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Sex Pheromone of the Longtailed Mealybug: A New Class of Monoterpene Structure

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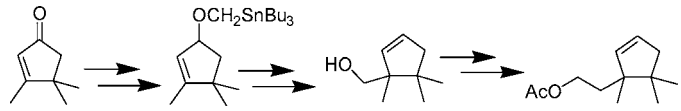
ABSTRACT



The sex pheromone of the longtailed mealybug, identified as 2-(1,5,5-trimethylcyclopent-2-en-1-yl)ethyl acetate, represents the first example of a new monoterpenoid skeleton. A [2,3]-sigmatropic rearrangement was used in a key step during construction of the sterically congested tetraalkylcyclopentene framework.

The longtailed mealybug, *Pseudococcus longispinus*, is a widely distributed polyphagous insect species that infests crops such as grapes, citrus, apples, pears, and a wide variety of ornamental plants.[1] The sessile females produce a pheromone that is highly attractive to the ephemeral, winged males. As part of an ongoing study of the unusual chemistry of the terpenoid pheromones of mealybugs in the Pseudococcidae family,[2] we report here the identification and synthesis of the sex pheromone of this species.

To collect sufficient pheromone to identify, large numbers of unmated female mealybugs were required as sources of pheromone. Thus, colonies of immature mealybugs reared on squash fruit were treated with a discriminating dose of pyriproxifen, an insect growth regulator, to selectively kill the males,[3] which must undergo a complete metamorphosis to the winged adult form. The females, which do not undergo full metamorphosis, are much less susceptible to pyriproxifen. The resulting cohorts containing only unmated females were placed in glass chambers swept with a continuous flow of clean air, with activated charcoal traps at the air outlets being used to collect the headspace odors. Comparisons of the gas chromatography profiles of the resulting extracts from cohorts of sexually mature and immature female mealybugs on squash, and from squash fruit controls, showed one compound (Kovat's index [KI] 1327 on an HP-5MS GC column)[4] that was consistently present only in the headspace volatiles from sexually mature females. The 70 eV EI mass spectrum of the compound showed a base peak at *m*/*z* 109 amu, with *m*/*z* 136 amu being the largest mass ion detected, suggestive of a monoterpenoid structure. The compound was hydrolyzed by treatment with NaOH in EtOH, indicative of an ester, and the resulting alcohol showed an EI-MS base peak at *m*/*z* 109 amu and a small molecular ion at *m*/*z* 154 amu, suggestive of a monoterpene alcohol with molecular formula $C_{10}H_{18}O$, with two rings or unsaturations. The KI

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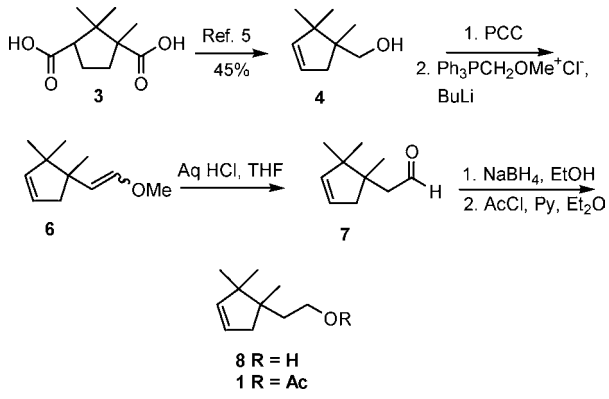
value of the alcohol (1197), different by only 130 KI units from the parent compound, indicated that the ester had to be an acetate. Catalytic reduction of the parent compound or the alcohol (5% Pd on carbon, H_2) resulted in the uptake of two hydrogens, indicating one carbon−carbon double bond present; thus, the final site of unsaturation had to be a ring. Furthermore, the facts that reduction of either the parent compound or its alcohol produced only a single product in each case suggested that the double bond was endocyclic and 1,2-disubstituted, because reduction of a tri- or tetra-substituted endocyclic double bond or a double bond exo to the ring would likely have produced more than one stereoisomeric product in each case.

To complete the identification, headspace extracts collected from multiple cohorts of virgin female mealybugs over more than one year were pooled, concentrated, and fractionated by normal phase HPLC (2% ether in pentane isocratic). The fraction enriched in the pheromone compound was further purified by preparative gas chromatography (SP-1000 packed column, 135 °C isothermal), yielding a few micrograms of the pure compound. The sample in deuterobenzene was analyzed by microprobe NMR spectroscopy. The resulting proton spectrum showed four methyl singlets, with the first at 1.72 ppm corresponding to the acetate methyl, and the other three at 0.86, 0.84, and 0.77 attributed to methyls on quarternary carbons. A broadened singlet integrating for two protons at 1.98 ppm suggested an isolated allylic CH_2 group. Two geminally coupled, one-proton ddds at 1.66 and 1.48 ppm, which were further coupled to two, one-proton ddds at 4.19 and 4.09 ppm suggested a $-CH_2CH_2OAc$ subunit attached to a chiral quaternary carbon, which rendered the protons in each of the CH_2 groups diastereotopic. The final two, one-proton multiplets at 5.51 and 5.46 were assigned to alkene protons on a 1,2-disubstituted alkene in the ring. In total, these data suggested two possible structures, **1** and **2** (Schemes 1 and 2). The available NMR data did not allow

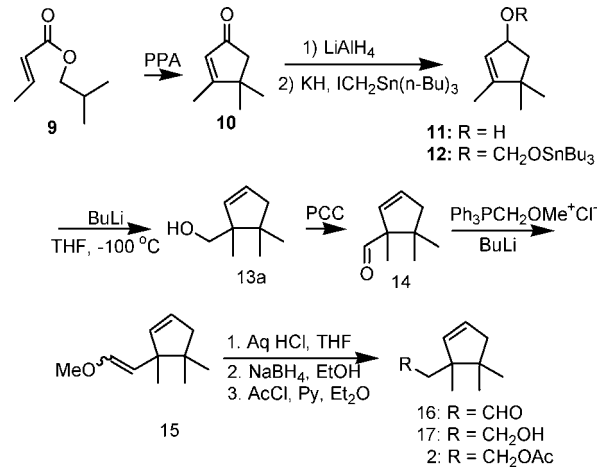
Scheme 1. Synthesis of First Pheromone Candidate (**1**) from Camphoric Acid



definitive determination as to which was the correct structure, so both were synthesized.

Examination of the literature revealed that either enantiomer of (1,2,2-trimethylcyclopent-3-enyl)-methanol **4**, a

Scheme 2. Synthesis of Natural Pheromone (**2**)



plausible late-stage intermediate in a synthesis of **1**, was readily available from camphoric acid **3**,[5] and so this synthesis was carried out first (Scheme 1). Thus, alcohol **4** was obtained from camphoric acid **3** in 45% yield over four steps, according to the published procedure.[5] Oxidation of **4** to aldehyde **5**, followed by Wittig reaction with (methoxymethylene)triphenylphosphorane gave methylvinyl ether **6**, simultaneously adding the final carbon and an oxygen functional group to the skeleton. The straightforward sequence of hydrolysis to aldehyde **7**, reduction to alcohol **8**, and acetylation provided acetate ester **1**. The spectra did not match those of the insect-produced compound but this isomer still provided valuable information, because catalytic reduction of acetate **1** and alcohol **8** produced the same two compounds that had been obtained by reduction of the insect-produced analogs, proving that the carbon skeleton was correct, and that isomer **2** must therefore be the natural product.

Because the structure of acetate **1** was so close to that of the target compound, we first attempted to simply transpose the double bond to the desired position, by the sequence of allylic oxidation to an α,β-unsaturated ketone, reduction of the alkene, conversion of the ketone to the corresponding enol triflate, and reduction. However, the final product of this sequence was not the desired compound **2**, but the starting material **1**, indicating that the initial allylic oxidation occurred at the sp^2 carbon adjacent to the geminal dimethyl group with transposition of the double bond, rather than at the desired allylic carbon. Throughout this sequence, the exact position of the double bond and functional group could not be determined with any degree of certainty, and so it was not obvious that the oxidation had occurred with transposition until the end of the sequence.

The target compound **2** was synthesized successfully by the route shown in Scheme 2, using a [2,3] sigmatropic rearrangement to place the double bond in the desired

(5) Fuganti, C.; Serra, S. *J. Org. Chem.* **1999**, *64*, 8728–8730.

position.[6] Thus, the known cyclopentenone **10,** prepared in one step from isobutyl 2-butenoate **9** by treatment with hot polyphosphoric acid,[7] was converted into the key allyl stannane **12** by reduction of **10** with $LiAlH_4$ to give alcohol **11** (89%), followed by deprotonation with KH in THF at 0 °C, and treatment with $(n\text{-}Bu)_3SnCH_2I$ (91%).[6] Upon treatment with n-BuLi at −100 °C, stannane **12** underwent [2,3] sigmatropic rearrangement in low yield (25%), furnishing cyclopentenol **13a** with the double bond and alkyl substituents correctly placed.

The low yield in this step was a result of competing reactions, specifically, [1,2] rearrangement to give **13b** (4%), elimination to give **13c (**61%), and reduction to give methyl ether **13d (**10%**)** (Figure 1).

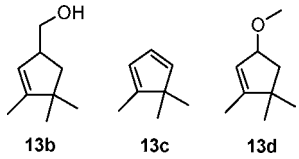


Figure 1. Side products from [2,3] sigmatropic rearrangement.

The synthesis was completed by straightforward one-carbon homologation as described for **1** above. Thus, alcohol **13a** was oxidized to aldehyde **14**, which was immediately reacted with $(Ph)_3P{=}CHOMe$ to give methyl vinyl ether **15** in 53% yield over two steps. Acid hydrolysis of **15**, reduction of the resulting aldehyde **16** with $NaBH_4$ (71% over two steps), and acetylation of alcohol **17** with acetyl chloride and pyridine gave (1,5,5-trimethylcyclopent-2-en-1-yl)-ethyl acetate **2** in 92% yield, the spectra of which matched those of the insect-produced compound.

It has not yet been possible to determine the absolute configuration of the naturally occurring pheromone. In particular, racemic **2**, the corresponding alcohol **17**, and the saturated analogs of **2** and **17** were not resolved on a Cyclodex B chiral stationary phase GC column. Diastereoisomeric derivatives of the alcohol produced by esterification of alcohol **17** with *O*-acetyl lactic acid chloride or Mosher's reagent also were not separable on polar DB-Wax or nonpolar DB-5 columns. Fortunately, preliminary results from field trials have shown that the racemic pheromone is highly attractive to male longtailed mealybugs.

To our knowledge, this pheromone structure constitutes the first example of the 1,2,2-trimethylcyclopentane skeleton in naturally occurring monoterpenoids, although this structural motif has been found in sesquiterpenes in the cuparene[8] and herbertene[9] families, and in higher terpenoids such as the carotenoids capsanthin and capsorubin.[10] The structure of the pheromone suggests that it might be assembled by the standard 4′−1 connection of two isoprene units to form a linear geranyl-type structure, followed by a 3′−3 connection to form the 1,1,2,2-tetraalkylcyclopentane core, and then functional group introduction and/or modification. Formation of the 5-membered ring might occur via cyclization of a linalyl cation, as occurs in biosynthesis of borneol, or via protonation of the distal olefin followed by cyclization, analogous to reactions mediated by abietadiene synthase. In either case, initial cyclization would proceed by an unusual anti-Markovnikov formation of the 5-membered ring. Thus, this structure continues the trend of unusual terpenoid pheromones within the mealybugs and scale insects. Field trials testing the biological activity of the racemic pheromone are in progress in California, South America, New Zealand, Australia, and South Africa. The results of these trials will be reported in due course.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **1**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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