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Sex pheromone of the aerial root mealybug, *Pseudococcus baliteus*: A unique monoterpenoid containing an α-hydroxyketone moiety

Jun Tabata [a,*], Tsunashi Kamo [a], Tomohiro Watanabe [b], Takeshi Kinsho [b]

[a] National Agriculture and Food Research Organization (NARO), 3-1-3 Kannondai, Tsukuba, Ibaraki Prefecture, Japan
[b] Shin-Etsu Chemical Co., Ltd., 28-1 Nishifukushima, Kubiki-ku, Joetsu, Niigata Prefecture, Japan

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ABSTRACT

A single compound that elicited an electrophysiological response from conspecific male antennae was isolated from volatiles emitted by adult females of the aerial root mealybug, *Pseudococcus baliteus*. Mass spectrometry and nuclear resonance spectroscopy analyses, as well as enantioselective syntheses, revealed the structure to be 2-((*S*)-1,2,2-trimethyl-3-cyclopentenyl)-2-oxoethyl (*S*)-2-methylbutyrate. This ester, which is a hitherto-unknown monoterpene with an α-hydroxyketone moiety, displayed attractiveness to adult males and was concluded to be the *P. baliteus* sex pheromone.

Mealybugs (Hemiptera: Pseudococcidae) are small insects, such as aphids and whiteflies, that feed on plant sap, and some members are notorious agricultural pests that parasitize and severely damage crop and fruit plants. Because mealybugs are often hard to find on plant tissues to which they are adhered, it is sometimes difficult to eliminate them during quarantine of live plant materials. Traps baited with sex pheromones that strongly and species-selectively attract pests would be useful for detecting and monitoring these targets in the global plant trade.

Adult female mealybugs hardly ever move, because they lack wings and have retrogressed legs. In contrast, adult males are winged but tiny and fragile with a limited lifespan of a few days at most because they do not feed as adults [1,2]. Sex pheromones emitted by sedentary females are essential for attracting ephemeral males and are considered to be under strong selection pressure to facilitate mating and reproduction by serving as a key navigation tool for copulation [3,4]. In fact, mealybug pheromones are highly divergent with strictly species-specific structures [5,6]. Thus, mealybug pheromones are both a useful tool for pest management and an intriguing model for studying the diversification of chemical communication channels in insects.

In this study, we discovered and completely characterized a hitherto-unknown monoterpenoid with an α-hydroxyketone moiety as the sex pheromone of the aerial root mealybug, *Pseudococcus baliteus*, which was originally described from the Philippines. First, headspace volatiles, which included the pheromone released from virgin adult females that infested a squash in a glass chamber (1 L) were pulled at a flow rate of 1 L/min and collected through a Haye-Sep Q adsorbent (1 g; Alltech Inc., Deerfield, IL, USA). The volatiles were extracted with 15 mL of hexane every 3–4 days, concentrated with an evaporator at room temperature, and kept at −20 °C. A total of approximately 294,000 female-day equivalents of volatiles were collected. When an aliquot of the crude extract was analyzed by a gas chromatograph (GC) equipped with an electroantennographic detector (EAD) using an antenna of a fresh adult male, a single compound (**1**) that elicited a response of the antenna was found as a pheromone candidate with a Kovat's index of 1608 on an apolar DB-1 column (Fig. 1a). The total amount of **1** in the crude extract was approximately 0.2 mg.

Compound **1** was analyzed using a GC-mass spectrometer (MS), and the high-resolution mass spectrum in electron-impact (EI; 70 eV) mode showed that the molecular formula of **1** was $C_{15}H_{24}O_3$ (observed, 252.17804; calculated, 252.17255), with four double bonds or rings. Micro-scale hydrogenation of **1** generated one product (**2**) that had a molecular ion at *m*/*z* 254, which indicated the presence of one olefinic double-bond. The base peaks of the mass fragment spectra of **1** and **2** were observed at *m*/*z* 109 and 111 (Fig. 1b), respectively, which was suggestive of a trimethylcyclopentenyl (C_8H_{13}) structure in **1** [7]. Basic ethanolysis and transesterification of **1** produced two products (**3** and **4**), the molecular ions of which were observed at *m*/*z* 168 and 130,

* Corresponding author.
E-mail address: jtabata@affrc.go.jp (J. Tabata).

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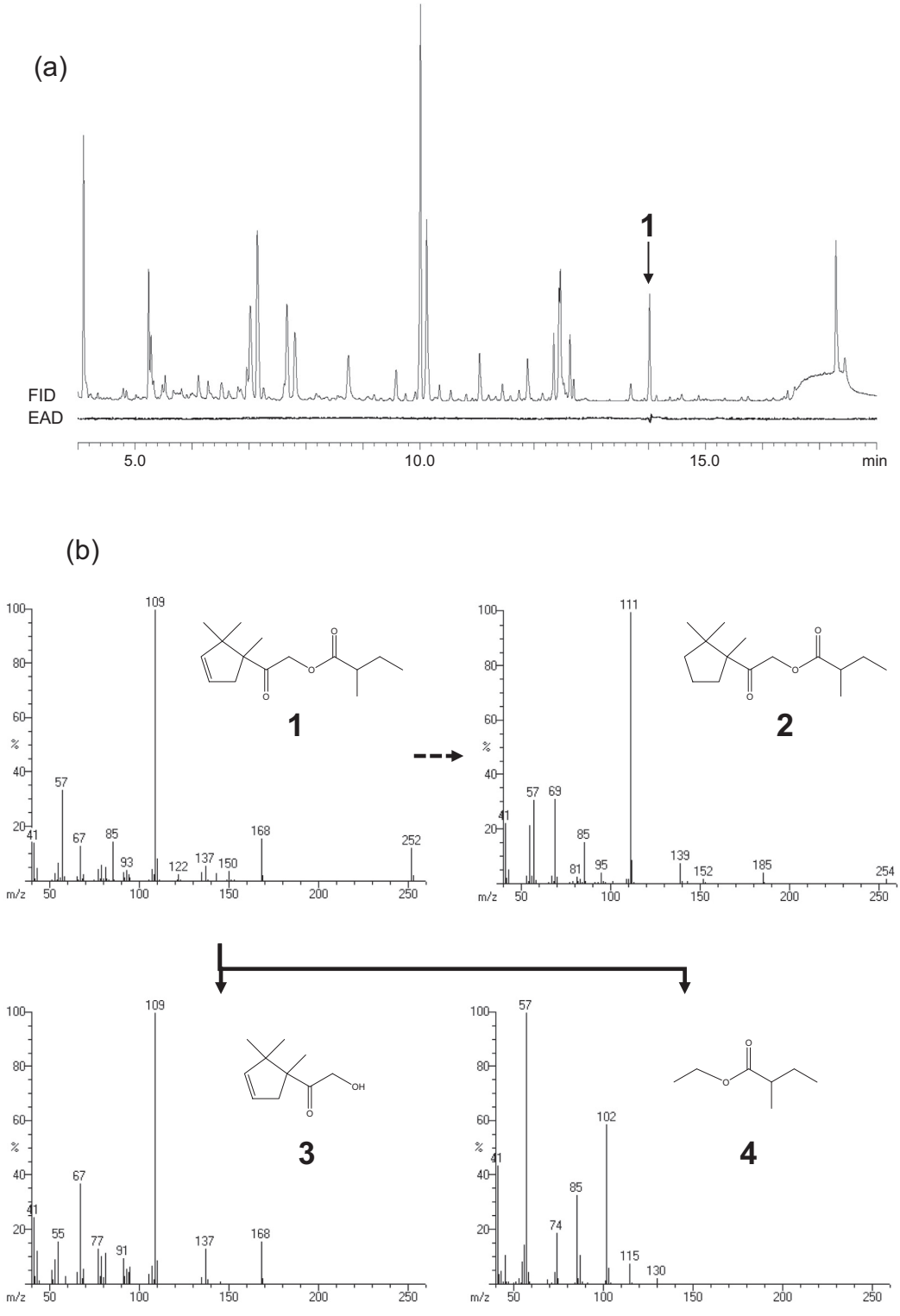

Fig. 1. (a) GC-flame ionization detection (FID) and GC-electrophysiological antenna detection (EAD) chromatograms of the headspace volatile extracts of virgin female *Pseudococcus baliteus*. A single compound (**1**) that elicited a clear male EAD signal was selected as the pheromone candidate. (b) Mass spectra of **1**, its hydrogenated product (**2**), and basic ethanolysis products (**3** and **4**).

respectively (Fig. 1b). The EI-MS and the GC-retention time of **4** were identical to those of authentic ethyl 2-methylbutyrate, which indicated that **1** is an ester of 2-methylbutyric acid and an alcohol with a trimethylcyclopentenyl structure.

Approximately 0.1 mg of **1** was purified to >99% using preparative liquid and gas chromatography, and was subjected to nuclear magnetic resonance (NMR) analyses using a micro-bottom tube and 35 μl of C_6D_6 solvent ESI. The ^{1}H NMR spectra indicated the

presence of two olefinic and five sets of methyl protons. Moreover, the ^{13}C NMR signals and heteronuclear single-quantum coherence (HSQC) analyses indicated four quaternary carbons including two carbonyl carbons. One of the signals of the carbonyl carbons (175.9 ppm) was suggested to be that of the carboxylic ester. According to heteronuclear multiple-bond coherence (HMBC) analyses, this carbonyl carbon was coupled with a methine proton (2.47 ppm), which was further coupled with a methyl group (1.21 ppm, d, J = 7.0 Hz) and a set of geminal protons (1.46 ppm and 1.83 ppm) linked to another methyl group (0.965 ppm, t, J = 7.4 Hz); thus, the micro-scale transesterification product indicates that **1** is a 2-methylbutyrate. The signal of the other carbonyl carbon (205.5 ppm) was correlated with a pair of doublet proton signals at 4.54 and 4.59 ppm (J = 16.7 Hz) that were suggestive of a neighboring ester oxygen. Among the other parts, which included two quaternary carbons and three sets of methyl protons (singlet signals at 0.917, 0.949, and 0.961 ppm) revealed a trimethylcyclopentenyl structure, the two olefin protons (5.11 ppm, ddd, J = 1.4, 2.6, 5.7 Hz; 5.29 ppm, ddd, J = 2.2, 2.7, 5.7 Hz) were determined to be coupled with the other pair of geminal protons (1.71 ppm, ddd, J = 1.4, 2.7, 16.3 Hz; 2.93 ppm, ddd, J = 2.2, 2.6, 16.3 Hz) by decoupling analyses of the ^1H NMR spectra; these findings indicate that the ring has a 1,2,2-trimethyl-3-cyclopentenyl or 1,2,2-trimethyl-4-cyclopentenyl structure. The former structure was more plausible, because correlations between a pair of geminal methyl groups (δ_H 0.917 and 0.949 ppm) and a double bond (δ_C 140.6 ppm) were observed in the HMBC analysis. Thus **1** was determined to be 2-(1,2,2-trimethyl-3-cyclopentenyl)-2-oxoethyl 2-methylbutyrate.

This compound contains two chiral centers and therefore four possible stereoisomers. We therefore synthesized all of the four possible stereoisomers, i.e. 2-((*R*)-1,2,2-trimethyl-3-cyclopentenyl)-2-oxoethyl (*R*)-2-methylbutyrate, 2-((*R*)-1,2,2-trimethyl-3-cyclopentenyl)-2-oxoethyl (*S*)-2-methylbutyrate, 2-((*S*)-1,2,2-trimethyl-3-cyclopentenyl)-2-oxoethyl (*R*)-2-methylbutyrate, and 2-((*S*)-1,2,2-trimethyl-3-cyclopentenyl)-2-oxoethyl (*S*)-2-methylbutyrate (Fig. 2). Our synthesis started from enantiomerically pure (*R*)- and (*S*)-1,2,2-trimethyl-3-cyclopentenyl methyl ketone, which were prepared from (+)- and (−)-camphor, respectively [8]. The methyl ketone was first treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of a weak non-nucleophilic base to give the corresponding silyl enol ether, which was then halogenated to bromomethyl 1,2,2-trimethyl-3-cyclopentenyl ketone (**5**) using *N*-bromosuccinimide (NBS) in moderate yield (52–56% over two steps). Subsequently, **5** was esterified with (*R*)- or (*S*)-2-methylbutyric acid (89.3% ee and 98.6% ee, respectively) by nucleophilic substitution under mild conditions to give >99.5% pure **1** in fair to high yield (54–94%). No obvious epimerization was observed during the synthesis.

The ^1H NMR patterns of the diastereotopic methylene protons neighboring the keto group of the natural product were identical to those of (*R**,*R**)-**1** but were different from those of (*R**,*S**)-**1** (Fig.3). The retention times of synthetic (*R*)- and (*S*)-**3** were 25.2 and 25.3 min, respectively, in GC analyses equipped with a chiral resolution column (β-DEX™ 120), and the latter was identical to the retention time of a hydrolyzed product of the natural **1**. Moreover, both synthetic (*S*,*S*)-**1** and natural showed levo-rotatory ($[\alpha]_D^{24}$ −65 (c = 1.01, $CHCl_3$) and $[\alpha]_D^{25}$ −69 (c = 0.0135, hexane), respectively), which supports *S*,*S* as the absolute configuration of the natural product.

Finally, the attractiveness of the four synthetic isomers of **1** was tested in a trap bioassay in a greenhouse (Fig. 4). The (*S*,*S*)-isomer, with the natural configuration, was the most attractive to males. The (*S*,*R*)-isomer also attracted some males, but it was unclear whether males responded to the (*S*,*R*)-isomer or the slightly contaminated (*S*,*S*)-isomer. The other isomers showed little attractiveness. The (*S*,*S*)-isomer and the natural pheromone attracted equivalent numbers of males (N = 32 and 29, respectively; P = 0.701, chi-squared test) in a choice test under a large glass dish (15 cm in diameter × 3.5 cm in height) where each 5 ng of the synthetic and natural samples were simultaneously presented. Based on these results and those of the chemical analyses, we concluded that (*S*,*S*)-**1** is the *P. baliteus* sex pheromone.

Relatively simple secondary alcohols of an α-hydroxyketone (α-ketol) are a known key component of the male-produced aggregation pheromones of several longicorn beetles; for example, 3-hydroxyhexan-2-one and its eight- and 10-carbon analogs are pheromones or attractants for more than 10 longicorn beetle

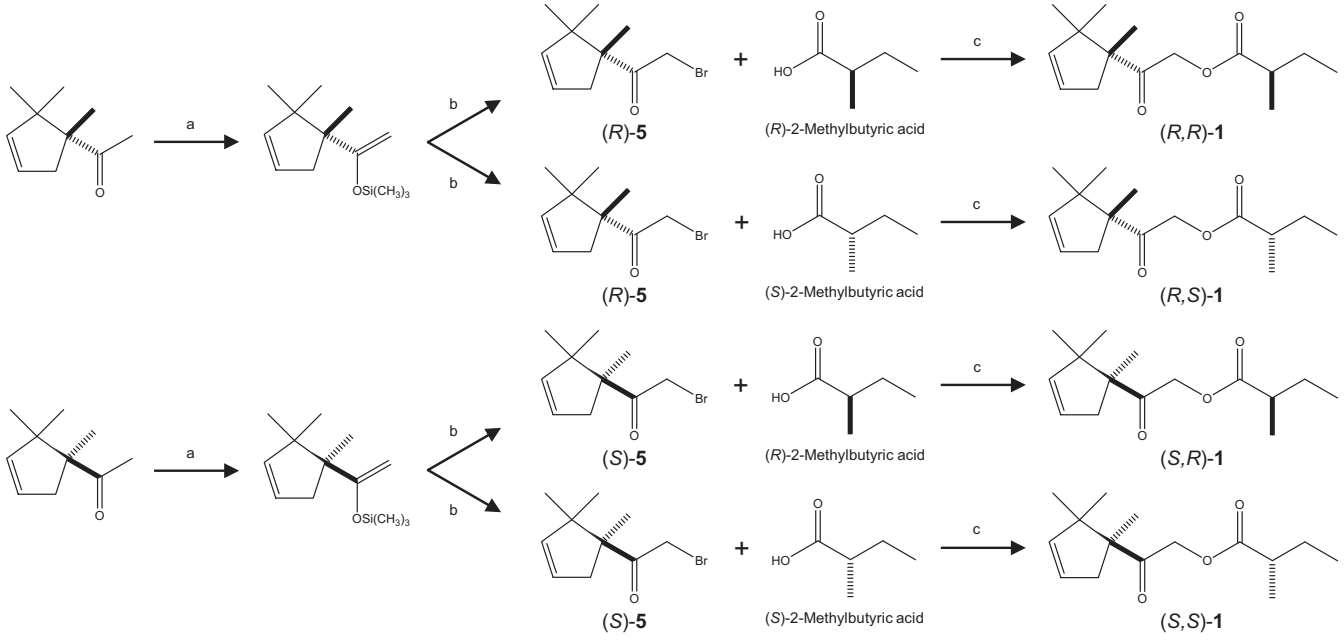

Fig. 2. Synthesis of **1**. Reagents and conditions: (a) TMSOTf, Et_2O, DIPEA, CH_2Cl_2, 0 °C to room temperature; (b) NBS, $NaHCO_3$, THF, −60 °C to 3 °C (52–56% yield over two steps); (c) K_2CO_3, DMF, room temperature (54–94% yield).

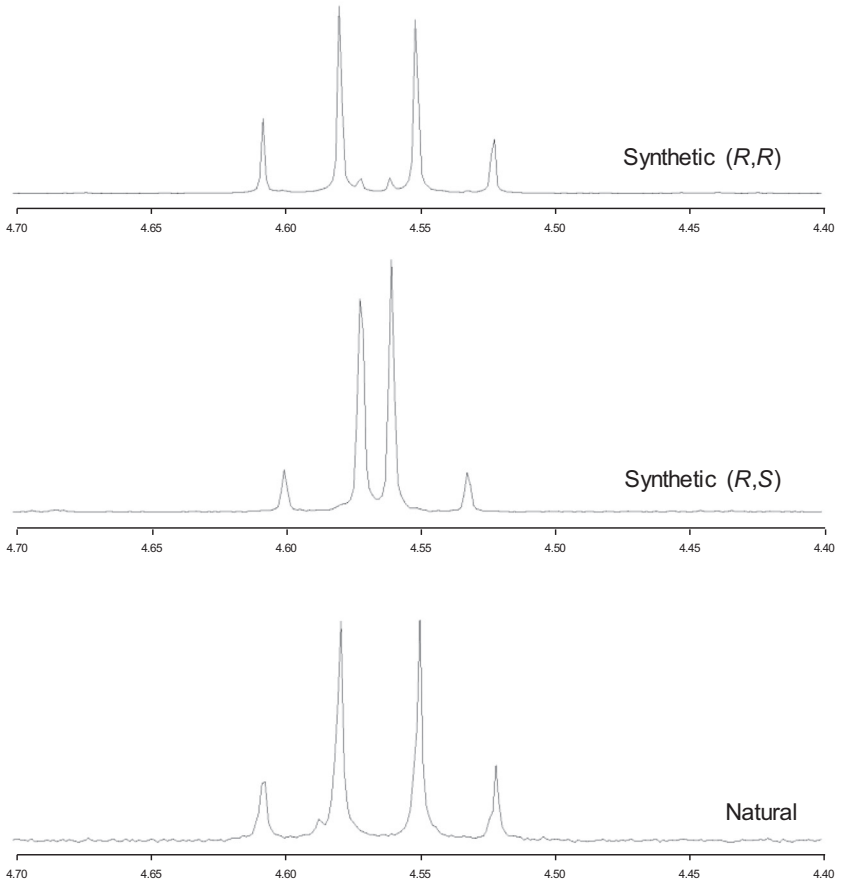

Fig. 3. ^{1}H NMR signals of the methylene protons neighboring the keto group of **1**.

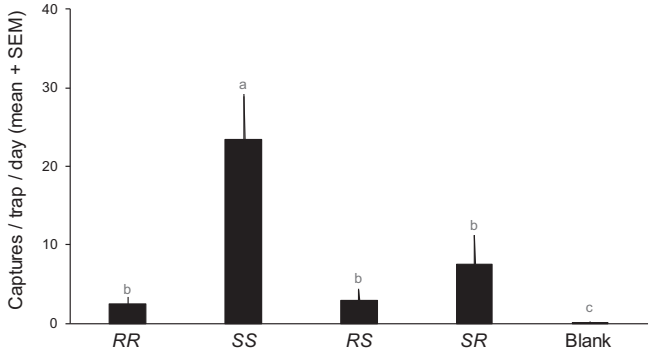

Fig. 4. Males of *Pseudococcus baliteus* captured by traps baited with stereoisomers of synthetic **1**. Blank: solvent (hexane) only. Significant differences are indicated by different lowercase letters (ANOVA followed by Tukey-Kramer HSD test).

species (Coleoptera: Cerambycidae) [9–13]. However, primary alcohols with a keto group at the α-position or their esters, such as **1**, appear to be relatively scarce in insect pheromones [14]. This study discovered a unique example of a monoterpenoid with an α-hydroxyketone moiety and revealed further diversity of mealybug pheromones. In addition, we demonstrated that traps baited with the synthetic pheromone were potentially useful for monitoring *P. baliteus* during quarantine. The isomers with unnatural configurations appear to have no obvious antagonistic effects on male responses, and rigid purity of the enantiomer is not necessary for male attraction.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.151802. These data include MOL files and InChiKeys of the most important compounds described in this article.

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