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Sex Pheromone of the Cotton Mealybug, *Phenacoccus solenopsis,* with an Unusual Cyclobutane Structure

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Abstract The cotton mealybug, *Phenacoccus solenopsis*, the distribution of which was formerly limited to Nearctic and Neotropical regions, recently invaded many countries in various regions including Asia, Africa, and the Pacific. More recently, *P. solenopsis* was newly recorded in Japan and is currently an emerging pest of agricultural crops. In this study, we determined the structure of a sex pheromone of *P. solenopsis* in order to develop an effective lure for monitoring this pest. From volatiles emitted by virgin adult females, we isolated a compound attractive to males. By means of coupled gas chromatography–mass spectrometry and nuclear magnetic resonance spectroscopy, we identified this as (2,2-dimethyl-3-isopropylidenecyclobutyl)methyl 3-methylbut-2-enoate. This compound was synthesized and shown to be attractive to male *P. solenopsis*. Analysis by gas chromatography using an enantioselective stationary phase and polarimetry analyses of the natural pheromone and synthetic enantiomers showed the natural compound to be the (*R*)-(−)-enantiomer. This compound is an ester of maconelliol, which has an unusual cyclobutane structure found in sex pheromones of other mealybug species, and senecioic acid, also found in the pheromones of other mealybug species. However, this is the first example of the ester of maconelliol and senecioic acid as a natural product.

Introduction

Mealybugs (Hemiptera: Pseudococcidae) are small sap-sucking insects that are covered with powdery wax. They exist worldwide and are parasites of various plants, including many agricultural crops (Ben-Dov 1994). Some mealybugs recently have expanded their distributions, probably as a result of increasing global commerce in live plant materials (Gullan et al. 2003) and/or climate change (Gutierrez et al. 2008; Jara et al. 2013). Exotic pests can cause serious damage in the area they invade, and the cotton mealybug, *Phenacoccus solenopsis* Tinsley, is one such example. Distribution of this species previously was limited to the Nearctic and Neotropical regions, but, since 2005, it has been known to have invaded cotton-growing areas of Pakistan and India (Hodgson et al. 2008). The infestation by *P. solenopsis*, which was described by the name of *Phenacoccus gossypiphilous* Abbas, Arif & Saeed in a *nomen nudum* (Abbas et al. 2005), was recorded in cotton fields of the Punjab and Sindh provinces of Pakistan. This outbreak occurred on both Bt and non-Bt cotton, leading to the use of large amounts of pesticides against this pest — more than 120 million US dollars' worth in two months in 2007 (Hodgson et al. 2008). The pest is known to have subsequently invaded many countries in various regions including Asia, Africa, and the Pacific (García Morales et al. 2016). More recently, this species has been recorded in Japan (García Morales et al. 2016; Tanaka and Tabata 2014; Tanaka and Uesato 2012) and has been reported to attack agricultural crops including tomato and eggplant (Inada 2016). Detection and monitoring of this potential agricultural pest is thus urgently required for worldwide quarantine. Traps baited with

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pheromones would be a useful monitoring tool that would be less time-consuming than other methods (Gut et al. 2004).

Sex pheromones have been isolated and identified in 15 mealybug species (Tabata and Ichiki 2015). Most of the isolated compounds contain irregular monoterpene moieties. In monoterpenes, the two isoprene units are generally coupled by a 1–4′ linkage ("head-to-tail" connection; Breitmaier 2006), whereas mealybug monoterpene moieties contain unusual non-head-to-tail connections, such as the 1–2′ linkage that is found in lavandulol (5-methyl-2-isopropenyl-4-hexenol), a characteristic component of lavender fragrance (e.g., Tabata et al. 2015). Although there are common motifs, the mealybug pheromone components previously examined differ among species, and each species produces a characteristic compound (Millar et al. 2005; Zou and Millar 2015). Thus, the sex pheromone systems of mealybugs are clearly species-specific and provide a good opportunity not only to develop a pest management tool but also to study the structural diversity of the chemicals used in communication.

In the present study, we used chromatography to isolate the sex pheromone compound of *P. solenopsis* from volatiles emitted by virgin female. Coupled gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectrometry were used to determine the chemical structure. The pheromone candidate was synthesized enantioselectively, and its attractiveness to males was confirmed. Moreover, the absolute configuration of the natural pheromone was determined by comparisons to synthetic enantiomers in GC analyses with an enantioselective stationary phase.

Methods and Materials

Analytical Instruments Analyses by coupled gas chromatography-mass spectrometry (GC-MS) analyses were performed with an HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with an SX-102 A mass spectrometer (JEOL, Tokyo, Japan). The temperatures of the GC interface and the ion source were 210 °C and 220 °C, respectively. A nonpolar column (DB-1; 0.25 mm internal diam × 30 m length, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) was used. Helium was the carrier gas at a constant flow rate of 1.0 ml/min. The column oven temperature was maintained at 60 °C for 1 min, raised to 220 °C at 8 °C/min, and held at the final temperature for 5 min. Mass spectra were obtained in the electron-impact mode at 70 eV. For certain runs, *n*-alkanes were added as references for calculation of retention indices (Kováts indices, KI).

An enantioselective cyclodextrin column (beta-DEX 120; 0.25 mm internal diameter × 30 m length, 0.25 μm film thickness; Restek, Bellefonte, PA, USA) also was used, and the GC

oven temperature was programmed to 100 °C for 5 min and then raised to 180 °C at 5 °C/min.

Nuclear magnetic resonance (NMR) spectra were obtained at 30 °C with a JNM-A600 spectrometer (^{1}H: 600.05 MHz, ^{13}C: 150.80 MHz; JEOL). Micro-bottom NMR tubes (SP-501; 1.7 mm outside diam; Shigemi Co., Tokyo, Japan) were used with 35-μl of deuterated benzene (min. 99.96 %; Sigma-Aldrich, St. Louis, MO, USA) as the solvent. Optical rotation was measured with a P-1020 polarimeter (JASCO, Tokyo, Japan).

Collection of Volatiles from Insects Mealybugs were collected from sunflowers (*Helianthus annuus*) growing on Ôhama beach (24.3°N, 124.2°E; Ishigaki City, Okinawa Prefecture, Japan) on 16 July 2015. The insects were reared on germinated broad bean seeds and maintained in a rearing room (16:8 h L:D; 23 °C; 50 % RH). Males and females were manually separated immediately after the males made cocoons, and females were transferred to a new container (10 cm diam × 4 cm height) with diet.

Volatiles from virgin female mealybugs were collected by placing germinated broad bean seeds with approximately 200 virgin females into a 0.2-L glass jar. Ambient air cleaned through activated charcoal (15 g) was pulled over the virgin females in the jar and then passed through an adsorbent (1.0 g of HayeSep Q; 60/80 mesh; Alltech, Deerfield, IL, USA) at a flow rate of 0.1 L/min using a vacuum pump. Every 3–4 d, the volatiles were extracted from the adsorbent with 15 ml of hexane, the extract was concentrated with an evaporator at room temperature, and the residue was kept at −20 °C. The collection of volatiles continued for 6 wk., and four rounds of the collection were performed to obtain approximately 33,600 female day equivalents (FDE) in total.

Voucher specimens are stored at the National Agriculture and Food Research Organization (Tsukuba, Japan). Partial mitochondrial DNA sequences of these were determined by a previously described method (Tabata et al. 2016) and deposited in the GenBank/DDBJ/EMBL database (accession number LC171400).

Preparative Liquid Chromatography Open-column chromatography was carried out on silica gel (0.2 g; Wakogel C-200; Wako Pure Chemicals, Osaka, Japan) by a method for separating lipids by class. The column was successively eluted with 2 ml each of 0, 5, 15, and 50 % diethyl ether in hexane. High-performance liquid chromatography (HPLC) was performed with an HP 1050 instrument (Hewlett-Packard) with a silica gel column (Inertsil SIL; 4.6 mm internal diam × 250 mm length, 5 μm particle size; GL Sciences Inc., Tokyo, Japan). The elution solvent was 5 % diethyl ether in hexane at a flow rate of 1 ml/min, and the eluate was monitored with a UV detector (λ = 210 nm).

Preparative Gas Chromatography Preparative GC was carried out with an Agilent 6890 N gas chromatograph (Agilent Technologies Inc., Wilmington, DE, USA) equipped with a flame ionization detector kept at 220 °C and a preparative fraction collection system (Gerstel GmbH & Co. KG, Mulheim an der Ruhr, Germany) cooled by a dry ice–acetone bath. A polar column (TC-FFAP; 0.53 mm internal diam × 15 m length, 1 μm film thickness; GL Sciences Inc.) was installed, and the gas chromatograph oven temperature was held at 50 °C for 1 min and then increased to 200 °C at 8 °C/min. A fully programmable injector (OPTIC 3; ATAS GL International, Eindhoven, The Netherlands) maintained at 40 °C for 10 s and then raised to 200 °C at 5 °C/s was used for large-volume injection; 10 μl of the sample were injected in splitless mode for 1 min. Helium was used as the carrier gas at 5 ml/min.

Bioassays The attractiveness to males of each chromatographic fraction was assayed as described previously (Arai 2000). Adult males (10 individuals) were released into a glass dish (9 cm diam, 2 cm height). A sample (0.1 FDE) dissolved in 5 μl of hexane was loaded onto a piece of filter paper (Toyo Roshi Co. Ltd., Tokyo, Japan) and placed in the dish. After 5 min, the number of males mounted on the sample as a percentage of the number of males released was used as an index of attractiveness. Three replicates were performed for each sample in the glass dish assay.

Attractiveness of the synthetic pheromones was further confirmed by a trap bioassay in a greenhouse (3.0 × 4.8 × 2.8 m) located at NARO (Tsukuba, Ibaraki Prefecture, Japan), where mealybugs naturally occurred on potted potato plants. Males were captured by a white delta trap with a sticky board (12 × 22 cm) baited with 0.05 mg of the synthetic pheromone impregnated into a red rubber septum (8 mm outside diam × 19 mm height; Wheaton, Millville, NJ, USA). A trap baited with a blank (solvent only) septum was prepared as a control. One set of traps for each sample was placed 0.6 m above the ground at approximately 1.5 m intervals, and the assay was performed seven times with randomly exchange of the trap locations from 11 to 17 June 2016. Data were analyzed by using generalized linear mixed models with a Poisson error distribution to assess the effects of treatments and random effects to account for overdispersion of scores among the tests. The calculations were performed by the function 'glmer' of the lme4 package in the R statistical software package (version 3.2.5; R Development Core Team 2016). Multiple comparisons were performed with Bonferroni corrections.

Micro-Chemical Reactions The compound proposed as pheromone (100 ng) was dissolved in 1 ml of 0.5 M KOH in ethanol solution for ethanolysis. The reaction mixture was held overnight at room temperature, poured into 1 ml of H_2O, and extracted with hexane (3 × 1 ml). The combined extracts were concentrated to approximately 30 μl with an evaporator

at room temperature, and an aliquot (2 μl) was used for GC-MS analysis. The residue was dissolved in 1 ml of CH_2Cl_2 and was re-esterified with 10 μl of 0.1 mM solution of 3-methyl-2-butenoic acid (senecioic acid; Tokyo Chemical Industry Co., Tokyo, Japan) in the presence of 10 μl each of 0.1 mM solution of 4-dimethylaminopyridine (DMAP; Tokyo Chemical Industry Co.) and N,N′-diisopropylcarbodiimide (DIC; Wako Pure Chemicals). The reaction mixture was stirred overnight at 40 °C, poured into 1 ml of H_2O, and extracted with CH_2Cl_2 (3 × 1 ml). The combined extracts were dried over Na_2SO_4 and concentrated to approximately 30 μl with an evaporator at room temperature, and an aliquot (2 μl) was used for GC-MS analysis. For hydrogenation, the proposed pheromone (100 ng) was dissolved in 50 μl of ethanol, and the solution was stirred in the presence of platinum black catalyst in a H_2 atmosphere. After 10 min, the solution was centrifuged, and an aliquot (2 μl) of the supernatant was used for GC-MS analysis.

Synthesis of Maconelliyl Senecioate (R)- and (S)-(2,2-Dimethyl-3-isopropylidenecyclobutyl)methanol (maconelliol) were prepared from (+)- and (−)-α-pinene (>99 % ee; Sigma-Aldrich), respectively, by previously described methods (Zhang and Nie 2005; Zhang et al. 2004b). To a solution of each enantiomer of the alcohol (7.7 mg, 50 μmol) in CH_2Cl_2, senecioic acid (7.5 mg, 75 μmol), DIC (12 μl, 75 μmol), and DMAP (9.2 mg, 75 μmol) were added. After the reaction mixture was stirred at 40 °C for 24 h, the solvent was removed with an evaporator. The residue was poured into 2 ml of H_2O and extracted with hexane (3 × 2 ml). The combined extract was washed with 4 ml of brine, dried over Na_2SO_4, concentrated, and purified by using silica gel chromatography with 5 % diethyl ether in hexane as an eluent to give maconelliyl senecioate (9.6–10.8 mg, 81–92 % yield); GC-MS (EI 70 eV): Rt 13.87 min (on DB-1), m/z 55 (9 %), 67 (4 %), 83 (33 %), 93 (15 %), 105 (10 %), 121 (100 %), 136 (37 %), 236 (0.3 %, M^+); ^1H–NMR δ_H: 1.14 [3H, s, $(CH_3)(CH_3)C<$], 1.25 [3H, s, $(CH_3)(CH_3)C<$], 1.40 [3H, s, $(CH_3)(CH_3)C = C<$], 1.43 [3H, s, $(CH_3)(CH_3)C = CH$], 1.51 [3H, s, $(CH_3)(CH_3)C = C<$], 2.12 [1H, br, $CHCHHC=$], 2.13 [3H, s, $(CH_3)(CH_3)C = CH-$], 2.23 [1H, m, $CHCH_2OCO$], 2.51 [1H, br, $CHCHHC=$], 4.23 [2H, dd, J = 1.8, 8.4 Hz, CH_2OCO], 5.75 [1H, s, $CH = C(CH_3)_2$]; ^{13}C–NMR δ_C: 18.6, 19.6, 20.0, 21.1, 26.9, 28.0, 28.6, 39.8, 44.4, 64.7, 116.7, 122.4, 137.7, 155.9, 166.3; (R)-maconelliyl senecioate: $[\alpha]^{22.0}_D$ = −14.6 (observed, 99.8 % ee, c 0.181, hexane); (S)-maconelliyl senecioate: $[\alpha]^{22.0}_D$ = +11.9 (observed, 71.8 % ee, c 0.130, hexane).

Results

Isolation of Pheromone The crude collection of volatiles emitted by virgin female *P. solenopsis* (Fig. 1a) was

fractionated into four fractions (0, 5, 15, and 50 % diethyl ether in hexane) by means of open-column chromatography on silica gel. Only the 5 % diethyl ether/hexane fraction exhibited attractiveness to male mealybugs (86.7 ± 13.3 %, mean $\pm$ SEM of males attracted). Esters are predicted to elute in this fraction, and it was separated further into seven fractions (Lc. 1–7) by means of HPLC (Fig. 1b). Males showed the strongest attraction toward Lc. 3 (93.3 ± 6.7 %). Lc. 3 was subjected to preparative GC and separated into three fractions (Gc. 1–3; Fig. 1c). Only Gc. 2, which consisted of a single compound, showed significant attractiveness (100 %), and this compound was assumed to be a component of sex pheromone. The amount of pheromone was approximately 0.2 ng per FDE.

Chemical Analyses In GC-MS analyses, the isolated compound appeared at KI = 1598 on a DB-1 column. High-resolution electron-impact MS data (m/z 236.17845) showed that the molecular formula was $C_{15}H_{24}O_2$ (calculated 236.17763) and indicated that four units of unsaturation (double-bonds or rings) were present. Characteristic mass fragments were observed at m/z 136 [$M^+ - 100$ (C_4H_7COOH)], 121 [136–15 (CH_3)], and 93 [136–43 ((CH_3)$_2CH$)] (Fig. 2a). Platinum-catalyzed heterogeneous hydrogenation generated a pair of diastereoisomers in approximately 3:1 ratio. These products had indistinguishable mass spectra with the molecular ion at m/z 240 (236 + 4), indicating that the original compound had two olefinic double bonds (Fig. 2b). The fact that the hydrogenation generated a pair of diastereomers suggests that one of the double bonds was either in a ring or attached to a ring containing an asymmetric carbon. Platinum-catalyzed heterogeneous hydrogenation of olefins proceeds via *syn* addition of H_2, and one face of a double bond in or attached to a ring is usually favored over the other. Moreover, a unique fragment ion at m/z 170 [240–70 (C_5H_{10})] was observed in the mass spectrum after

Fig. 1 Total ion chromatogram (TIC) from GC-MS analysis of collection of volatiles from virgin female *Phenacoccus solenopsis* (**a**); preparative HPLC chromatogram of 5 % diethyl ether/hexane fraction from silica gel open-column chromatography of the collection of volatiles (**b**); preparative GC chromatogram of HPLC fraction Lc. 3 (**c**); the candidate pheromone compound was isolated from GC fraction Gc. 2
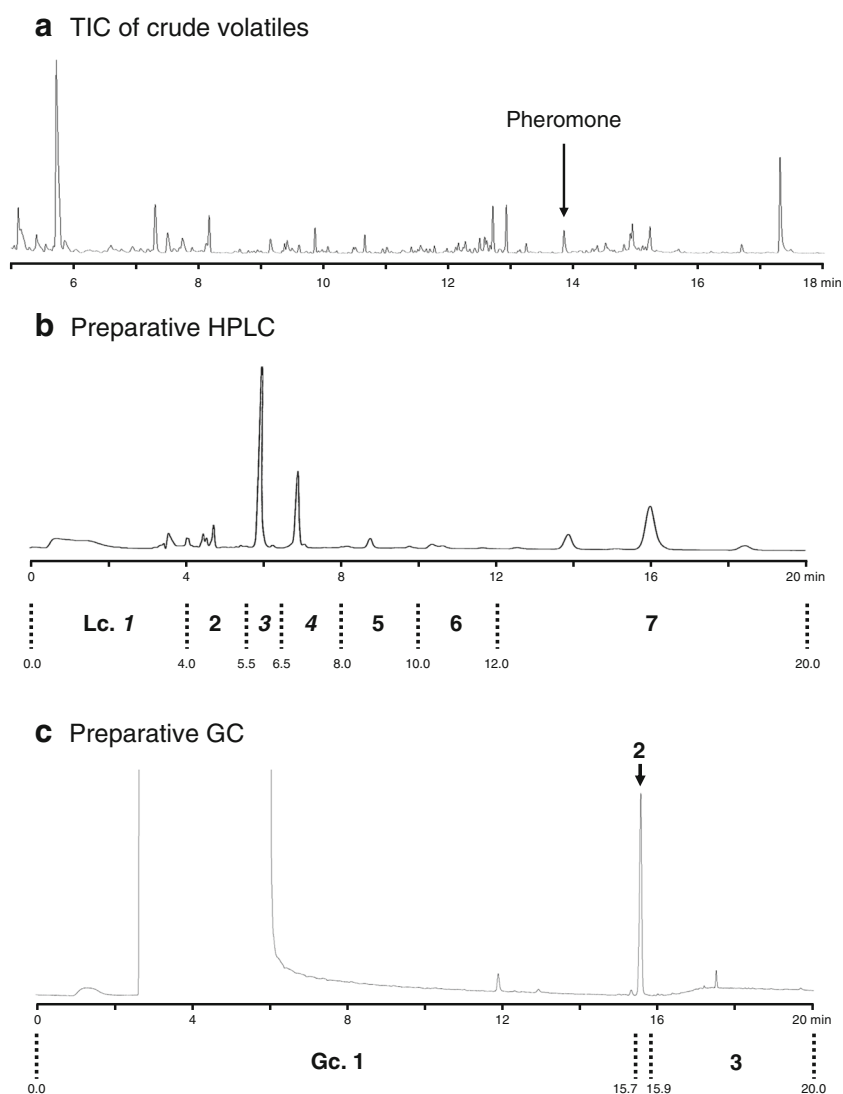
Fig. 2 Mass spectra of the candidate pheromone (a), its hydrogenated product (b), and ethanolysis products (c, d), measured in electron-impact mode
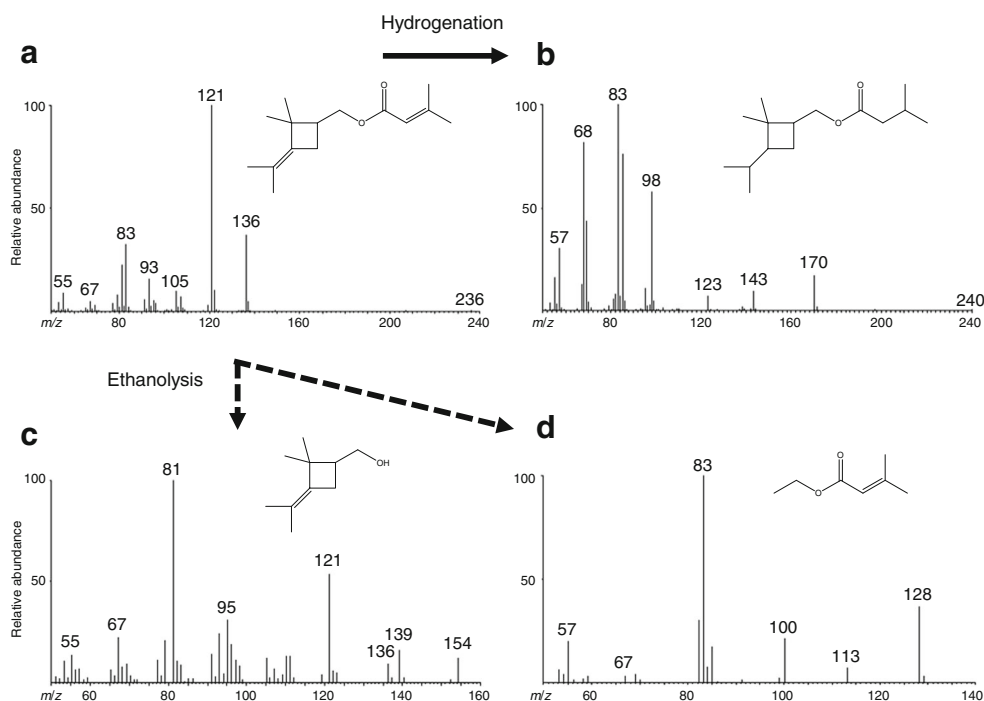

hydrogenation. The same fragmentation pattern also was reported in the hydrogenation product of (2,2-dimethyl-3-isopropylidenecyclobutyl)methyl 2-methylbutanoate (maconelliyl 2-methylbutanoate), and this fragmentation was interpreted as 2 + 2 retro-cleavage of a cyclobutane structure (Zhang et al. 2004a). Thus, the present candidate pheromone of P. solenopsis was suggested to have two olefinic double bonds and one C4 ring.

After basic ethanolysis, an alcohol with a molecular ion at m/z 154 [136 + 18 (H_2O)] (Fig. 2c) and a transesterification product of an acid with a molecular ion at m/z 128 (Fig. 2d) were generated. The mass spectrum of the alcohol was almost identical to that reported for maconelliol by Zhang et al. (2004a). The GC-retention time (4.42 min) and the mass spectrum (Fig. 2d) of the esterified acid were identical to those of ethyl senecioate (Tokyo Chemical Industry Co.). Re-esterification of the ethanolysis product with senecioic acid restored the retention time and the mass spectrum pattern of the original compound. These results indicate that the compound was a senecioate of an alcohol similar to maconelliol.

Approximately 4 μg of the isolated candidate pheromone dissolved in 35 μl of C_6D_6 were placed in a micro-bottom NMR tube, and ^1H–NMR spectra and ^1H–^1H correlation spectra were measured. ^1H–NMR analysis showed a characteristic spectrum indicating one olefinic proton, six sets of methyl protons, two sets of methylene protons, and one methine proton (Fig. 3). Four of the methyl groups (singlets at 1.40, 1.43, 1.51, and 2.13 ppm) were indicated to be on double bonds and

to form two isopropylidene groups, one of which was assumed to be part of a senecioyl group. The signal downfield at 2.13 ppm probably was affected by a spatially-close carbonyl oxygen and was characteristic of a senecioyl group. The other two methyl groups (singlets at 1.14 and 1.25 ppm) were consistent with being on a quaternary carbon. One olefinic proton (singlet at 5.75 ppm) was considered to be attached to a double bond in a senecioyl group. One set of methylene protons was observed at a characteristic chemical shift of 4.23 ppm, indicating that it was attached to a senecioyl group. This signal was coupled with the methine proton signal (2.23 ppm). The methine proton also was coupled with the other set of methylene protons. Because no other protons were observed, these methine and methylene were considered to be part of a C4 ring with quaternary carbons. Accordingly, the pheromone was deduced to be either (2,2-dimethyl-3-isopropylidenecyclobutyl)methyl senecioate or (3,3-dimethyl-2-isopropylidenecyclobutyl)methyl senecioate. We considered the former to be more plausible, because long-range homoallylic coupling (CH–C = C–CH) was observed for the methylene protons in the four-membered ring; that is, we interpreted the broad signals of these two protons (2.12 and 2.51 ppm) as indicating long-range homoallylic coupling between them and the terminal methyl groups on an isopropylidene group. This idea was supported further by GC-MS data of the hydrogenated product (Fig. 2b); a characteristic fragment ion was observed at m/z 170 [240–70 (C_5H_{10})], which was presumably generated by 2 + 2 retro-cleavage and was assumed to be m/z 184 [240–56 (C_4H_8)] if the compound was 3,3-dimethyl.

Fig. 3 ^{1}H–NMR spectrum (600.05 MHz) of the synthetic and natural pheromones. Chemical shifts are expressed in parts per million (ppm) by frequency relative to residual C_6H_6 (δ 7.15 ppm) in the solvent
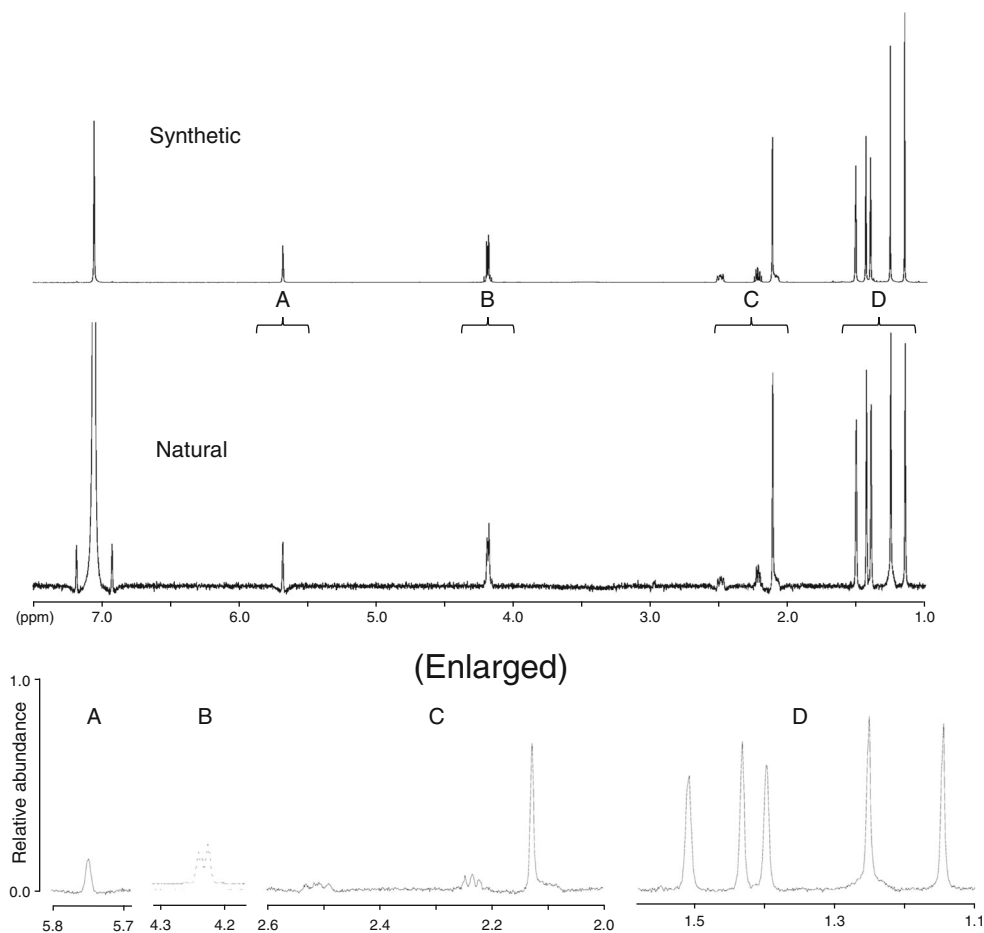
Synthesis, Absolute Configuration, and Activity The pheromone candidate, (2,2-dimethyl-3-isopropylidenecyclobutyl) methyl senecioate (maconelliyl senecioate), was synthesized via condensation of maconelliol and senecioic acid in the presence of DMAP and DIC. The chemical data including GC retention time, mass spectrum, and ^{1}H–NMR spectrum of the synthetic candidate were identical to those of the natural pheromone.

The pheromone compound has a chiral center at the second carbon of the alcohol moiety (maconelliol). We therefore determined the absolute configuration by comparative analyses of the hydrolysis product of the natural pheromone and synthetic (R)- and (S)-maconelliol by GC on an enantioselective column. The GC retention time of the natural maconelliol (15.48 min) was identical to that of (R)-maconelliol rather than that of (S)-maconelliol (15.63 min). The pheromone therefore was concluded to be (R)-(−)-maconelliyl senecioate. The hydrolysis and re-esterification reactions used in this study did not result in any epimerization, as indicated by repeated analyses by enantioselective GC.

Finally, the biological activities of synthetic (R)- and (S)-maconelliyl senecioate were examined by a trap experiment in a greenhouse occupied by mealybugs. (R)-Maconelliyl

senecioate (99.8 % ee) elicited strong and significant attractiveness to males; more than 30 males per day (mean value) were attracted and captured by (R)-enantiomer-baited traps. (S)-Maconelliyl senecioate (71.8 % ee) showed significant but relatively weak activity; the number of males attracted to the (S)-enantiomer was less than half the number that were attracted to the (R)-enantiomer (Fig. 4).

Discussion

We isolated the sex pheromone from volatiles emitted by virgin adult females of the cotton mealybug, *P. solenopsis*, and determined it to be (2,2-dimethyl-3-isopropylidenecyclobutyl)methyl 3-methybut-2-enoate (maconelliyl senecioate). To the best of our knowledge, the ester of maconelliol and senecioic acid has not been described previously as a natural product. Moreover, we confirmed the pheromone to be (R)-(−)-maconelliyl senecioate by comparisons of the natural and synthetic compounds in GC analyses by using a chiral stationary phase column. The synthetic (R)-enantiomer (99.8 % ee) showed strong attractiveness to male mealybugs. This study will, therefore, open the way for the
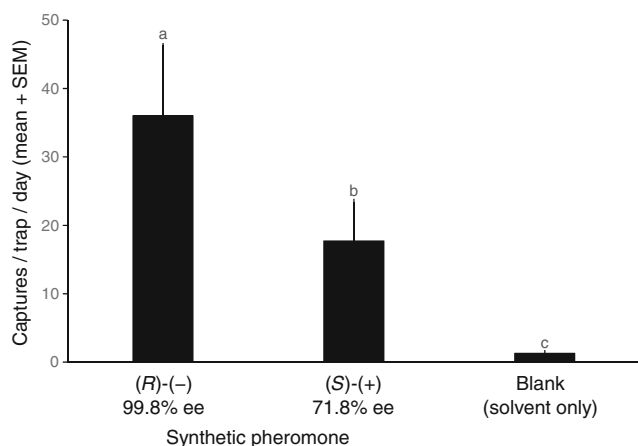
Fig. 4 Attractiveness of (*R*)- and (*S*)-enantiomers of the synthetic pheromone to males in a greenhouse. Each of the enantiomers (0.05 mg) was released from a rubber septum. Significant differences are indicated with lowercase letters (multiple comparisons using generalized linear mixed models followed by the Bonferroni method, $N = 7$, $P < 0.05$)

development of a useful attractant for monitoring the emergence or invasion of *P. solenopsis*.
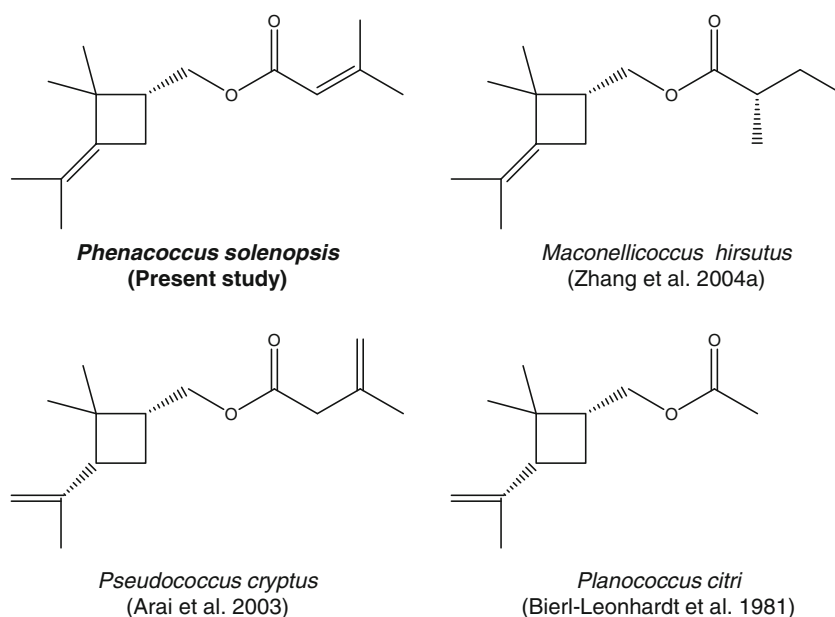
Traps baited with the other synthetic enantiomer, (*S*)-maconelliyl senecioate, attracted a substantial number of males. This suggests that (*S*)-enantiomer would not negatively affect male attraction, although whether the pure (*S*)-(+)-enantiomer has attractiveness remains unclear because the (*S*)-enantiomer prepared in this study was not enantiomerically pure (71.8 % ee). Partial epimerization was considered to have occurred during exothermic reactions for our preparation of (*S*)-maconelliol. Similar epimerization is reported in an original synthesis of maconelliol (Zhang et al. 2004b). However, this suggests it is not necessary to remove the opposite

enantiomer potentially generated during synthesis of (*R*)-maconelliol for its development as a pest management tool, which is thus economically favorable.

Nineteen pheromone compounds from 16 mealybug species have now been reported. All of the compounds are esters of alcohols and carboxylic acids, and the alcohol moieties are generally monoterpenes, with one exception - isoprenol in *Crisicoccus matsumotoi* (Tabata et al. 2012). Four of the alcohols include cyclobutane structures (Fig. 5). *Planococcus citri* (Bierl-Leonhard et al. 1981) and *Pseudococcus cryptus* (Arai et al. 2003) use (2,2-dimethyl-3-isopropenylcyclobutyl) methyl esters, and *Maconellicoccus hirsutus* (Zhang et al. 2004a) and *P. solenopsis* use (2,2-dimethyl-3-isopropylidenecyclobutyl)methyl (maconelliyl) esters. Both of these moieties have the same unusual non-head-to-tail (1–2′) linkage of isoprene units that is found in lavandulol (Sugie et al. 2008; Zhang et al. 2004a). Moreover, the second carbon of the alcohol moiety in each of these four pheromones is in the (*R*)-configuration. Thus, these four mealybugs may share a mechanism for biosynthesis of unusual cyclobutane structures via a unique connection of isoprene units, a mechanism that differs from the general monoterpene biosynthesis mechanism found in other organisms. Other than mealybugs, monoterpenes related to lavandulol with non-head-to-tail linkages are found in a few plants including *Lavandula angustifolia* (Lamiaceae) (Da Porto et al. 2009), *Seseli indicum* (Apiaceae) (Logani et al. 1967), and *Artemisia* spp. (Asteraceae) (Gunawardena et al. 2002).

Although the pheromones of *P. solenopsis* and *M. hirsutus* include maconelliol as the alcohol moiety in common, their acid moieties are different; the former is senecioate, and the latter is (*S*)-2-methylbutanoate (Fig. 5). The difference in the acid moiety is expected to act as a key for species recognition

Fig. 5 Unusual cyclobutanoid structures found in mealybug pheromones

Phenacoccus solenopsis
(Present study)

Maconellicoccus hirsutus
(Zhang et al. 2004a)

Pseudococcus cryptus
(Arai et al. 2003)

Planococcus citri
(Bierl-Leonhardt et al. 1981)

between the two species when they occur simultaneously on the same host plants. It is unlikely, however, that these two pheromones have co-evolved along with interspecies interferences, because *P. solenopsis* seems to be of Nearctic or Neotropical origin (Hodgson et al. 2008), whereas *M. hirsutus* is considered to be native to Indo-Malayan or Australasian regions (Williams 1996). A senecioate pheromone, (*S*)-lavandulyl senecioate, is reported in another mealybug species, *Planococcus ficus* (Hinkens et al. 2001). Further comparative studies of pheromone chemistry in mealybugs would provide interesting insights into the diversification of insect pheromone communication systems.

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