)-enantiomer was highly attractive to male mealybugs (Figure 2), strongly suggesting that female longtailed mealybugs produce this enantiomer. The (*R*)-(−)-enantiomer was

Scheme 2



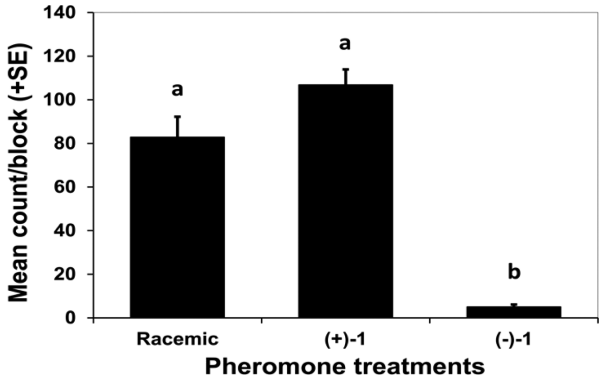


Figure 2. Male longtailed mealybugs caught in traps baited with each of the enantiomers of the pheromone, and the racemate. No mealybugs were caught in solvent treated controls.

only slightly more attractive than solvent-treated controls. This very slight attraction may have been due to the contamination of this enantiomer with 1.7% of the bioactive (*S*)-enantiomer. Furthermore, as seen with many other mealybug species,[2] the racemate was as attractive as the pure enantiomer, indicating that the (*R*)-(−)-enantiomer is not inhibitory. Thus, the cheaper and more readily synthesized racemic pheromone should be entirely adequate for practical applications in pest management.

EXPERIMENTAL SECTION

General Methods. All reactions were carried out in oven-dried glassware under argon or nitrogen unless otherwise specified, with magnetic stirring. Air sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus via rubber septa. All reagents, starting materials, and solvents were obtained from commercial suppliers and used without further purification. Reactions were monitored by thin layer chromatography (TLC) with 0.25 mm precoated silica gel plates (60 F254). Visualization was accomplished with either UV light, iodine vapors, or by immersion in ethanolic solutions of phosphomolybdic acid, *para*-anisaldehyde, or $KMnO_4$ followed by heating with a heat gun for ∼15 s. Flash column chromatography was performed on silica gel (100−200 or 230−400 mesh size). High resolution mass spectra (HRMS, ESI) were recorded with an ORBITRAP mass analyzer (Q Exactive). Mass spectra were measured with electrospray ionization with an MSQ LCMS mass spectrometer. Infrared (IR) spectra were recorded on a FT-IR spectrometer as thin films. Optical rotations were recorded on a polarimeter at 589 nm.

CSP Esters (3a and 3b). A solution of 3,4,4-trimethyl-3-vinyl-hept-6-en-1-ol **2** (50 mg, 0.27 mmol) and *N*-(2-carboxybenzoyl)-(−)-10,2-camphorsultam (131 mg, 0.36 mmol, prepared according to literature procedures[7a,b,8]) in CH_2Cl_2 (5 mL) was treated with dimethylaminopyridine (DMAP, 44 mg, 0.36 mmol) followed by dicyclohexylcarbodiimide (DCC, 61 mg, 0.30 mmol) at 0 °C. After stirring at rt for 24 h, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography yielding the mixture of diastereomers (105 mg, 74%). R_f = 0.2 (20% EtOAc:hexanes). The mixture was resolved by chiral preparative HPLC on a Daicel CHIRALPAK AD-H column (amylose tris(3,5-dimethylphenyl carbamate) coated on 5 μm silica gel; 30 mm id × 250 mm length), eluting with *n*-hexane/ethanol/methanol (90/10/2, v/v/v) at 40 mL/min and monitoring with a UV detector at 225 nm. The diastereomeric mixture was dissolved in the mobile phase (10 mg/mL), and 3 mL aliquots were injected. Diastereomers **3a** and **3b** eluted at 9 and 12 min, respectively. The purity of the collected fractions was checked on a 4.6 × 250 mm Chiralpak IA-3 column (3 μm particle size) eluted with *n*-hexane:EtOH (95/5), flow 1.0 mL/min, 25 °C, monitoring by UV at 224 nm.

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Diastereomer-I (3a): $[\alpha]^{26}_{D}$ −106.6° (*c* 0.2, $CHCl_3$); IR γ_{max} (thin film applied as $CHCl_3$ solution) 2963, 1721, 1686, 1635, 1336, 1300, 1168, 1112, 1083, 912, 776 cm^{-1}; HRMS (ESI) *m*/*z* calculated for $C_{30}H_{42}NO_5S$ $[M + H]^+$ 528.2778, found 528.2787; Chiral HPLC purity = 98.3% (96.6% ee); t_R:10.57 min. ^1H and ^{13}C NMR data are provided in Supporting Information.

Diastereomer-II (3b): $[\alpha]^{24}_{D}$ −71.0° (*c* 0.2, $CHCl_3$); IR γ_{max} (thin film applied as $CHCl_3$ solution) 2963, 1720, 1685, 1635, 1333, 1299, 1168, 1137, 1082 cm^{-1}; HRMS (ESI) *m*/*z* calculated for $C_{30}H_{42}NO_5S$ $[M + H]^+$ 528.2778, found 528.2787; Chiral HPLC purity = 99.3% (98.6% ee); t_R:13.63 min. ^1H and ^{13}C NMR data are provided in Supporting Information.

X-ray Crystal Structure Details. Single crystals of compound **3b** were obtained from petroleum ether. X-ray intensity data were collected on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (Mo Kα = 0.71073 Å) radiation at low temperature, 150(2) K. The X-ray generator was operated at 50 kV and 30 mA. Diffraction data were collected with a ω scan width of 0.5° and at different settings of φ and 2θ. The sample-to-detector distance was fixed at 5.00 cm. The X-ray data acquisition was monitored by the APEX2 program suite.[9] All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in the APEX2 program package.[9] The structures were solved by the direct method and refined by full matrix least-squares, on the basis of F^2, using SHELX-97.[10] Molecular diagrams were generated using XSHELL program integrated in SHELXTL package.[11] All the H-atoms were placed in geometrically idealized position (C−H = 0.95 Å for phenyl H-atoms, C−H = 0.99 Å for methylene H-atoms, C−H = 1.00 Å for methine H-atoms, and C−H = 0.98 Å for methyl H-atoms) and constrained to ride on their parent atoms [U_{iso}(H) = 1.2U_{eq}(C) for the phenyl, methylene, and methine group, and U_{iso}(H) = 1.5U_{eq}(C) for the methyl group]. Crystallographic data for **3b** ($C_{30}H_{41}NO_5S$): *M* = 527.70, Crystal dimensions 0.40 × 0.22 × 0.02 mm^3, monoclinic, space group $P2_1$, *a* = 9.8209(13), *b* = 11.2394(16), *c* = 13.1369(18) Å, β = 107.284(10)°, *V* = 1384.6(3) $Å^3$, *Z* = 2, ρ_{calcd} = 1.266 gcm^{-3}, μ(Mo Kα) = 0.157 mm^{-1}, *F*(000) = 568, $2\theta_{max}$ = 50.00°, *T* = 150(2) K, 8872 reflections collected, 4482 unique, 3055 observed ($I > 2\sigma(I)$) reflections, 340 refined parameters, *R* value 0.0524, *wR2* = 0.0902, (all data *R* = 0.0954, *wR2* = 0.1053), *S* = 0.996, minimum and maximum transmission 0.940 and 0.997; maximum and minimum residual electron densities +0.26 and −0.23 e $Å^{-3}$. The absolute configuration was established by anomalous dispersion effect (Flack parameter of 0.07(11)) in X-ray diffraction measurements, caused by the presence of the sulfur atom in the molecule.

(+)-3,4,4-Trimethyl-3-vinyl-hept-6-en-1-ol (*S*)-2. K_2CO_3 (282 mg, 2.04 mmol) was added to a solution of **3a** (90 mg, 0.170 mmol) in methanol at rt. After stirring for 2 h, the reaction mixture was concentrated under reduced pressure and purified by column chromatography to give (+)-3,4,4-trimethyl-3-vinyl-hept-6-en-1-ol (*S*)-**2** (28 mg, 90%): R_f = 0.4 (20% EtOAc:hexanes); $[\alpha]^{25}_{D}$ +1.7° (*c* 0.38, $CHCl_3$). The ^1H NMR spectrum was identical to that of the racemate. ^1H NMR: (400 MHz, $CDCl_3$) δ 0.82 (s, 6H) (gem-dimethyl), 0.98 (s, 3H) (quaternary methyl), 1.64−1.71 (m, 1H) (-$\underline{CH_2}$-CH_2−OH), 1.78−1.85 (m, 1H) (-$\underline{CH_2}$-CH_2−OH), 2.02 (d, *J* = 7.3 Hz, 2H) (allylic CH_2), 3.60 (t, *J* = 7.3 Hz, 2H) (CH_2-$\underline{CH_2}$−OH), 4.93−5.03 (m, 3H) (terminal olefin), 5.12 (dd, *J* = 10.8, 1.5 Hz, 1H) (terminal olefin), 5.76−5.95 (m, 2H) (internal olefin).

(+)-2-(1,5,5-Trimethylcyclopent-2-en-1-yl)ethyl acetate (*S*)-1. A solution of (+)-(*S*)-3,4,4-trimethyl-3-vinyl-hept-6-en-1-ol (*S*)-**2** (24 mg, 0.13 mmol) and Et_3N (73 μL, 0.52 mmol) in dry CH_2Cl_2 (3 mL) was treated with acetic anhydride (26 μL, 0.26 mmol) and a catalytic amount of DMAP (2.5 mg) at rt. After stirring for 2 h, the reaction mixture was concentrated under reduced pressure and directly purified by column chromatography to give (−)-(3,4,4-trimethyl-3-vinyl-hept-6-enyl) acetate (28 mg, 95%): R_f = 0.75 (20% EtOAc:hexanes); $[\alpha]^{23}_{D}$ −4.6° (*c* 0.1, $CHCl_3$). The ^1H NMR was identical to that of the racemate. ^1H NMR: (500 MHz, $CDCl_3$) δ 0.82 (s, 6H), 0.97 (s, 3H), 1.70−1.83 (m, 2H), 2.01−2.04 (m, 5H), 3.95−4.02 (m, 2H), 4.93−5.02 (m, 3H), 5.13 (dd, *J* = 11.0, 1.5 Hz, 1H), 5.77−5.84 (m, 2H).

A solution of (−)-(3,4,4-trimethyl-3-vinyl-hept-6-enyl) acetate (22 mg, 0.10 mmol) in dry CH_2Cl_2 (5 mL) was degassed for 10 min with a stream of argon and then treated with Grubbs' second generation catalyst (9 mg, 10 mol %; Aldrich, cat# 569747) in one portion. After stirring at 40 °C for 16 h, the mixture was treated with a drop of DMSO, and stirring was continued for 1 h. Evaporation of the solvent and purification by column chromatography furnished the (*S*)-(+)-2-(1,5,5-trimethylcyclopent-2-en-1-yl)ethyl acetate (*S*)-**1** (14.2 mg, 75%): R_f = 0.7 (20% EtOAc: hexanes); $[\alpha]^{25}_{D}$ +27.8° (*c* 0.16, CH_2Cl_2). The ^1H NMR matched that of the racemate. ^1H NMR: (400 MHz, $CDCl_3$) δ 0.89 (s, 3H), 0.94 (s, 3H), 0.95 (s, 3H), 1.52−1.58 (m, merged with $CDCl_3$ moisture, 1H), 1.66−1.74 (m, 1H), 2.04 (s, 3H), 2.13 (t, *J* = 1.6 Hz, 2H), 4.05−4.23 (m, 2H), 5.55−5.64 (m, 2H).

Diastereomer **3b** was converted to (*R*)-**1** in analogous fashion. Spectral data matched those of the corresponding enantiomers described above. Yields and optical rotations were as follows. Alcohol **2b**: Yield 94%; $[\alpha]^{25}_{D}$ −2.0° (*c* 0.15, $CHCl_3$). Acetate (*R*)-**2**: yield 94%; $[\alpha]^{25}_{D}$ +3.3° (*c* 0.15, $CHCl_3$). Pheromone (*R*)-**1**: yield 83%; $[\alpha]^{25}_{D}$ −24.0° (*c* 0.13, CH_2Cl_2).

Field Trial of the Pheromone Enantiomers and the Racemate. A field bioassay of the pheromone was conducted at a nursery in Bonsall, California, USA, in a 0.49 ha plot of *Ruscus hypoglossum* L. (plot coordinates: 33°17′18.36″ N, 117°16′55.61″ W elev 113 m) that was known to be infested with *P. longispinus*. The plot was divided into seven hoop houses (63 m long × 7 m wide), six of which were used in this study. Each house (block) was covered in plastic with open ends. Airflow between houses was not restricted because the plastic cover began 1 m above the plant canopy. Four delta sticky traps were spaced every 12.5 m along a transect within each house, suspended directly above the ruscus canopy. Each trap contained an 11 mm gray rubber septum impregnated with a hexane solution of one of four treatments: solvent control, 5 μg of (*S*)-(+)-enantiomer, 5 μg of (*R*)-(−)-enantiomer, 10 μg of the racemate. Treatments were assigned randomly along each transect. Traps were replaced, and treatments were repositioned once after 6 d. Traps remained in place for another 11 d. Trap count data were analyzed by analysis of variance after $\sqrt{(x + 0.5)}$ transformation of the data to meet the assumptions of normality and equal variances. Differences among means were tested using Tukey's honestly significant differences (HSD) test. There was no significant interaction between the two sampling periods (date) and the treatments (F = 3.23, df = 2, 30, and P = 0.054). Thus, data for each date were combined for the final analysis. There was both a significant effect of date (F = 10.19, df = 2, 32, P = 0.0032) and treatment (F = 130.04, df = 2, 32, P < 0.0001). Controls were not included in the analysis because their zero values and lack of variance violate the assumptions of ANOVA. Instead, confidence intervals were constructed, showing that the low trap counts for the (*R*)-(−)-enantiomer were significantly different than zero, i.e., that the (−)-enantiomer was slightly more attractive than controls.[12]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of chiral HPLC traces demonstrating enantiomeric purities of chiral intermediates; NMR data comparison of **3a** and **3b**; copies of NMR spectra and CIF file for the X-ray crystal structure of compound **3b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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