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RESEARCH

Sex Pheromone of the Azalea Mealybug: Absolute Configuration and Kairomonal Activity

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Abstract

The sex pheromone of the azalea mealybug, *Crisicoccus azaleae* (Tinsley, 1898) (Hemiptera: Pseudococcidae), includes esters of a methyl-branched medium-chain fatty acid, ethyl and isopropyl (*E*)-7-methyl-4-nonenoate. These compounds are exceptional among mealybug pheromones, which are commonly monoterpenes. Determination of the absolute configuration is challenging, because both chromatographic and spectrometric separations of stereoisomers of fatty acids with a methyl group distant from the carboxyl group are difficult. To solve this problem, we synthesized the enantiomers via the Johnson–Claisen rearrangement to build (*E*)-4-alkenoic acid by using (*R*)- and (*S*)-3-methylpentanal as chiral blocks, which were readily available from the amino acids L-(+)-alloisoleucine and L-(+)-isoleucine, respectively. Each pure enantiomer, as well as the natural pheromone, was subsequently derivatized with a highly potent chiral labeling reagent used in the Ohrui–Akasaka method. Through NMR spectral comparisons of these derivatives, the absolute configuration of the natural pheromone was determined to be *S*. Field-trap bioassays showed that male mealybugs were attracted more to (*S*)-enantiomers and preferred the natural stereochemistry. Moreover, the synthetic pheromones attracted *Anagyrus* wasps, indicating that the azalea mealybug pheromone has kairomonal activity.

Keywords Mealybug pheromone · Methyl-branched medium-chain fatty acid · Chiral derivatization · Pest monitoring · Kairomonal activity · Parasitoid · *Anagyrus* wasp

Introduction

The Asia–Pacific region is biogeographically one of the most diverse and rich regions in the world (Karki et al. 2018). As a reflection of this diversity, plant protection services in the Asia–Pacific are required to manage many pests and pathogens, including cryptic species, in both agricultural production and quarantine. Chemical ecologists have contributed to plant protection by, for example, providing selective monitoring tools based on insect pheromones, which are generally species-specific chemical communication agents. Such monitoring tools are particularly useful with mealybugs (Hemiptera: Pseudococcidae) (Dunkelblum 1999; Tabata 2020), which are hard to detect visually, and to identify to species level because of their cryptic behavior, tiny bodies in the nymphal stages, and limited morphological features.

Recently, we have found that different species of the genus *Crisicoccus* may coexist around Japan (Tanaka and Kamitani 2022). The Matsumoto mealybug *C. seruratus* (formerly *C. matsumotoi*) and the azalea mealybug *C. azaleae*, both of which are listed as pests of persimmons and pears in Japan (Japanese Society of Applied Entomology and Zoology 2006), have been potentially confused in plant protection, because their diagnostic features (e.g. shapes of the conical setae on the cerarii) cannot be observed without the inspection of slide-mounted specimens (Tanaka and Kamitani 2022). We were therefore challenged to determine the pheromones of *Crisicoccus* spp. in order to develop lures for monitoring traps that would provide convenient tools to

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detect their emergence with species-selectivity. The pheromone of *C. azaleae* was recently identified as a mixture of isopropyl (*E*)-7-methyl-4-nonenoate (**1**), isopropyl (*E*)-7-methyl-4-octenoate (**2**), and ethyl (*E*)-7-methyl-4-nonenoate (**3**) (Tabata and Yasui 2022), whereas the pheromone of *C. seruratus* is isoprenyl 5-methylhexanoate (Tabata et al. 2012). These esters, which include methyl-branched medium-chain fatty acid (MCFA) moieties, have been demonstrated to be an interesting exception among mealybug pheromones, because most of the other pheromone compounds so far identified from mealybugs are monoterpene alcohols esterified with short-chain fatty acids (Tabata and Yasui 2022). In addition, the MCFA esters (**1**, **2**, and **3**) are produced in a blend of approximately 10:5:1 by *C. azaleae* females, but each of them solely shows attractiveness to conspecific males without obvious synergistic effects, indicating a possible redundancy in the pheromone (Tabata and Yasui 2022).

The methyl-branched MCFA of the pheromone components of *C. azaleae* (**1** and **3**) is an anteiso-fatty acid that includes one asymmetric carbon. Its absolute configuration remains to be determined, but it is very difficult or impossible to separate enantiomers or diastereomeric derivatives with chiral centers more than three bonds remote from their carboxyl group by chromatographic or spectrometric methods (Akasaka and Ohrui 1999). To solve this intrinsic problem, Ohrui, Akasaka, and their coworkers developed a highly potent chiral derivatizing reagent, 2-(anthracene-2,3-dicarboximido)cyclohexanecarboxylic acid (Ohrui et al. 2002). The methyl groups of each enantiomer of the anteiso fatty compounds derivatized with this reagent are considered to exist in the region of different strength anisotropy from the anthracene ring (Ohrui et al. 2002). Here, by using this reagent, we set out to determine the absolute configuration of the natural pheromone components isolated from *C. azaleae* females.

We first synthesized enantiomers of **1** and **3** via the Johnson–Claisen rearrangement to build (*E*)-4-alkenoic acid, starting from (*R*)- and (*S*)-3-methylpentanal, which are readily available from the amino acids L-(+)-alloisoleucine and L-(+)-isoleucine, respectively, as chiral synthons (Fig. 1). We then compared the ^{1}H-NMR spectra of chiral-labeled derivatives of the synthetic enantiomers and the natural pheromone to determine the natural absolute configuration. Finally, we assayed the biological activities of synthetic enantiomers of **1** and **3**, as well as of **2**, in attracting males as a single component and confirmed them in field-trap experiments. We expected that our study would offer key insights into the development of pheromone-based tactics for specific management of the *Crisicoccus* species complex, as well as into the exceptional methyl-branched MCFA esters of mealybug pheromones which are produced in an apparently redundant manner.

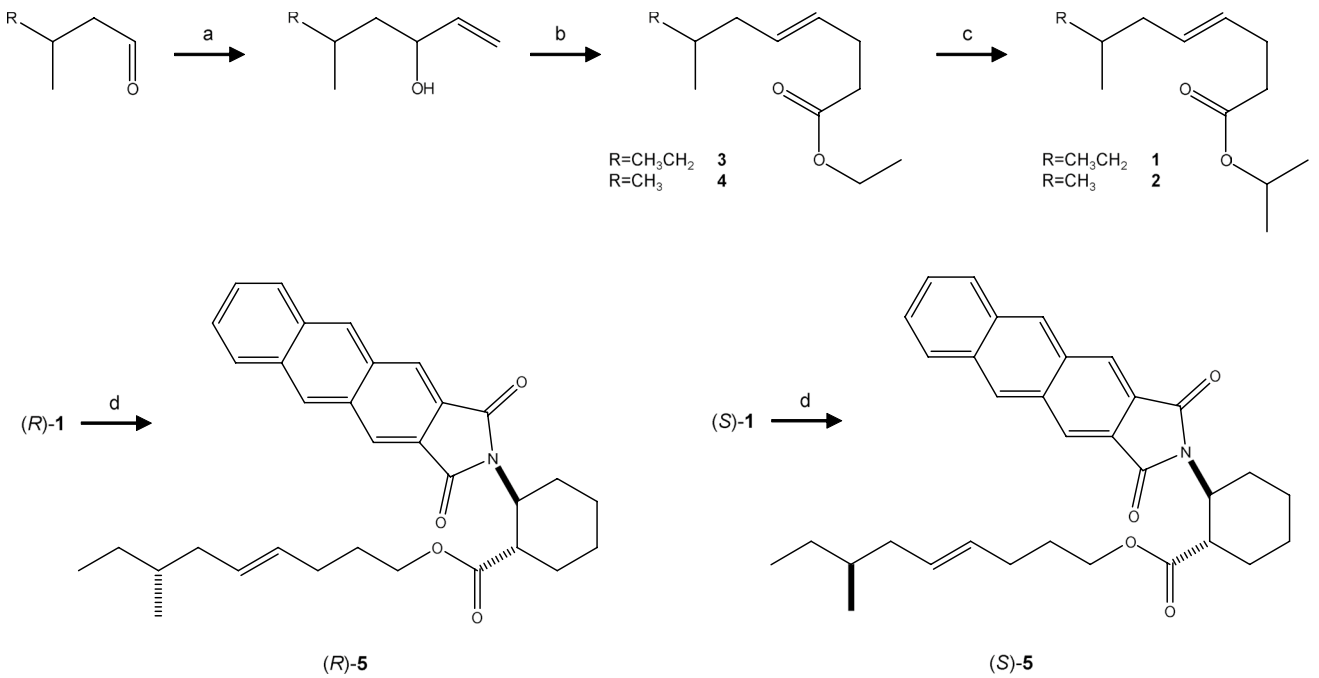

Fig. 1 Synthesis routes and reagents. a: (CH_2=CH)MgBr, THF (tetrahydrofuran), 0 °C to room temperature, overnight, 79%; b: $CH_3C(OC_2H_5)_3$, C_3H_7COOH, 138 °C, overnight, 83%; c: HCl, IPA (isopropyl alcohol), 52 °C, 24 h, 85%; d: (1) $LiAlH_4$, Et_2O, room temperature, overnight; (2) (1*R*,2*R*)-2-(anthracene-2,3-dicarboximido) cyclohexanecarboxylic acid, CH_2Cl_2, DMAP (4-dimethylaminopyridine), EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), 40 °C, overnight, 47% over two steps

Methods and Materials

Analytical Instrument

Gas chromatography – mass spectrometry (GC-MS) analyses were conducted on an Agilent 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a 5973 N mass selective detector. The injector temperature was 220 °C and the interface temperature was 230 °C. An apolar DB-1 column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) was used in constant flow mode (1.0 mL/min) with helium as the carrier gas. The column oven temperature was maintained at 60 °C for 1 min and then raised to 220 °C at 10 °C/min.

Nuclear magnetic resonance (NMR) spectra were obtained with a JNM-A600 spectrometer (JEOL, Tokyo, Japan; ^{1}H: 600.05 MHz; ^{13}C: 150.80 MHz). Standard 5-mm-O.D. tubes (Sigma-Aldrich, St. Louis, MO, USA) were used. Chemical shifts were reported in ppm as the differences from those of the solvent ($CDCl_3$; δ_C 77.0 ppm) and residual $CHCl_3$ (δ_H 7.24 ppm) in the solvent.

Optical rotations were measured with a P-1020 polarimeter (JASCO, Tokyo, Japan). After derivatization with a chiral labeling reagent (described below), enantiomeric excess was calculated from the ^{1}H-NMR spectra.

Synthesis

Ethyl (*E*)-7-methyl-4-octenoate (4)

Isovaleraldehyde (860 mg; 10 mmol; Tokyo Chemical Industry, Tokyo, Japan) was dissolved in 3 mL of dry tetrahydrofuran (THF) and added dropwise over 15 min to 11 mL of a 1 M solution of vinylmagnesium bromide in THF (Tokyo Chemical Industry) under argon at 0 °C. The mixture was stirred overnight at room temperature, and the reaction was quenched by adding 10 mL of saturated NH_4Cl aqueous solution. The organic phase was collected, and 3 mL of 10% aqueous H_2SO_4 was added to the aqueous phase, which was then extracted three times with 10 mL of diethyl ether. The combined ethereal solution was washed with brine, dried over Na_2SO_4, concentrated by rotary evaporation, and purified by silica gel column chromatography with elution with 20% diethyl ether in hexane to give 5-methyl-1-hexen-3-ol (903 mg; 79% yield). This allyl alcohol (627 mg; 5.5 mmol) was dissolved in triethyl orthoacetate (6.29 g; 39 mmol; Tokyo Chemical Industry) along with propionic acid (25 mg; 0.33 mmol) as a catalyst, and the mixture was heated to 138 °C with distillative removal of ethanol for the Johnson–Claisen rearrangement. After 3 h, the mixture was poured into 10 mL of H_2O and extracted three times with 10 mL of hexane. The extract was dried over $MgSO_4$, concentrated, and purified by silica gel column chromatography with elution with 5% diethyl ether in hexane to give **4** (843 mg; 83%). The amount of (*Z*)-isomer in the product was < 5%. The GC-MS and NMR data were identical to those reported by Tabata and Yasui (2022).

Ethyl (*E*)-7-methyl-4-nonenoate (3)

(*R*)-(+)-3-Methylpentanal and (*S*)-(−)-3-methylpentanal were prepared from L-(+)-alloisoleucine and L-(+)-isoleucine (Tokyo Chemical Industry Co., Tokyo, Japan), respectively, in accordance with a previously described method (Overberger and Cho 1968; see online Supplementary Information). These aldehydes were used instead of isovaleraldehyde in the Grignard reaction for the synthesis of **4**. The other conditions and procedures were the same as described above. The GC-MS and NMR data were identical to those reported by Tabata and Yasui (2022). The optical rotations were $[\alpha]^{19.5}_{D} = -2.61$ (observed, 97% ee, *c* 1.08, hexane) for ethyl (*R*,*E*)-7-methyl-4-nonenoate [(*R*)-**3**] and $[\alpha]^{19.5}_{D} = +2.74$ (observed, 98% ee, *c* 1.55, hexane) for ethyl (*S*,*E*)-7-methyl-4-nonenoate [(*S*)-**3**].

Isopropyl (*E*)-7-methyl-4-octenoate (2)

The ethyl ester (**4**) (110 mg; 0.60 mmol) was dissolved in 9 mL of isopropyl alcohol with 1 mL of concentrated aqueous HCl (~30%). The mixture was stirred overnight at 52 °C, then poured into 10 mL of H_2O and extracted three times with 10 mL of hexane. The combined hexane solution was washed with 10 mL each of saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography with elution with 5% diethyl ether in hexane to give isopropyl ester (**2**) (101 mg; 85%). The GC-MS and NMR data were identical to those reported by Tabata and Yasui (2022).

Isopropyl (*E*)-7-methyl-4-nonenoate (1)

Transesterification of **3** to **1** was performed in the same procedure as conversion of **4** to **2**, as described above. The GC-MS and NMR data were identical to those reported by Tabata and Yasui (2022). The optical rotations were $[\alpha]^{20.4}_{D} = -2.32$ (observed, 97% ee, *c* 1.54, hexane) for isopropyl (*R*,*E*)-7-methyl-4-nonenoate [(*R*)-**1**] and $[\alpha]^{20.4}_{D} = +2.44$ (observed, 98% ee, *c* 1.97, hexane) for isopropyl (*S*,*E*)-7-methyl-4-nonenoate [(*S*)-**1**].

Derivatization to Form Diastereomeric Derivatives

Each enantiomer of the anteiso-fatty acid ester (**1**; 2.5 mg) was dissolved in 1 mL of dry diethyl ether, and 5 mg of lithium aluminum hydride was added. The mixture was stirred overnight at room temperature, then quenched by adding 1 mL of saturated aqueous Rochelle salt solution on ice and extracted three times with 1 mL of diethyl ether. The combined extract was dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography with elution with 20% diethyl ether in hexane to give the corresponding alcohol. This alcohol was dissolved in 0.5 mL of dry dichloromethane, and a chiral derivatizing reagent, (1*R*,2*R*)-2-(anthracene-2,3-dicarboximido)cyclohexanecarboxylic acid (15 mg; >98.0% purity; Tokyo Chemical Industry), along with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (7.6 mg; Tokyo Chemical Industry) and 4-dimethylaminopyridine (3.0 mg; Tokyo Chemical Industry) as catalysts, was added (Akasaka et al. 2002). The mixture was incubated overnight at 40 °C and was then concentrated under a nitrogen stream. The residue was dissolved in 1 mL of ethyl acetate, washed with 1 mL of H_2O, dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography with elution with 20% ethyl acetate in hexane to give the derivatized product (**5**; 3.3 mg; 47% over 2 steps).

(*R*)-7-Methyl-4-nonen-1-yl (1*R*,2*R*)-2-(anthracene-2,3-dicarboximido)cyclohexanecarboxylate [(*R*)-**5**]: $[\alpha]^{26.0}_{D} = -13.2$ (*c* 0.11, $CHCl_3$); ^{1}H-NMR δ_H: 0.63 (3 H, d, *J* = 6.6 Hz, branched C*H*$_3$), 0.72 (3 H, t, *J* = 7.3 Hz, terminal C*H*$_3$), 0.90–0.96 (1H, m), 1.07–1.15 (2 H, m), 1.29–1.90 (12 H, m), 2.12–2.15 (1H, m), 2.21–2.28 (1H, m), 3.50 (1H, ddd, *J* = 3.7, 11.9, 12.0 Hz, >C*H*(C = O)–), 3.88 (2 H, t, *J* = 6.6 Hz, –C*H*$_2$O–), 4.44 (1H, ddd, *J* = 4.0, 11.8, 11.9 Hz, >C*H*N<), 5.01 (1H, ddd, *J* = 6.5, 6.8, 15.1 Hz, –CH = C*H*C$_3$H$_6$O–), 5.08 (1H, ddd, *J* = 6.8, 7.0, 15.1 Hz, –C*H* = CHC$_3$H$_6$O–), 7.60–8.60 (8 H, anthracene); ^{13}C-NMR δ_C :11.3, 18.8, 24.6, 25.3, 28.3, 28.6, 28.9, 29.3, 29.5, 34.6, 39.6, 44.9, 51.7, 64.0, 125.7, 126.5, 127.4, 128.4, 129.6, 129.8, 130.0, 130.5, 132.0, 133.2, 167.6, 173.7.

(*S*)-7-Methyl-4-nonen-1-yl (1*R*,2*R*)-2-(anthracene-2,3-dicarboximido)cyclohexanecarboxylate [(*S*)-**5**]: $[\alpha]^{26.0}_{D} = +15.3$ (*c* 0.05, $CHCl_3$); ^{1}H-NMR δ_H: 0.64 (3 H, d, *J* = 6.6 Hz, branched C*H*$_3$), 0.72 (3 H, t, *J* = 7.3 Hz, terminal C*H*$_3$), 0.90–0.95 (1H, m), 1.07–1.15 (2 H, m), 1.28–1.90 (12 H, m), 2.12–2.15 (1H, m), 2.21–2.28 (1H, m), 3.50 (1H, ddd, *J* = 3.8, 11.5, 12.3 Hz, >C*H*(C = O)–), 3.88 (2 H, t, *J* = 6.6 Hz, –C*H*$_2$O–), 4.43 (1H, ddd, *J* = 4.0, 11.5, 12.0 Hz, >C*H*N<), 5.01 (1H, ddd, *J* = 6.4, 6.7, 15.2 Hz, –CH = C*H*C$_3$H$_6$O–), 5.08 (1H, ddd, *J* = 6.8, 7.0, 15.2 Hz, –C*H* = CHC$_3$H$_6$O–), 7.60–8.61 (8 H, anthracene); ^{13}C-NMR δ_C :11.3, 18.8, 24.6, 25.3, 28.4, 28.6, 28.9, 29.4, 29.5, 34.6, 39.5, 45.0, 51.7, 64.0, 125.7, 126.5, 127.4, 128.4, 129.6, 129.8, 130.0, 130.6, 132.0, 133.2, 167.6, 173.7.

Natural pheromones (**1**, **2**, and **3**) were collected and isolated as a three-component mixture from the headspace volatiles emitted by female mealybugs, as described by Tabata and Yasui (2022). The natural pheromone esters, including ~ 80 µg of **1**, 40 µg of **2**, and 8 µg of **3**, were reduced to the corresponding alcohols and esterified with the chiral derivatizing agent as described above.

Field Trap Bioassay

The first field-trapping-bioassay experiment was performed in a persimmon orchard where mealybugs occurred in the city of Tsukuba, in Ibaraki Prefecture (36.0°N, 140.1°E), from 10 June to 5 August 2022, using delta traps with sticky boards (12 cm × 22 cm). Traps were baited with each compound (0.1 mg), which were dissolved in 0.2 mL of hexane and impregnated into red rubber septa (8 mm outside diameter, 19 mm height; Wheaton, Millville, NJ, USA). Two sets of traps with seven different lures (14 traps in total) were hung on trees at ~ 2-m intervals. Trap catches were counted and trap locations were rearranged every week, and the lure was exchanged every 2 weeks. To confirm reproducibility, a second experiment was performed in another persimmon orchard in the city of Higashi-Hiroshima, in Hiroshima Prefecture (34.3°N, 132.8°E), ~ 700 km from the site of the first experiment. The second experiment ran from 27 June to 12 September 2023. The other procedures were the same as those used in the first experiment. Because of the discrete distributions of the count data for mealybugs and parasitoid wasps, for which there were frequent zero counts, the data were analyzed by using a zero-inflated Poisson (ZIP) model to assess the fixed effects of the lures. ZIP models are used to analyze data with an excess of zero counts (Sileshi 2006). These were calculated with the *zeroinfl* function in the 'pscl' package of R v. 4.3.0 software, and each *P* value was calculated on the basis of likelihood ratio tests with the *Anova* function in the 'car' package. This was followed by adjustments in the false discovery rate control method for multiple comparisons by using the functions in the '*multicomp*' package of R.

Results

Absolute Configuration of the Natural Pheromone

Starting from L-(+)-alloisoleucine and L-(+)-isoleucine, almost enantiomerically pure pheromone components were successfully prepared; the methyl-branched (*E*)-4-alkenoate (**3**) was synthesized in good yield and in a highly stereoselective manner via two steps, including a Johnson–Claisen rearrangement, from readily available methyl-branched aldehydes (Fig. 1), in accordance with a previous report (Kaga

et al. 1996). Because the enantiomeric purities of our final products were high, no obvious racemization was likely to have occurred during our synthesis. Each enantiomer of the chiral-labeled derivative (**5**) showed different ^{1}H-NMR chemical shifts of the methyl branch, consistent with the spectral patterns reported by Ohrui et al. (2002); the doublet signal of the (*S*)-isomer (0.638 ppm) appeared downfield of that of the (*R*)-isomer (0.628 ppm), and that of the natural pheromone was identical to the former (Fig. 2). Thus, the absolute configuration of the anteiso-fatty acid structures in the *C. azaleae* pheromone was *S*.

Responses of Male Mealybugs to Synthetic Pheromones

The trends in the mealybug responses to synthetic pheromones were consistent between the two sites (Fig. 3). In the case of both the isopropyl ester and the ethyl ester, significantly more mealybugs were attracted to (*S*)-enantiomers than to (*R*)-enantiomers ($\chi^2 = 10.9$–17.1 at Tsukuba and 34.4–91.7 at Higashi-Hiroshima, df = 1, $P < 0.05$, Fig. 3). This result is consistent with that of the chemical analyses, indicating that the natural pheromones were (*S*)-enantiomers, as described above. The achiral homologs, **2** and **4**, attracted significantly fewer males than the corresponding (*S*)-enantiomers of **1** and **3**, respectively ($\chi^2 = 4.95$–8.95 at Tsukuba and 9.56–16.0 at Higashi-Hiroshima, df = 1, $P < 0.05$), but they still showed substantial activity.

Kairomonal Activity of Synthetic Pheromones

During the field bioassays, parasitoid wasps of the genus *Anagyrus* (Hymenoptera: Encyrtidae: Tetracneminae) were repeatedly captured in traps at both sites (Fig. 3). Most of the individuals were very similar to *A. sawadai* but had morphological differences in their antennae and ovipositors, suggesting that they belonged to an undescribed species. We tentatively named this *Anagyrus* species as *A.* sp. near *sawadai*. Only females were captured by the traps. The wasps were attracted to all of the compounds

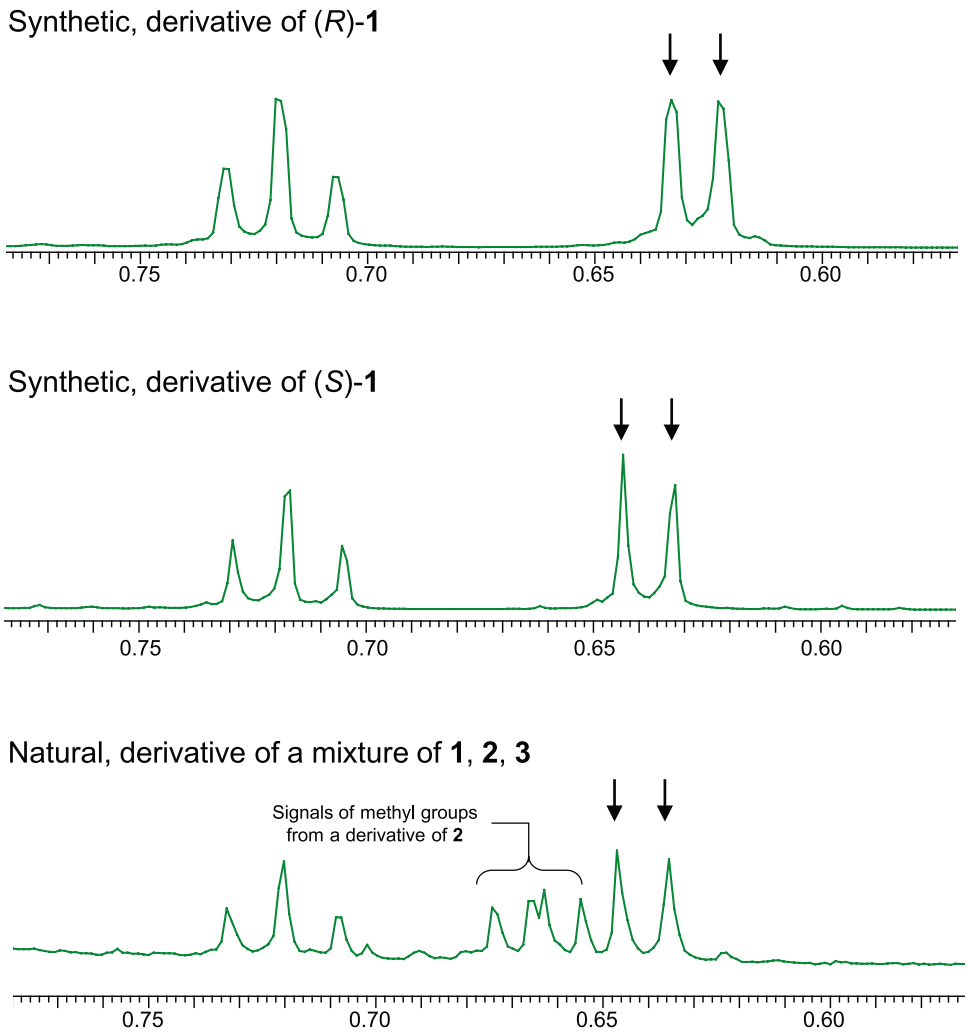

Fig. 2 ^{1}H-NMR spectra of methyl groups in synthetic (*R*)-**5**, (*S*)-**5**, and the derivative of natural anteiso-fatty acid with a chiral labeling reagent. Arrows indicates doublet signals of the methyl group attached to the asymmetric carbon of **5**

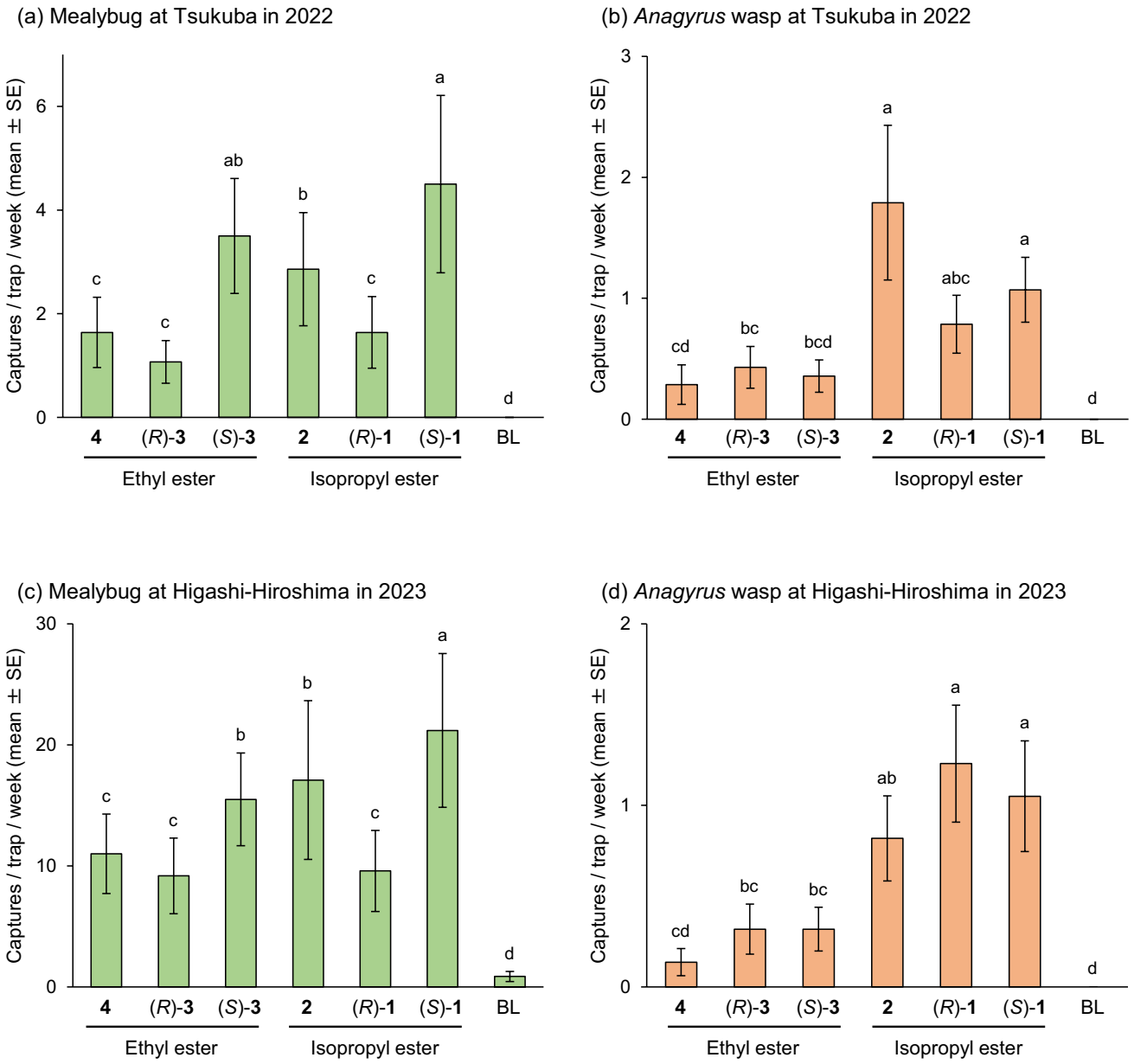

Fig. 3 Captures (means ± SEM) of male mealybugs of *Crisicoccus azaleae* and wasps of *Anagyrus* sp. nr. *sawadai* in traps baited with synthetic attractants (0.1 mg). **a**, **b** Bioassays performed in a persimmon orchard at Tsukuba by using two sets of traps with seven repetitions from 10 June to 5 August 2022. **c**, **d** Bioassays performed in a persimmon orchard at Higashi-Hiroshima using two sets of traps with 11 repetitions from 27 June to 12 September 2023. BL: blank (solvent only)

examined but significantly preferred the isopropyl esters to the corresponding ethyl esters ($\chi^2 = 4.53$–11.6 at Tsukuba and 7.59–9.31 at Higashi-Hiroshima, df = 1, $P < 0.05$), except for (*R*)-**1** versus (*R*)-**3** at Tsukuba ($\chi^2 = 1.43$, df = 1, $P = 0.232$). There were no obvious trends of differences in the responses of the wasps between the enantiomers of **1** and between the enantiomers of **3** (Fig. 3).

Discussion

Starting from methyl-branched aldehydes that were readily available from amino acids, the pheromone components of *C. azaleae* were successfully synthesized in two steps for **3** and three steps for **1** with acceptable yields and excellent enantiomeric excess values. The absolute configuration of

the natural pheromones was determined to be *S* through derivatization of each enantiomer with a highly potent chiral derivatizing reagent and through NMR spectral comparisons. Our study therefore provides a route for the highly selective synthesis of (4*E*,7*S*)-stereoisomers (**1** and **3**), which are likely to be the most efficient tools for monitoring the emergence of *C. azaleae*. The opposite (7*R*)-enantiomers also appear to elicit male-attraction, although, in a generally accepted view, unnatural enantiomers are usually inactive and sometimes even antagonistic (Mori 2011). Achiral homologs (**2** and **4**) synthesized from a relatively inexpensive material (isovaleraldehyde) can attract substantial numbers of males and may also be valuable for practical use such as a broad range monitoring.

Methyl-branched structures are found in the short-chain fatty acid moieties of monoterpene esters of several mealybug pheromones, and their complete stereochemistry has been determined. The Madeira mealybug (*Phenacoccus madeirensis*) produces esters of (*R*)-2-methylbutanoic acid (Ho et al. 2009), whereas the pink hibiscus mealybug (*Maconellicoccus hirsutus*) (Zhang et al. 2004) and the aerial root mealybug (*Pseudococcus baliteus*) uses esters of (*S*)-2-methylbutanoic acid (Tabata et al. 2020). The stereochemistry of methyl-branched structures in pheromones of mealybugs and other insects including moths and beetles (Ando and Yamakawa 2015) appears inconsistent and might have evolved to differentiate pheromonal signals at the time of species radiation.

During our field bioassay, significant numbers of a parasitoid wasp *Anagyrus* sp. nr. *sawadai* were attracted to the synthetic pheromones at the two test sites. The biological and taxonomic characters of this species suggest that it is an undescribed species, and are currently under investigation in our laboratory. We found that the same species emerged from a wild colony of *C. azaleae* on azalea bushes (*Rhododendron* × *pulchrum*) planted around the persimmon orchard in Higashi-Hiroshima (Y. Sugawara, unpublished). Thus, *A.* sp. nr. *sawadai* probably uses the *C. azaleae* pheromones as a kairomonal cue to locate host mealybugs.

In support of this hypothesis, a previous study demonstrated that an *Anagyrus* parasitoid showed kairomonal responses to the sex pheromone of a host mealybug (Franco et al. 2008): *Anagyrus vladimiri* (called *Anagyrus* sp. nov. near *pseudococci* in the referenced paper) was strongly attracted to lavandulyl senecioate, the major pheromone component of the vine mealybug *Planococcus ficus*. Franco et al. (2008) also demonstrated that another component of the *P. ficus* pheromone, lavandulyl isovalerate, was less attractive than lavandulyl senecioate. Kol-Maimon et al. (2010) inferred that the blend variations in the *P. ficus* pheromone components, lavandulyl senecioate and lavandulyl isovalerate, might be promoted by their different kairomonal activities in attracting the wasps. In the case of the *C. azaleae* pheromone, the isopropyl ester components are likely to elicit stronger kairomonal responses of *Anagyrus* wasps than the ethyl esters (Fig. 3), although the variations in the isopropyl and ethyl esters in the *C. azaleae* pheromone remain to be examined. Further studies of pheromone structures and their kairomonal functions in Asia–Pacific regions rich in biodiversity may provide essential insights into the whole scenario of the evolution of pheromone communication systems in mealybugs.

Another important practical implication of our findings is the potential application of synthetic pheromones as kairomones and the use of parasitoids in biological control programs against pest mealybugs. In our previous studies, we showed that two closely related *Anagyrus* wasps, *A. sawadai* and *A. subalbipes* (Sugawara et al. 2020), which attack a broad range of mealybugs, could be recruited by a mealybug-pheromone analogous attractant for generalist parasitoids and used as biological agents to suppress mealybug population increases (Teshiba and Tabata 2017; Teshiba et al. 2012). It may be possible to use the *C. azaleae* pheromones as a similar attractant for a specialist parasitoid of *C. azaleae* to increase the numbers and performance of *Anagyrus* wasps that attack azalea mealybugs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10886-024-01473-2.

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Data Availability The data are available from the corresponding author upon reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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