# Synthesis and Bioassay of Racemic and Chiral *trans*-α-Necrodyl Isobutyrate, the Sex Pheromone of the Grape Mealybug *Pseudococcus maritimus*

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A concise synthesis of the racemic form of the female-produced pheromone of the grape mealybug was developed. The synthesis was readily adapted to production of both enantiomers of the pheromone via lipase-catalyzed kinetic resolution of an intermediate in the synthesis. Replicated field trials revealed that, contrary to a preliminary report, the (*R,R*)- rather than the (*S,S*)-enantiomer is the attractive stereoisomer. Lithium aluminum hydride reduction of the insect-produced compound to α-necrodol followed by analysis on a chiral stationary phase GC column showed that the insect-produced material was actually an 85:15 mixture of the (*R,R*)- and (*S,S*)-enantiomers. The racemic form of the pheromone was highly attractive to male mealybugs, and in one of two field bioassays, the racemic material was significantly more attractive than the pure (*R,R*)-enantiomer, suggesting that the (*S,S*)-enantiomer might act synergistically.

KEYWORDS: α-Necrodol; *β*-necrodol; kinetic resolution; enantiomers; asymmetric synthesis

## INTRODUCTION

The grape mealybug, *Pseudococcus maritimus*, is a widely distributed pest of grapes and pome fruit in the United States (*1*, *2*). In 2007, we identified the irregular monoterpenoid *trans*-α-necrodyl isobutyrate as the female-produced sex pheromone of this species (*3*). The structure was confirmed by preparation of milligram quantities of the ester, via acylation of a sample of α-necrodol obtained as a gift (*4*). To further study this pheromone and determine its potential for practical applications in management of the insect, we required an efficient synthesis capable of producing the compound on gram scale.

Although the pheromone itself was a compound new to science, the core monoterpenoid structure α-necrodol was first isolated from another insect, the redlined carrion beetle *Necrodes surinamensis* (*5*). In light of its interesting structure as an irregular monoterpenoid, α-necrodol and its isomers have been the object of several synthetic efforts. It was first synthesized by Meinwald and co-workers in 1990, by a long and difficult route that used camphoric anhydride as a starting material, with the cyclopentane ring and several alkyl substituents already in place (*6*). Taber and Yu reported a shorter synthesis, using intramolecular insertion of an alkylidene into a C—H bond as a key transformation to build the multiply substituted cyclopentane ring (*7*). Both of these syntheses suffered from low yield and poor selectivity, with mixtures of *trans*- and *cis*-α-necrodol being obtained. Pamingle et al. reported an efficient method of isomerizing the exocyclic double bond of *β*-necrodol to the endocyclic double bond of

α-necrodol (*8*), but their synthesis of *β*-necrodol was inefficient (*9*). However, *β*-necrodol has been synthesized by several other routes (*10*−*15*), with that reported by Monti et al. being both short and proceeding with complete 1,3-diastereoselection (*14*). Here, we describe a convenient synthesis of the racemic grape mealybug sex pheromone by combination and further optimization of elements from several of these syntheses. We also describe syntheses of each of the enantiomers, based on the kinetic resolution of one of the intermediates in that synthesis (*14*), followed by taking each of the resolved enantiomers of the intermediate through the remainder of the steps in the synthesis. We also report the results of field trials testing both enantiomers and the racemate as lures in pheromone-baited traps.

## MATERIALS AND METHODS

**Synthesis of the Racemic Pheromone (Figure 1).** *General.* All solvents were Optima grade (Fisher Scientific, Pittsburgh, PA). Tetrahydrofuran (THF) was distilled from sodium/benzophenone under argon atmosphere. ^{1}H and ^{13}C NMR spectra were recorded with a Varian INOVA-400 (400 and 100.5 MHz respectively) spectrometer (Palo Alto, CA), as CDCl₃ solutions. ^{1}H NMR chemical shifts are expressed in ppm relative to residual CHCl₃ (7.27 ppm) and ^{13}C NMR chemical shifts are reported relative to CDCl₃ (77.16 ppm). Unless otherwise stated, solvent extracts of reaction mixtures were dried over anhydrous Na₂SO₄ and concentrated by rotary evaporation under reduced pressure. Crude products were purified by vacuum flash chromatography or column flash chromatography on silica gel (230−400 mesh; Fisher Scientific). Yields refer to isolated yields of chromatographically pure products. Mass spectra were obtained with a Hewlett-Packard (HP) 5890 GC (Avondale, PA) interfaced to an HP 5970 mass selective detector, in EI mode (70 eV) with helium carrier gas. The GC was equipped with an HP5-MS column (25 m × 0.20 mm i.d. × 0.33 μm film). Reactions with air- or water-sensitive

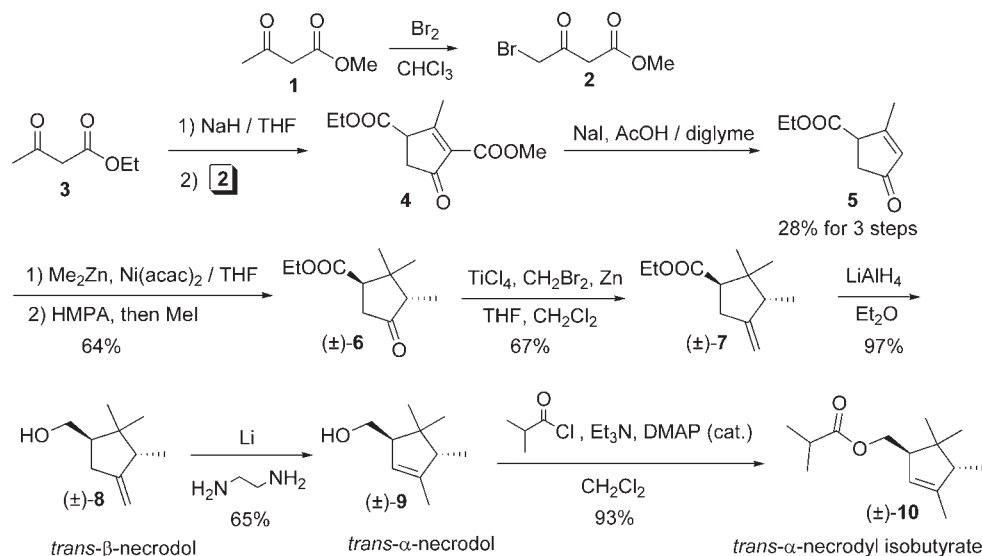*Corresponding author. Tel: 951 827 5821. Fax: 951 827 3086. E-mail: millar@ucr.edu.

**Figure 1.** Synthesis of racemic grape mealybug pheromone.

reagents were carried out in oven-dried glassware under argon atmosphere.

**2-Methyl-5-oxocyclopent-1-ene-1,3-dicarboxylic Acid 3-Ethyl Ester 1-Methyl Ester (4).** Methyl 4-bromoacetoacetate **2** was made by the method described for synthesis of ethyl 4-bromoacetoacetate (*16*, *17*). Thus, bromine (2.6 mL, 50 mmol, in 5 mL of CHCl₃) was added slowly to a solution of methyl acetoacetate **1** (5.81 g, 50 mmol; Aldrich Chemical Co., Milwaukee, WI) in CHCl₃ (45 mL) at 0 °C, and the mixture was stirred overnight while gradually warming to room temperature. Nitrogen was then passed through the solution for 30 min to flush off most of the HBr, and the solution was poured into ice−water. The organic layer was washed with brine, dried, and concentrated to give 9.28 g of **2** as a yellow oil. The crude product was used without further purification.

2-Methyl-5-oxocyclopent-1-ene-1,3-dicarboxylic acid 3-ethyl ester 1-methyl ester (**4**) was made by the method described for synthesis of 2-methyl-5-oxocyclopent-1-ene-1,3-dicarboxylic acid 1-ethyl ester 3-methyl ester (*18*). A three-neck round-bottomed flask under argon was charged with NaH (60% dispersion in mineral oil, 2.15 g, 53.7 mmol; Aldrich Chemical Co.) and THF (75 mL). The suspension was cooled in an ice−salt bath to −10 °C, and ethyl acetoacetate **3** (6.99 g, 53.7 mmol; Aldrich Chemical Co.) was added dropwise. The mixture was stirred for 1 h while gradually warming to room temperature, then **2** (5.24 g, 26.9 mmol) was added dropwise and the mixture was stirred for 2 h. The solvent was removed in vacuo, and ether was added to the residue. The resulting sodium salt of the enolate was collected by vacuum filtration and washed several times with ether to remove the mineral oil from the NaH dispersion and other neutral impurities. The salt then was taken up in 1 M HCl, and the mixture was extracted twice with ether. The combined organic extracts were washed with water and brine, dried, and concentrated to give 3.13 g of **4** as a pale yellow semisolid, as a mixture of the keto and enol forms. The crude product was used without further purification.

**2-Methyl-4-oxo-cyclopent-2-enecarboxylic Acid Ethyl Ester (5).** A mixture of **4** (3.13 g, 13.8 mmol), NaI (8.30 g, 55.3 mmol), acetic acid (1.5 mL) and diglyme (15 mL) was refluxed under Ar for 30 min. The mixture was cooled to room temperature, poured into water, and extracted with ether. The combined organic layer was washed with water and brine, dried, and concentrated. The crude product was purified by Kugelrohr distillation (0.25 Torr, forerun at 40 °C to remove residual diglyme, then 80 °C to collect desired product) to give 1.32 g (28% for 3 steps) of **5** as a colorless liquid. The ¹H NMR spectrum was in agreement with that reported in the literature (*19*).

**2,2,3-Trimethyl-4-oxo-cyclopentanecarboxylic Acid Ethyl Ester (6).** Me₂Zn (1.2 M in PhMe, 30 mL, 36 mmol; Alfa-Aesar, Ward Hill, MA) was added dropwise to a stirred suspension of **5** (2.00 g, 11.9 mmol) and Ni(acac)₂ (0.31 g, 1.19 mmol; Aldrich Chemical Co.) in THF (50 mL) at 0 °C under argon, and the mixture was stirred for 8 h while gradually warming to room temperature. The solution was cooled to −78 °C, and

dry HMPA (15 mL, 88 mmol) was added, followed after 15 min by MeI (7.4 mL, 119 mmol) in THF (20 mL). The solution was warmed to room temperature overnight, then poured into saturated aqueous NH₄Cl and extracted with ether. The combined organic layer was washed with water and brine, dried, and concentrated. The crude product was purified by flash chromatography (3.8 × 22.5 cm column eluted with hexanes/EtOAc, 9:1) to give 1.51 g (64%) of **6** as a colorless liquid. The ¹H NMR spectrum was in agreement with that previously reported (*14*).

**2,2,3-Trimethyl-4-methylene-cyclopentanecarboxylic Acid Ethyl Ester (7).** TiCl₄ (1.1 mL, 10 mmol; Acros Organics, Morris Plains, NJ) was added dropwise to a suspension of Zn dust (2.79 g, 42.7 mmol; Spectrum Chemical Co., Gardena, CA) and CH₂Br₂ (0.99 mL, 14 mmol; Acros Organics) in THF (25 mL) under argon at −40 °C. The mixture was warmed to 4 °C and stirred at this temperature for 3 days to produce a thick gray slurry of the active reagent. To this slurry was added CH₂Cl₂ (5 mL), then **6** (0.79 g, 4 mmol) in CH₂Cl₂ (5 mL). The mixture was stirred for 2 h while gradually warming to room temperature, then diluted with ether and poured into cold saturated aqueous NaHCO₃. The resulting mixture was filtered through Celite, and extracted with ether. The combined organic layer was washed with water and brine, dried, and concentrated. The crude product was purified by flash chromatography (1.9 cm × 18 cm column, eluted with 5% Et₂O in hexanes) to give 0.53 g (67%) of **7** as a colorless liquid. The ¹H NMR spectrum was in agreement with that previously reported (*14*).

**(2,2,3-Trimethyl-4-methylene-cyclopentyl)methanol (β-Necrodol, 8).** A solution of **7** (0.39 g, 2 mmol) in ether (5 mL) was added to a suspension of LiAlH₄ (0.19 g, 5 mmol) in ether (15 mL), and the mixture was stirred at room temperature for 1 h. The mixture was cooled in an ice bath, and water (0.19 mL), 15% NaOH solution (0.19 mL), and water (0.57 mL) were added successively dropwise. After stirring 10 min, the granular precipitate was removed by filtration through Celite, the filtrate was dried and concentrated, and the crude product was purified by vacuum flash chromatography (30 mL sintered glass funnel, eluting with hexanes/ EtOAc, 19:1 to 5:1) to give 0.30 g (97%) of **8** as a colorless liquid. The ¹H NMR spectrum was in agreement with that reported in the literature (*14*).

**(3,4,5,5-Tetramethylcyclopent-2-enyl)methanol (α-Necrodol, 9).** Ethylenediamine (5.0 mL) was heated at 90 °C and Li wire (0.27 g, 38.9 mmol; Aldrich Chemical Co.) was added. After the initial deep blue solution turned light yellow, the temperature was lowered to 70 °C and **8** (1.00 g, 6.48 mmol) in ethylenediamine (1 mL) was added. After 9 min, the mixture was poured into ice, and extracted with ether. The combined organic layer was washed with saturated aqueous NH₄Cl and brine, dried, and concentrated. GC analysis indicated that the crude product consisted of α-necrodol (81%), γ-necrodol (18%), and the starting material β-necrodol (1%). The crude product was purified by flash chromatography (3.1 cm × 22.5 cm column eluted with hexanes/EtOAc, 9:1 (α-necrodol
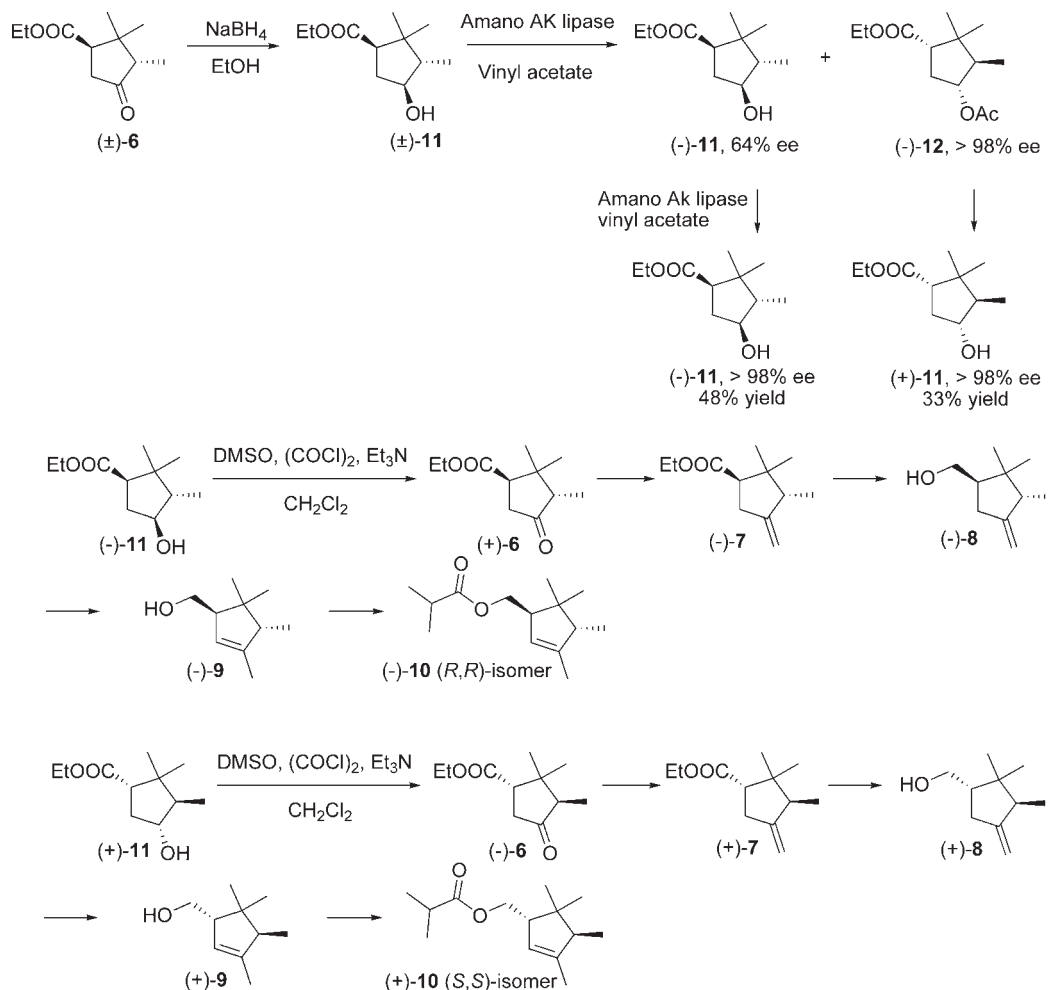
**Figure 2.** Synthesis of the (*R,R*)- and (*S,S*)-enantiomers of the grape mealybug pheromone via kinetic resolution of a key intermediate.

$R_f$ = 0.17, γ-necrodol $R_f$ = 0.13) to give 0.65 g (65%) of **9** as a colorless liquid. The $^1$H NMR spectrum was in agreement with that previously reported (*8*).

**Isobutyric Acid 3,4,5,5-Tetramethylcyclopent-2-enylmethyl Ester (*trans*-α-Necrodyl Isobutyrate, 10).** Isobutyryl chloride (0.53 mL, 5.0 mmol) was added dropwise to a solution of **9** (0.31 g, 2.0 mmol), Et₃N (0.84 mL, 6.0 mmol), and DMAP (0.024 g, 0.2 mmol) in CH₂Cl₂ at 0 °C. The mixture was stirred at room temperature overnight, then poured into water and extracted with ether. The combined organic layer was washed with saturated aqueous NaHCO₃, 1 M HCl, and brine, then dried and concentrated. The crude product was purified by vacuum flash chromatography (30 mL sintered glass funnel, eluted with 3% Et₂O in hexanes) to give 0.42 g (93%) of **10** as a colorless liquid. The $^1$H NMR spectrum was in agreement with that reported in our preliminary, milli-gram scale synthesis (*3*). $^{13}$C NMR (CDCl₃, 100 MHz): δ 177.3 (C), 145.4 (C), 123.2 (CH), 64.7 (CH₂), 52.7 (CH), 52.6 (CH), 43.1 (C), 34.2 (CH), 24.7 (CH₃), 24.1 (CH₃), 19.13 (CH₃), 19.09 (CH₃), 15.3 (CH₃), 12.5 (CH₃).

**Synthesis of the Pheromone Enantiomers (Figure 2).** *Ethyl 4-Hydroxy-2,2,3-trimethylcyclopentanecarboxylate (11).* This compound was made from reduction of (±)-**6** with sodium borohydride following the literature procedure (*14*).

*General Procedure for Lipase-Catalyzed Acylation of (±)-11.* The enzymatic transesterification was carried out following the literature procedure (*14*) with the exception that 44% conversion after 8 h was reported, whereas in our hands, only 40% conversion was achieved after 12 h. Separation by flash chromatography afforded the less reactive alcohol (−)-**11** (64% ee) and the acetate (−)-**12**, which was hydrolyzed with K₂CO₃/MeOH to afford alcohol (+)-**11** (>98% ee, determined by chiral stationary phase GC). The enantiomerically enriched alcohol (−)-**11** was subjected to the enzymatic transesterification conditions again. After 24 h, the more reactive enantiomer of the starting material had been

completely consumed and, after purification, (−)-**11** was obtained in >98% ee. Thus, 0.46 g of the racemate yielded 0.15 g of (+)-**11** (33%) and 0.22 g of (−)-**11** (48%). Enantiomeric purities were determined by analyses on a Cyclodex B chiral stationary phase GC column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA) at 110 °C isothermal.

*(+)-(1R,3S)-Ethyl 2,2,3-Trimethyl-4-oxocyclopentanecarboxylate ((+)-6).* This compound was prepared by Swern oxidation of (−)-**11** following the literature procedure (*14*). The $^1$H NMR spectrum was identical to that of (±)-**6**.

*(−)-(1S,3R)-Ethyl 2,2,3-Trimethyl-4-oxocyclopentanecarboxylate ((−)-6).* This was made by Swern oxidation of (+)-**11**. The $^1$H NMR spectrum was identical to that of (±)-**6**.

Each of the chiral ketoesters **6** was then carried through the synthesis described above for the racemate, producing the enantiomers of the pheromone in comparable yields. Because the pheromone enantiomers were not resolved on the Cyclodex B chiral stationary phase column, the enantiomeric purities of the penultimate intermediates, the α-necrodol enantiomers, were determined instead, using a temperature program of 50 °C/1 min, then 3 °C/min to 200 °C/4 min.

**Determination of Absolute Configuration of the Insect-Produced Pheromone.** Approximately 500 ng of grape mealybug pheromone, isolated from aerations of the insects, was recovered from the NMR sample used in the identification of the compound (*3*), and was transferred to a 1 mL glass culture tube in 50 μL of pentane. Most of the solvent was evaporated under a gentle stream of nitrogen, and LiAlH₄ solution (20 μL, 5 mg/mL in ether) was added. The tube was sealed, and vortexed periodically over 2 h. Pentane (100 μL) was added, followed by aqueous HCl (100 μL, 0.1M). The mixture was vortexed vigorously, then let stand for 5 min for the layers to separate. The pentane layer was removed with a syringe into a conical vial, and the aqueous residue was extracted again with pentane. The combined pentane extracts were carefully concentrated

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to ~50 μL under a gentle stream of nitrogen while the vial was chilled in an ice bath. Equivalent samples of the synthetic racemic, (*R*,*R*)-, and (*S*,*S*)-enantiomers of the pheromone were submitted to the same procedure to verify that no side products were generated that might interfere with the subsequent analyses of the samples.

Aliquots of the resulting samples were analyzed by splitless injections on the chiral stationary phase Cyclodex B column, using the temperature program described above. The sample from the insect-produced compound was found to be an 85:15 mixture of the (*R*,*R*)-, and (*S*,*S*)-enantiomers of α-necrodol. The results were confirmed by coinjection of the insect-produced compound with synthetic racemic α-necrodol.

**Field Bioassay of the Racemic and Chiral Pheromone.** Stock solutions of the racemic pheromone (1 mg/mL) and each of the enantiomers (0.5 mg/mL) were made up in hexane, and dispensed onto 11 mm gray rubber septa (West Pharmaceutical Services, Lionville, PA) (25 μL/septum, or 25 μg of racemic pheromone and 12.5 μg of each of the enantiomers), allowing the solvent to evaporate and the pheromone to be adsorbed into the septum matrix in a fume hood. Control lures were treated with 25 μL of clean hexane. Each treatment was replicated 5 times, with lures being deployed in sticky Delta traps (Trécé Inc., Stillwater, OK), in each of two Crimson Seedless grape variety vineyards near Delano, CA. One vineyard had a high density mealybug population (traps were hung from 14 to 27 August, 2008), whereas the other had a relatively low mealybug population level (traps were hung from 28 August to 26 September, 2008). At both sites, traps were spaced ~10 m apart, with the order of treatments being randomized in each block of four treatments. Trap catch data were analyzed by analysis of variance (ANOVA), followed by Tukey's pairwise comparison to separate means (α = 0.05).

## RESULTS AND DISCUSSION

The synthesis commenced with bromination of methyl acetoacetate to give methyl 4-bromoacetoacetate, using conditions previously reported for the synthesis of the analogous ethyl ester (*16, 17*). This procedure proved superior to those in earlier reports (*19, 20*), which resulted in a higher percentage of side products. The crude bromoester was coupled with the enolate of ethyl acetoacetate, prepared from ethyl acetoacetate and NaH in THF, and the resulting diketodiester underwent intramolecular Knoevenagel condensation in the same pot, producing diester **4** (*18, 19*). Fortuitously, after the condensation reaction was complete, the weakly acidic diester product was obtained as the sodium salt of the enolate, which was relatively insoluble in ether. Thus, removal of the THF solvent followed by trituration of the resulting solids with ether effectively removed both the neutral organic side products and the mineral oil from the NaH dispersion. The remaining solids were then taken up in 1 M HCl, and the neutralized diester was readily extracted with ether, as a mixture of the keto and enol forms. The mixture was then decarboxylated with NaI and AcOH in refluxing diglyme (*19*), giving ketoester **5** in 28% purified yield over three steps. In the above reaction sequence, the relative positions of the methyl and ethyl esters were critically important; during optimization of the reaction parameters, it was found that if the methyl and ethyl ester components were switched by starting the sequence with ethyl 4-bromoacetate and methyl acetoacetate respectively, the decarboxylation reaction gave only a complicated mixture of products.

Conjugate addition of a methyl group to ketoester **5** using dimethylzinc with nickel catalysis occurred smoothly, but the conditions for methylation of the resulting enolate in the same pot with methyl iodide proved to be critically dependent on solvent. Methylation occurred readily using HMPA as cosolvent (*14*), whereas use of the less toxic DMPU in place of HMPA resulted in no methylation of the enolate after the conjugate addition. The stereochemical course of the reaction was also very sensitive to the reaction conditions. In the literature report of this reaction sequence (*14*), the desired *trans*-isomer was apparently obtained as a single product. In our hands, using commercial Me$_2$Zn

(2.0 M in toluene, from Aldrich Chemical Co.), an ~80:20 mixture of *trans:cis* isomers was obtained. However, when the reaction was repeated using Me$_2$Zn from a different source (1.2 M in toluene, from Alfa-Aesar), with the volume of THF being adjusted to keep the ratio of THF to toluene constant, the *trans*-isomer was obtained as >95% of the product mixture. The reason for this improved selectivity is not clear.

The final carbon of the skeleton was attached by methylenation of ketone **6** in 67% yield, using the CH$_2$Br$_2$−Zn−TiCl$_4$ reagent as previously described (*14*), with the exception that the ketone was added to the thick slurry of the preformed reagent rather than *vice versa* because the thick slurry was difficult to transfer by cannula. Reduction of ester **7** in almost quantitative yield with LiAlH$_4$ in ether then gave β-necrodol **8**. The subsequent isomerization of the *exo* double bond to give α-necrodol was carried out with the lithium salt of ethylenediamine (*8*). The yellow solution of the salt was most easily produced at elevated temperatures (90 °C); at room temperature, a deep blue solution of the dissolved metal was obtained, rather than the strongly basic salt required for the isomerization. The isomerization reaction also required careful timing, because α-necrodol slowly isomerized further to the tetrasubstituted γ-necrodol, which became the sole product after 15 h. The initial preference for formation of α-necrodol is presumably due to the more facile removal of an allylic proton on C(5) rather than on C(3), because the CH$_2$O$^-$Li$^+$ group on C(1) is *cis* to C(3)-H, making that proton less accessible than C(5)-H. This first isomerization is then followed by the slower, second isomerization to the thermodynamically most stable tetrasubstituted alkene. The synthesis was completed by acylation of α-necrodol with isobutyryl chloride and Et$_3$N, with a catalytic amount of *N,N*-dimethylaminopyridine. The overall yield for the entire synthetic sequence was 7%.

In our initial report of the identification of the grape mealybug pheromone, an authentic sample of the pheromone was prepared in milligram amount by acylation of a sample of α-necrodol obtained as a gift (*4*), and thought to be the (*S*,*S*)-enantiomer (*3*). The resulting isobutyrate ester gave a single peak when analyzed on a chiral phase Cyclodex B GC column, but without having either the racemate or the (*R*,*R*)-enantiomer available for comparison, it was not possible to conclusively determine the enantiomeric purity of the synthesized pheromone. In a small-scale trial in a greenhouse, this material appeared to be attractive to male grape mealybugs (*3*). Because of the uncertainty with regard to the enantiomeric purity of the synthetic pheromone sample, we decided that an unequivocal synthesis of the two enantiomers would be prudent to verify these preliminary results. Fortunately, both enantiomers were readily accessible via a known synthesis of the enantiomers of β-necrodol that employed an enzyme-catalyzed kinetic resolution (*14*) of alcohol **11** derived from racemic ketoester **6** (**Figure 2**). Furthermore, the relative and absolute configurations of the enantiomers of **11** had been unequivocally demonstrated by reaction of the (+)-enantiomer with (−)-(1*S*,4*R*)-camphanic acid chloride, followed by determination of the crystal structure of the resulting camphanate ester by X-ray crystallography (*14*).

Thus, racemic ketoester **6** was stereoselectively reduced to (±)-alcohol **11**, which was then subjected to lipase-assisted kinetic resolution with Amano AK lipase and vinyl acetate as previously described (*14*). Galano et al. had reported that this reaction resulted in 44% conversion after 8 h, whereas in our hands, only 40% conversion was achieved after 12 h. Thus, longer reaction time, or use of a higher proportion of lipase was required in order to get the reaction to go to completion. After adjustment of the reaction conditions, satisfactory yields of unreacted (−)-**11** (>98% ee) and the acetate (−)-**12** (>98% ee) were obtained.
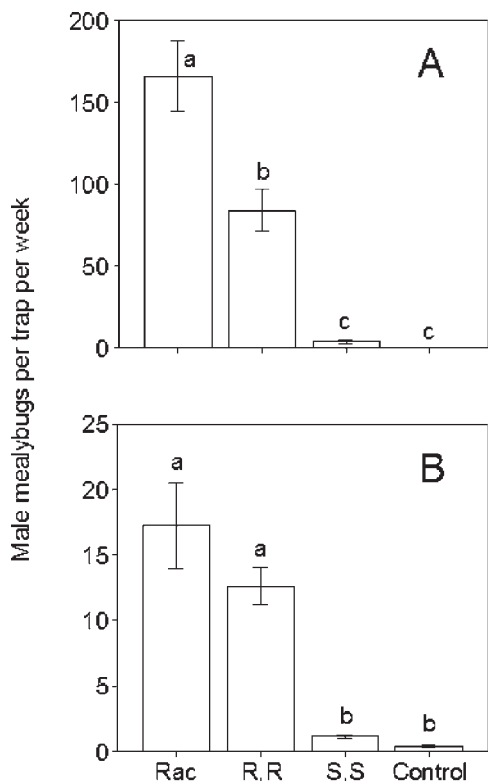
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**Figure 3.** Mealybugs (±SE) caught in pheromone traps baited with the racemic pheromone (25 $\mu$g), the ($R,R$)- and ($S,S$)-enantiomers (12.5 $\mu$g), or control lures. Treatments surmounted by different letters are significantly different. Vineyard **A**, high population density: $F_{3,16}$ = 38.9, $P$ < 0.001. Vineyard **B**, low population density: $F_{3,16}$ = 21.7, $P$ < 0.001.

Swern oxidation of (−)-**11** gave (+)-**6**, which was then carried through the synthesis described above for the racemate to give ($R,R$)-(−)-**10**. Similarly, after hydrolysis of acetate (−)-**12**, alcohol (+)-**11** was used to produce ($S,S$)-(+)-**10**.

In two field bioassays of racemic, ($R,R$)-(−)-**10**, and ($S,S$)-(+)-**10**, the racemate was clearly as attractive as ($R,R$)-(−)-**10** (**Figure 3**), whereas the numbers of male mealybugs caught in traps baited with ($S,S$)-(+)-**10** were no different from the numbers caught in untreated controls. The fact that the insects responded to ($R,R$)-(−)-**10** rather than ($S,S$)-(+)-**10** appeared contrary to the earlier results described above (*3*), which had suggested that the insect might produce ($S,S$)-(+)-**10**. Thus, the bioassay was repeated, with analogous results, demonstrating unequivocally that ($R,R$)-(−)-**10** was the attractive enantiomer. To determine the cause of the apparently anomalous results from the preliminary bioassays done with the small amount of pheromone prepared from a donated sample of α-necrodol thought to be the ($S,S$)-enantiomer, and with both enantiomers of the pheromone and α-necrodol in hand, the enantiomers of the pheromone and of α-necrodol were analyzed on a chiral stationary phase Cyclodex B GC column. Whereas the enantiomers of the pheromone were not resolved, the enantiomers of α-necrodol separated readily. Analysis of the donated sample of α-necrodol revealed that, in fact, it consisted of a 92:8 ratio of the ($S,S$)- and ($R,R$)-enantiomers. Because the unnatural ($S,S$)-enantiomer is not antagonistic (**Figure 3**), the small amount of ($R,R$)-**10** in this sample of the synthesized pheromone had apparently been sufficient to attract some male mealybugs in our 2007 trial (*3*).

To further confirm these results, a sample of pheromone isolated from headspace volatiles of virgin female mealybugs was reduced with LiAlH$_4$. Analysis of the resulting α-necrodol on the Cyclodex B GC column revealed that the insect-produced compound appeared to be an 85:15 mixture of the ($R,R$)- and ($S,S$)-enantiomers. Thus, the fact that the insect produces a scalemic mixture of the pheromone enantiomers rather than one pure enantiomer may provide an explanation for the racemic material being significantly more attractive than the ($R,R$)-enantiomer in the first field trial.

In summary, the syntheses described above provide access to both the racemic grape mealybug pheromone and its enantiomers. In greenhouse bioassays, the racemate and the ($R,R$)-enantiomer were similar in biological activity, indicating that the more easily made racemate is satisfactory for detection and monitoring of this insect with pheromone-baited traps. The pheromone is currently being used in surveys of vineyards throughout the western United States, to detect and delineate populations of this pest species.

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