Sex pheromone of the pink hibiscus mealybug, *Maconellicoccus hirsutus*, contains an unusual cyclobutanoid monoterpene

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Two compounds that together constitute the female sex pheromone of the pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus*, were isolated, identified, and synthesized. They are (*R*)-2-isopropenyl-5-methyl-4-hexenyl (*S*)-2-methylbutanoate [common name is (*R*)-lavandulyl (*S*)-2-methylbutanoate] and [(*R*)-2,2-dimethyl-3-(1-methylethylidene)cyclobutyl]methyl (*S*)-2-methylbutanoate [which we refer to as (*R*)-maconelliyl (*S*)-2-methylbutanoate]. Maconelliol is an unusual cyclobutanoid monoterpene, and its structure has been established by enantioselective synthesis from precursors of known structure and configuration. A 1:5 synthetic mixture of the two *RS* esters (1 μg per rubber septum) proved to be a potent attractant of males in field bioassays. The pheromone component, maconelliyl 2-methylbutanoate, represents a heretofore undescribed natural product.

electroantennogram | (*R*)-lavandulyl (*S*)-2-methylbutanoate | (*R*)-maconelliyl (*S*)-2-methylbutanoate | field bioassay

The pink hibiscus mealybug (PHM), *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae), causes severe economic problems throughout tropical and subtropical regions. It seems to be native to Australia or Southern Asia (1). This exotic insect pest has been spreading through the entire Caribbean region since it was first detected on the island of Grenada in 1994 (2, 3). Since then, it has spread to Southern California, Mexico, Central America, and, in 2002, to Florida (www.doacs.state.fl.us/pi/enpp/ento/pink.htm).

PHM feeds on a wide range of host plants, inflicting severe damage by injecting toxic saliva into the host plant, leading to malformation of fruit, leaves, and shoots, stunting of plant growth, and eventual plant death (4). Agricultural crops in the United States expected to be at greatest economic risk to PHM invasion include ornamental crops, vegetable crops, citrus, grapes, avocados, and many other plants (www.aphis.usda.gov/lpa/pubs/fsheet_faq_notice/fs_phphmealybug.html). Potential losses of $750 million per year have been estimated if the insect cannot be controlled (www.aphis.usda.gov/lpa/news/1999/09/MELBUGCA.HTM). To date, detection of PHM infestations relies on visual inspection although live virgin females can be used as an attractant source (5, 6); however, limited availability and low survivorship of live virgin females made this application impractical. A synthetic pheromone would provide a much more economical, convenient, and useful survey tool. An artificial lure might also enable the development of mass trapping and mating disruption technology for managing this pest, which would complement ongoing biological control eradication efforts in the Caribbean (4), California (www.aphis.usda.gov/lpa/news/1999/09/MELBUGCA.HTM), and Florida (www.doacs.state.fl.us/pi/enpp/ento/pink.htm). The present research was launched to help manage the PHM infestation in the continental U.S.

Materials and Methods

Insects Mass-Rearing. Virgin female mealybugs, *M. hirsutus*, were mass-reared on Japanese pumpkins in the U.S. Department of Agriculture (USDA)-Animal and Plant Health Inspection Service laboratory in Puerto Rico. Japanese pumpkins, *Cucurbita moschata* L., were field-grown at the USDA-Agricultural Research Service Experimental Station at St. Croix, U.S. Virgin Islands. Infestations of *M. hirsutus* were achieved by brushing 24-hr-old crawlers with a camel's hair brush onto the pumpkins twice a week. Infested pumpkins were maintained in an incubator at 27 ± 1°C, 70% relative humidity (RH), in total darkness. When the first molt occurred, the infested pumpkins were completely submerged for 30–40 s in a 100-ppm solution of an insect growth regulator, pyriproxifen (Distance, Valent, Walnut Creek, CA), which prevents development of males (7). After air-drying for 1 hr, pumpkins were placed in male-exclusion cages and kept in a rearing room at 26 ± 2°C, 60 ± 10% RH in darkness. If ovisacs were observed, the treated pumpkins were considered contaminated with mated females and were rejected for pheromone collection purposes.

Pheromone Collection and Purification. The pheromone was collected by using two groups of 14-day-old females (≈3,000 virgin females per group) in San Juan, PR. The females with white flocculent wax filaments were separately brushed into two 1-liter, four-necked glass containers (8). Humidified air was drawn into the container through 6-14 mesh-activated charcoal (Fisher Scientific) and out of the container through two traps (15 × 1.5-cm o.d.) containing Super Q (200 mg each; Alltech Associates) by vacuum (≈1 liter/min). Females were fed with 10% sugar solution on cotton balls and aerated continuously for 47 days at room temperature and 16 light:8 dark photoperiod. The adsorbent traps were changed every 5 days, and the adsorbents were eluted with methylene chloride (4 × 0.5 ml per each sample). The eluates were stored in −30°C freezer after eluting and then shipped to Beltsville, MD, by an express carrier. Each sample was concentrated to ≈100 μl under nitrogen for further analyses. Seven collections were then combined and concentrated. The combined extracts were subjected to either fractionation by micropreparative GC or microreaction. The micropreparative GC fractionation was carried out on a Hewlett–Packard 6890 GC equipped with a Gerstel preparative fraction collector (Gerstel, Baltimore) by using a 60 m × 0.53-mm i.d., 0.50-μm film-thickness DB-1 capillary column (J & W Scientific, Folsom, CA). Injector temperature was 32°C at injection and

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Abbreviations: PHM, pink hibiscus mealybug; EAD, electroantennographic detection; EI, electron impact; CI, chemical ionization.

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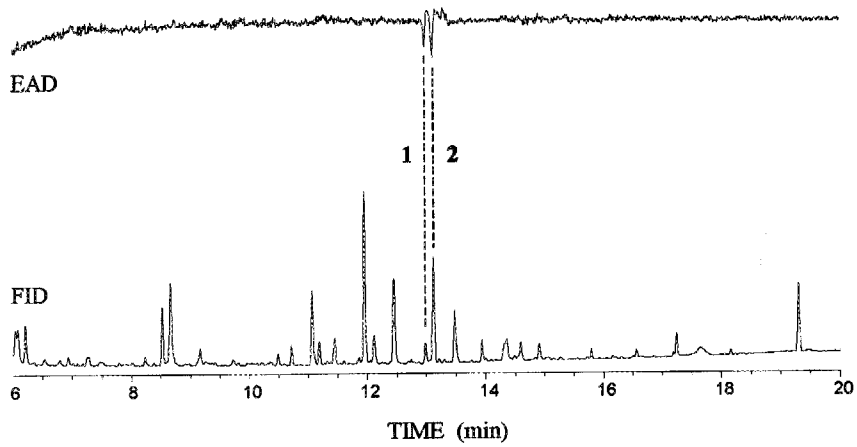


Fig. 1. Simultaneous responses of flame ionization detection (FID) and EAD of an adult male *M. hirsutus* to the volatiles of female PHM on a DB-WAXETR capillary column. Two EAD active compounds were marked as **1** and **2**.

programmed to 230°C at 60°C/min to transfer solute onto the column, which was held at 80°C for 2 min, then programmed to 220°C at 30°C/min and held for 30 min. Split ratio of column effluent to flame ionization detection and fraction collector was set at 4:96. The collector was cooled to −20°C by circulating MeOH from a benchtop refrigeration unit (Julabo F25-MP, Julabo, Mertztown, PA), and the collection efficiency was ≈70%.

Analytical Methods. The coupled GC-electroantennographic detection (GC-EAD) system used was as described (9–11). A Hewlett–Packard 6890 GC equipped with a 60 m × 0.25-mm i.d., 0.25-μm film-thickness DB-WAXETR (J & W Scientific) capillary column in the splitless mode with hydrogen (1.4 ml/min) as carrier was used for analysis. The column temperature program was held at 80°C for 2 min, then heated to 250°C at 15°C/min and held for 15 min. Electron impact (EI) MS was conducted on a Hewlett–Packard 6890 GC coupled to a Hewlett–Packard 5973 Mass Selective Detector by using an a 60 m × 0.25-mm i.d., 0.25-μm film-thickness DB-WAXETR capillary column at 50°C for 2 min, then programmed to 230°C at 15°C/min and held for 15 min or a 30 m × 0.25-mm i.d., 0.25-μm film-thickness DB-1 capillary column (50°C for 2 min, then programmed to 300°C at 15°C/min and held for 15 min) with helium as carrier gas. A 70-eV electron beam was used for sample ionization. Chemical ionization (CI) MS spectra were obtained from a Finnigan 4510 GC-MS with ammonia (NH_3) or with deuteroammonia (ND_3) as reagent gases by using the same above columns and conditions. GC-Fourier transform IR spectra were recorded on a Hewlett–Packard GC coupled to a Bio-Rad IRD II infrared detector by using a 60-m DB-WAXETR capillary column with conditions as described above. Chiral GC analyses were carried out isothermally at 100°C (55 cm/s) on a Hewlett–Packard 6890 GC, equipped with a 30 m × 0.25-mm i.d., 0.25-μm film-thickness β-DEX 120 capillary column (Supelco) in the split mode (100:1) with hydrogen as carrier. NMR spectra were recorded in C_6D_6 solution on a Bruker (Billerica, MA) QE Plus spectrometer at 300 MHz for ^1H. The chemical shifts are expressed in ppm relative to the residual solvent for ^1H (C_6H_6 at δ 7.15 ppm).

Microreactions. A pheromone aeration sample was hydrolyzed by treating a 50-μl (≈200 ng of pheromone) aliquot of combined extracts with 100 μl of 95% methanol and two drops of 2 M NaOH in a 2-ml vial for 1.5 hr at room temperature. Water (0.5 ml) was then added, and the aqueous phase was extracted with hexane (0.5 ml twice). The combined organic layers were concentrated under nitrogen (neutral part). The aqueous phase was acidified to pH < 2 by adding 2 M HCl and extracted with hexane (0.5 ml twice). The combined organic layers were concentrated under a stream of nitrogen to ≈50 μl (acid fraction).

Microhydrogenation was carried out by a catalytic amount of 10% palladium on carbon (Pd-C, Aldrich) in a 2-ml vial, which was sealed by a hydrogen-filled balloon. Samples included 50 μl of concentrated pheromone extract, hydrolyzed alcohol, and silanized alcohol (≈100 ng each). Each sample was diluted with 100 μl of ethyl acetate and stirred with a micro magnetic stirrer bar for 10 min. The solution was then transferred to another vial by a micropipette, and the reaction vial was washed with 50 μl of hexane several times. The resulting organic solution was concentrated to ≈20 μl.

Microsilylation of hydrolyzed alcohols was performed in 1-ml vials. An aliquot containing ≈50 ng of hydrolyzed or hydrogenated alcohol solution was evaporated to near dryness, and the residue was redissolved in 20 μl of silylation reagent, *N,O*-bis(trimethysily)-trifluoroacetamide (BSTFA, Aldrich). The reaction vial was maintained at 65°C for 2 hr, and excess reagent was evaporated by nitrogen, a few drops of hexane were added, the solvent was again evaporated, and the sample was redissolved in ≈10 μl of hexane.

Field Bioassays. Gray halo-butyl rubber septa (5 mm, West Pharmaceutical Services, Kearney, NE) loaded with 1 μg of *RS*, *SR*, *RR*, and *SS* binary diastereoisomeric mixtures of two candidate pheromone compounds placed on white sticky cards (Pherocon V traps, Trécé, Salinas, CA) were used for the field trails in Florida. Treatments were arranged in a randomized complete block design with nine replicates and about ≈1 m between traps within replicates. The cards were attached to the top of metal rods and placed 1 foot above hibiscus plants in a hedgerow. Cards were collected, put separately in transparent plastic bags, and brought back to the laboratory to count the trapped male PHM under a microscope. Although it would have been ideal to have PHM virgin females deployed in the field along the synthetic compounds, this experimental design was impossible because such females were not available at the Florida test site. Therefore, comparisons were made between candidate synthetic mixtures and a blank control trap.

Results

Coupled GC-EAD analyses of female airborne extracts demonstrated that male *M. hirsutus* antennae consistently responded to two compounds, **1** and **2** (Fig. 1). The EI MS spectra of both compounds (Fig. 2 *A* and *B*) contained parent ions at m/z 238. The assumption of m/z 238 as the molecular ion was confirmed by CI MS spectra (relative intensities in parentheses) {m/z 239 ($[M + H]^+$, 25) and 256 ($[M + NH_4]^+$, 100) for **1**; and 239 ($[M + H]^+$, 28) and 256 ($[M + NH_4]^+$, 100) for **2**} with ammonia (NH_3) as the

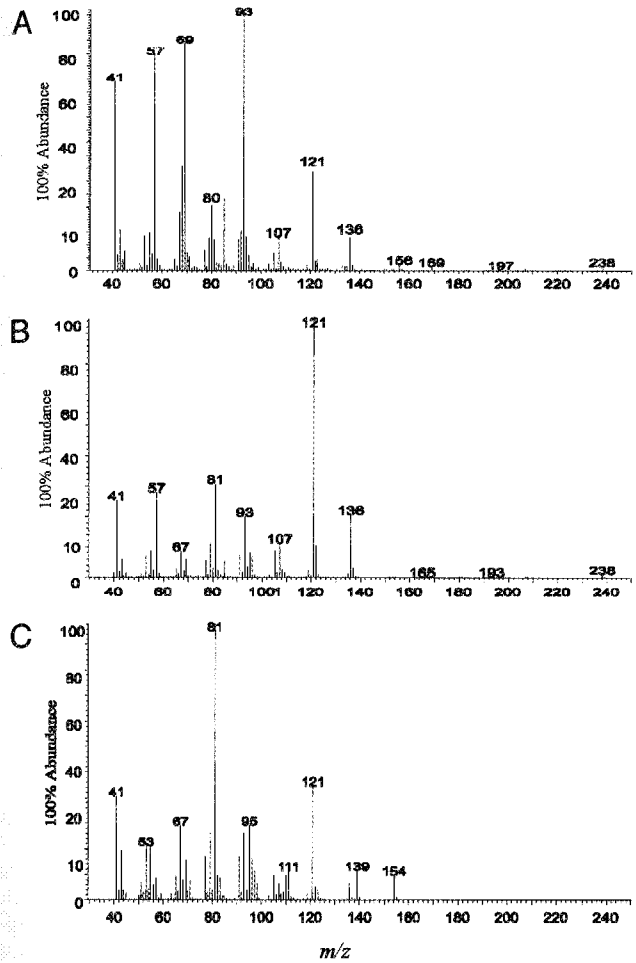


Fig. 2. The EI MS spectra of the natural pheromone and alcohol. (*A*) Compound **1**. (*B*) Compound **2**. (*C*) Maconelliol.

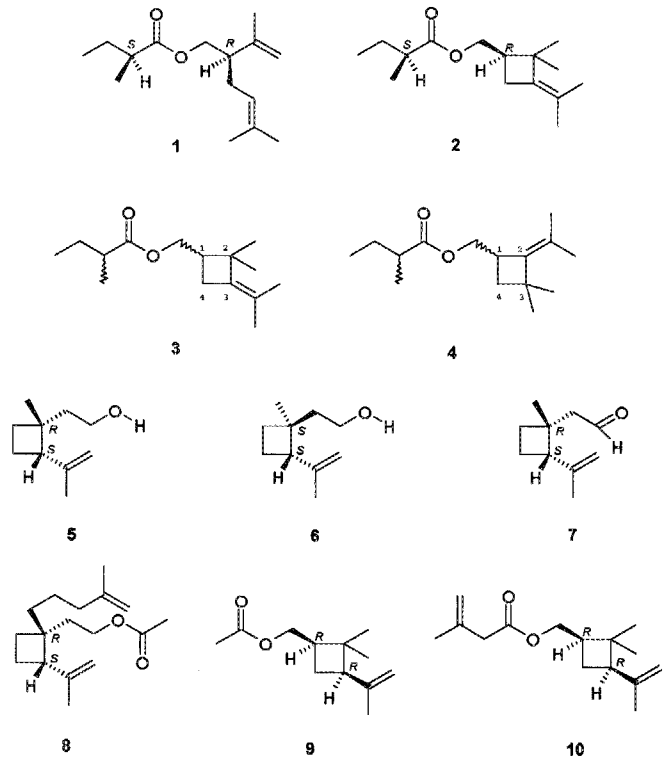


Fig. 3. Structures of *M. hirsutus* pheromone components **1** and **2** and other known semiochemicals that contain cyclobutane as a substructure.

reagent gas. The corresponding ions (M + 2 and M + 22) were obtained with deuteroammonia (ND_3), indicating that neither compound contained exchangeable protons. Both compounds produced fragment ions at m/z 136 (M − 102) without any other significant fragments in the high-mass region (between m/z 238 and 136, Fig. 2 *A* and *B*), suggesting that a neutral molecule was easily eliminated during fragmentation. The ions of m/z 136 (M − 102) could be interpreted as $[M - C_4H_9COOH]^{+\bullet}$ or $[M - C_6H_{13}OH]^{+\bullet}$. This hypothesis was supported by GC-Fourier transform IR data because **1** and **2** showed strong absorptions at 1,747 cm^{-1} (C=O) and hydrocarbon absorption bands but few other features. Thus, both compounds were proposed to be esters of either a C_5 acid or a C_6 alcohol.

In addition, presence of two double bonds in compound **1** and one double bond and one ring in compound **2** could be deducted from the CI MS ions of compounds produced by catalytic microhydrogenation. The ions exhibited at m/z 243 ($[M + H]^+$, 5) and 260 ($[M + NH_4]^+$, 100) indicated that 242 was the molecular weight and four hydrogens had been added to compound **1**. Similarly, ions at m/z 241 ($[M + H]^+$, 3) and 258 ($[M + NH_4]^+$, 100) indicated that 240 was the molecular weight and that only two hydrogens had been added to compound **2**. Because both compounds initially possessed the same molecular weight, the uptake of only one mole of hydrogen suggested the existence of a ring as the remaining element of unsaturation in compound **2**. Moreover, the hydrogenation product of compound **2** produced two GC peaks (3:1 ratio) with indistinguishable spectra, suggesting that the single double bond had been located either in the ring or connected to the ring with other chiral centers existing in the molecule so that the catalytic microhydrogenation occurred in favor of one side of the molecule and generated a pair of diastereoisomers in different amounts. It was also noticed that these two derivatives underwent distinctly different fragmentations during MS, producing ions at m/z M − 70 (instead of M − 102), resulting in m/z 170 being the highest mass ions of moderate abundance in the spectra. This fragmentation pathway was not observed before hydrogenation.

Alkaline hydrolysis was performed to confirm the existence of an ester. MS of the acid fractions from both compounds closely matched that of a spectrum of 2-methylbutyric acid (Wiley 275 mass spectral database). The MS of the alcohol (neutral fraction) from compound **1** closely matched a spectrum of lavandulol, suggesting that compound **1** was the 2-methylbutyrate of lavandulol ($C_{15}H_{26}O_2$) (Fig. 3). It seemed reasonable to assume that compound **2** also had the same molecular formula because it contained the same acid moiety and possessed the same molecular weight. A number of other microreactions were then performed to get more structural information about the remaining unknown alcohol, which we refer to as maconelliol (EI MS spectrum, see Fig. 2*C*). Microhydrogenation confirmed the existence of a double bond and a ring in the alcohol. This reaction gave two widely separated GC peaks with the same MS, demonstrating that maconelliol was a cyclic, unsaturated chiral alcohol {M − 70 fragmentation [m/z 86 (47)]}. These hydrogenated alcohols were then subjected to trimethylsilylation. The unique M − 70 fragmentation [m/z 158 (21)], was observed again from the MS, and molecular weights (m/z 228) were confirmed by CI MS [m/z 229 (15) and 246 (100)]. In contrast, trimethylsilylation of the natural alcohol (maconelliol) generated only one GC peak, which exhibited a MS similar to that of the natural pheromone **2**. Hydrogentation produced two isomers with indistinguishable {EI MS (M = 228), CI MS [m/z 229 (15), 246 (100)]}. Thus, maconelliol was proposed to be a monocyclic, monounsaturated alcohol.

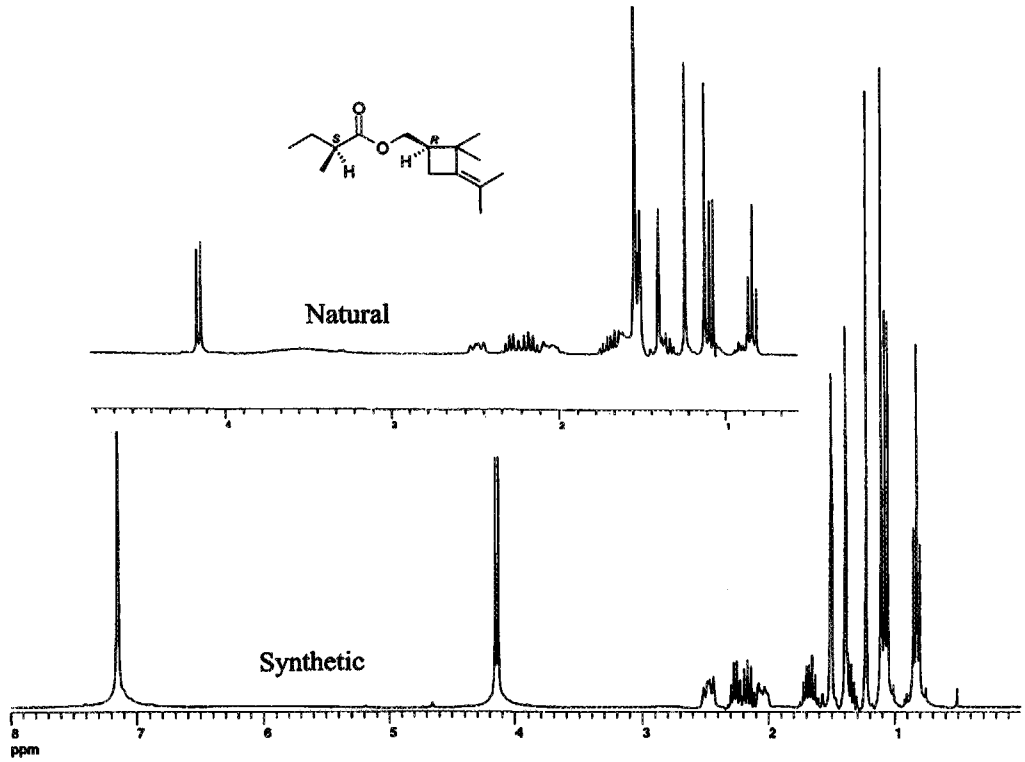


Fig. 4. ^{1}H NMR spectra (300 MHz) and structure of (*R*)-maconelliyl (*S*)-2-methylbutanoate.

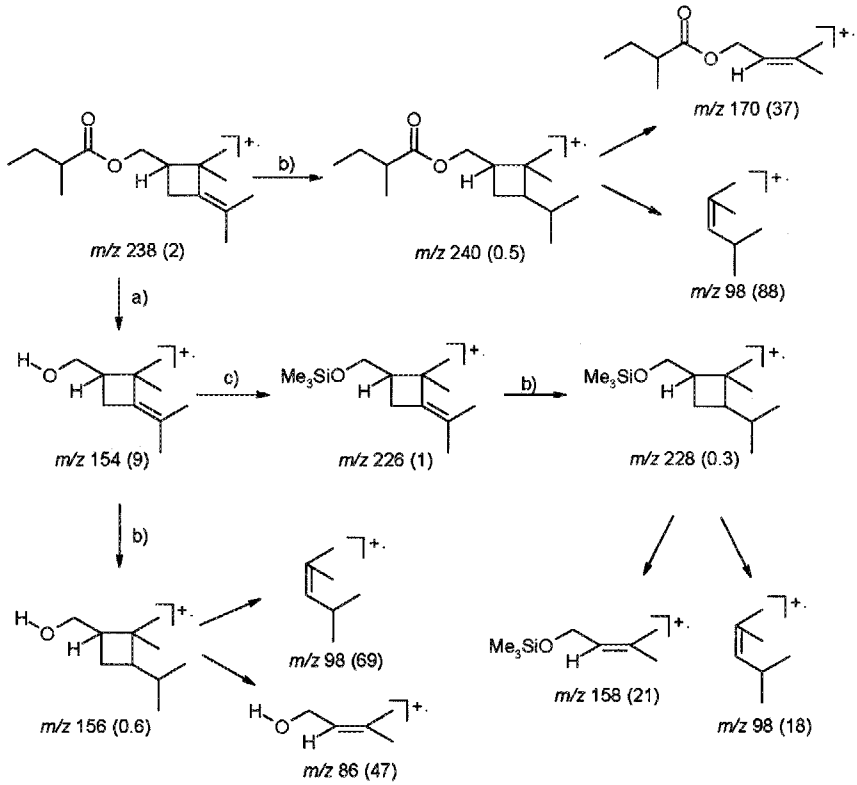
The ^{1}H NMR spectrum (Fig. 4) of purified compound **2** (≈30 μg obtained by preparative GC) contained resonance for 26 protons, including six methyl groups, consistent with the assumed molecular formula $C_{15}H_{26}O_2$. A doublet at 4.15 ppm (d, 2 H, J = 7.57 Hz) corresponded to a methylene in an ester (—O—CH_2—CH—). Four distinct methyl signals were clearly displayed; two signals at 1.51 (s, 3 H) and 1.40 (s, 3 H) ppm from two methyl groups on the double bond (CH_3—C═C), and another two signals at 1.24 (s, 3 H) and 1.12 (s, 3 H) ppm corresponding to a geminal dimethyl [$(CH_3)_2C$—] or two methyl groups on quaternary carbons. The remaining two methyl groups supported the presence of a 2-methyl butyrate moiety $CH_3CH_2CH(CH_3)CO$ (δ 0.83, t, 3 H, J = 7.19 Hz; 1.077, d, 3 H, J = 6.82 Hz). Accordingly, in the absence of any olefinic protons, the tetra-substituted cyclobutane 2-methylbutanoate (compound **3** or **4**, Fig. 3) were considered as reasonable pheromone candidates. The structure **3** was more consistent with the long-range homoallylic coupling (H—C—C═C—CH) observed for the methylene group in the four-member ring. The two protons at the 4 position were not equivalent. Broad signals at δ 2.47 and 2.05 could be interpreted as the homoallylic long-range coupling with the terminal methyl groups on the double bond. Correspondingly, these two methyl groups (δ 1.51 and 1.40) also exhibited the same coupling pattern (coupling constant < 1 Hz). This homoallylic long-range coupling was confirmed by a ^{1}H-^{1}H COSY spectrum and decoupling experiments.

The structure of **3** was also supported by MS data obtained from products of microreactions performed on the natural pheromone. The unique M − 70 fragmentation observed in hydrogenated products could be interpreted as a 2 + 2 *retro*-cleavage (Scheme 1). The fragment ion, m/z 184 (M − 56, C_4H_8), would be expected from compound **4** rather than the ion of m/z 170 (M − 70, C_5H_{10}). At least two types of 2 + 2 *retro*-cleavage ions had been detected in EI MS spectra in all of hydrogenated products. The second type of degradation resulted in an ion at m/z 98. Therefore, the 2 + 2 *retro*-cleavage pathway could serve as a diagnostic fragmentation in the characterization of analogues and/or derivatives of the present pheromone. This degradation pathway does not occur with the unsaturated alcohol and alcohol derivative ions because they would have to form unfavorable allene fragments. They instead undergo elimination of a neutral molecule to form the fragment ion, m/z 136 and the daughter ion, m/z 121 (m/z 136 − CH_3).

To confirm the pheromone structure and to define its stereochemistry, all four possible stereoisomers were synthesized for each of compounds **1** and **2**. (*S*)-(+)-2-Methylbutyric acid is commercially available (Aldrich) and (*R*)-(−)-2-methylbutyric acid was enantioselectively synthesized from (+)-ephedrine hydrochloride and urea (12, 13). (*S*)-(+)-Lavandulol and (*R*)-(−)-lavandulol were prepared by using the same strategy. (*S*)-(+)-Maconelliol and (*R*)-(−)-maconelliol were enantioselectively synthesized from (*S*)-(−)-α-pinene and (*R*)-(+)-α-pinene (Scheme 2). The described series of microreactions for maconelliol were carried out on the synthetic enantiomer separately. The four diastereoisomers of **1** and **2** produced mass spectra virtually identical to each other and indistinguishable from that of the natural pheromone.

The absolute configuration of the natural pheromone was unambiguously determined by comparison of retention times of natural 2-methylbutyric acid, lavandulol, and maconelliol with synthetic stereoisomers on a chiral β-DEX 120 GC capillary column (Table 1). Accordingly, the absolute configurations were determined to be *RS* for both compounds **1** and **2**. Furthermore, the ^{1}H NMR spectrum of synthetic maconelliyl 2-methylbutanoate was indistinguishable from that of the natural compound isolated from PHM (Fig. 4).

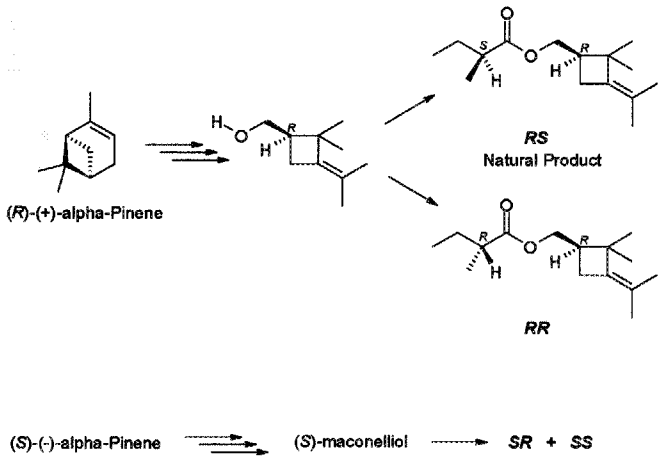
The field bioassays were conducted for three weeks in Key Biscayne, Florida from July 8 to July 28, 2003, by using *RS*, *SR*, *RR*, and *SS* binary mixtures of diastereoisomers of **1** and **2**. Field test results presented in Table 2 show that a 1-μg binary mixture of the *RS* isomers of compound **1** and compound **2** in their natural ratio (1:5) was significantly more attractive to PHM males than *SR*, *RR*, and *SS* binary mixtures. Neither *SR* nor *RR* mixtures were different from the control; however, *SS* mixture was significantly more attractive than the *SR* and *RR* mixtures.



Scheme 1. Microreactions performed on *M. hirsutus* natural pheromone extracts and possible 2 + 2 *retro*-cleavage of compound **2** according to EI MS data (relative intensities in parentheses). a, 2 M NaOH in 95% EtOH; b, H2/Pd-C in EtOAc; c, *N,O*-bis(trimethysily)trifluoroacetamide.

Discussion

The cyclobutane carbon skeleton is not rare among natural products. For example, the ubiquitous sesquiterpene, caryophyllene contains the fused cyclobutane ring (14, 15), as does viridianol, a rearranged sesquiterpene isolated from red seaweed, *Laurencia viridis* (16), and raoulic acid, an anti-leukemic and antibacterial compound found in the New Zealand plant, *Raoulia australis* (17). However, to date, only six cyclobutane derivatives have been discovered as pheromones or attractants (Fig. 3). Grandisol **5** was the first cyclobutane derivative identified in 1969 as the most important constituent of aggregation pheromone of the cotton boll weevil, *Anthonomus grandis* (18), and was later found in several other bark beetles, *Pityogenes quadridens*, *P. bidentatus*, and *P. calcaratus* (19) and pecan weevil *Curculio caryae* (20). Fragranol **6**, the trans isomer of grandisol, was identified in 1973 in the extracts of the plant *Artemisia fragrans* (21). Grandisal **7**, the oxidation product of grandisol, was found in 1983 as an aggregation pheromone component in two species of *Pissodes* bark weevils (22, 23). Compound **8** was identified in 1998 as the sex pheromone of the oleander scale, *Aspidiotus nerii* (24). Compound **9** was identified in 1981 as the sex pheromone of the citrus mealybug, *Planococcus citri* (25), and similar structure **10** was identified as *Pseudococcus cryptus* sex pheromone in 2003 (26, 27). Although the substituents are different, all of the above-mentioned semiochemicals contain an isopropenyl group on the ring. However, *M. hirsutus* pheromone component, maconelliyl 2-methylbutanoate **2**, contains a methyl-



Scheme 2. Enantioselective synthesis of compound **2** and other diastereoisomers.

Table 1. Determination of *M. hirsutus* sex pheromone absolute configurations from the alkaline hydrolyzed acid and alcohols by a chiral GC column

Compounds	Retention time,* min
From females	
2-Methylbutyric acid	9.118
Lavandulol	21.097
Maconelliol	25.897
Synthetic	
(*S*)-(+)-2-Methylbutyric acid	9.114
(*R*)-(−)-2-Methylbutyric acid	9.588
(*S*)-(+)-Lavandulol	19.737
(*R*)-(−)-Lavandulol	21.079
(*S*)-(+)-Maconelliol	27.667
(*R*)-(−)-Maconelliol	25.857

*A β-DEX 120 capillary column in the split mode (100:1) with hydrogen as carrier (55 cm/s, 100°C isothermal).

Table 2. Number (mean ± SEM) of male *M. hirsutus* captured in sticky traps baited with binary diastereoisomeric (1:5) of compounds of 1 and 2 in Florida

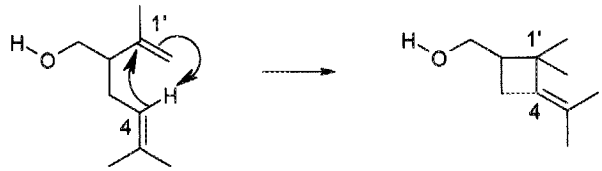
Stereoisomers	No. of males captured per trap
RS	1241 ± 378*
SR	90 ± 39†
RR	76 ± 31†
SS	547 ± 193‡
Control	78 ± 24†

The trial was conducted from July 8 to July 28, 2003. Total number of male *M. hirsutus* captured was 18,291. The data were converted to proportion and then transformed by the standard variance stabilizing transformation for proportions (arcsin $\sqrt{p}$), where p is the original proportion. (one-way ANOVA, Ryan–Einot–Gabriel–Welsch range test, $N = 9$, $F = 62.87$; df = 4, 40; $P < 0.001$). Means followed by the different symbols are significantly different at $\alpha = 0.05$.

ethylidene group on the ring, thus creating a type of cyclobutane derivative that has not been previously reported.

The biosynthetic origin of the maconelliol skeleton has not been established although it is speculated to be derived from the lavandulol carbon skeleton by cyclization between carbons C-4 and C-1′ to give a 2,2-dimethyl-3-(1-methylethylidene)cyclobutylmethanol (maconelliol) carbon skeleton (Scheme 3).

In summary, we have discovered, identified, and synthesized two compounds that together constitute the female sex pheromone of pink hibiscus mealybug, *M. hirsutus*: (*R*)-lavandulyl (*S*)-2-methylbutanoate) **1** and (*R*)-maconelliyl (*S*)-2-methylbutanoate **2**. The terpene alcohol lavandulol, and an incompletely characterized ester of lavandulol with 2-methylbutanoic acid have been described by other workers (28), but the specific ester with (*R*)-lavandulol and (*S*)-2-methylbutanoic acid has not previously been described. Maconelliol is an unusual cyclobutanoid monoterpene, and its structure has been established by enantioselective synthesis from precursors of known structure and configuration. A synthetic 1:5 mixture of the two *RS* pheromone components (1 μg per rubber septum) is an extremely potent attractant. The synthetic mixture will be a useful tool for monitoring PHM flight activity and tracking biocontrol efforts. The identification of the sex pheromone will also enable future development of mating disruption and attract-and-kill technologies for managing *M. hirsutus* populations.



Lavandulol Maconelliol

Scheme 3. Possible biosynthetic pathway of maconelliol.

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1. Williams, D. J. (1996) *Bull. Entomol. Res.* **86,** 617–628.
2. Matile-Ferrero, D. & Eitienne, J. (1996) *Rev. Fr. Entomol.* **18,** 38.
3. Etienne, J., Matile-Ferrero, D., Leblanc, F. & Marival, D. (1998) *Bull. Soc. Entomol. Fr.* **103,** 173–174.
4. Kairo, M. T. K., Pollard, G. V., Petetkin, D. D. & Lopez, V. F. (2000) *Int. Pest Manage. Rev.* **5,** 241–254.
5. Stibick, J. N. L. (1997) *New Pest Response Guidelines: Pink Hibiscus Mealybug Maconellicoccus hirsutus* (U.S. Department of Agriculture, Animal and Plant Health Inspection Service).
6. Serrano, M., S., Lapointe, S. L. & Meyerdirk, D. E. (2001) *Popul. Ecol.* **30,** 339–345.
7. Bierl-Leonhardt, B. A., Moreno, D. S., Schwarz, M., Fargerlund, J., Plimmer, J. R. & DeVilbiss, E. D. (1981) *Tetrahedron Lett.* **22,** 389–392.
8. Zhang, A., Facundo, H. T., Robbins, P. S., Linn, C. E., Jr., Hanula, J. L., Villani, M. G. & Roelofs, W. L. (1994) *J. Chem. Ecol.* **20,** 2415–2427.
9. Zhang, A., Robbins, P. S., Leal, W. S., Linn, C. E., Jr., Villani, M. G. & Roelofs, W. L. (1997) *J. Chem. Ecol.* **23,** 231–245.
10. Zhang, A., Linn, C. E., Jr., Wright, S., Prokopy, R., Reissig, W. & Roelofs, W. L. (1999) *J. Chem. Ecol.* **25,** 1221–1232.
11. Zhang, A. & Polavarapu, S. (2003) *J. Chem. Ecol.* **29,** 2153–2164.
12. Close, W. J. (1950) *J. Org. Chem.* **15,** 1131–1134.
13. Cardillo, G., D'Amico, A., Orena, M. & Sandri, S. (1988) *J. Org. Chem.* **53,** 2354–2356.
14. Budavari, S. (1989) *The Merck Index* (Merck, Rahway, NJ), 11th Ed., p. 287.
15. Hinkley, S. F., Perry, N. B. & Weavers, R. T. (1994) *Tetrahedron Lett.* **35,** 3775–3776.
16. Norte, M., Fernandez, J. J. & Souto, M. L. (1994) *Tetrahedron Lett.* **35,** 4607–4610.
17. Bloor, S. J. (1993) *Tetrahedron Lett.* **34,** 5617–5620.
18. Tumlinson, J. H., Hardee, D. D., Gueldner, R. C., Thompson, A. C., Hedin, P. A. & Minyard, J. P. (1969) *Science* **166,** 1010–1012.
19. Francke, W., Bartels, J., Krohn, S., Schulz, S., Baader, E., Tengo, J. & Schneider, D. (1989) *Pure Appl. Chem.* **16,** 539–542.
20. Hedin, P. A., Dollar, D. A., Collins, J. K., Dubois, J. G., Mulder, P. G., Hedger, G. H., Smith, M. W. & Eikenbary, R. D. (1997) *J. Chem. Ecol.* **23,** 965–977.
21. Bohlmann, F., Zedero, C. & Faass, U. (1973) *Chem. Ber.* **106,** 2904–2909.
22. Booth, D. C., Phillips, T. W., Claesson, A., Silverstein, R. M., Lanier, G. N. & West, J. R. (1983) *J. Chem. Ecol.* **9,** 1–12.
23. Phillips, T. W., West, J. R., Foltz, J. L., Silverstein, R. M. & Lanier, G. N. (1984) *J. Chem. Ecol.* **10,** 1417–1423.
24. Einhorn, J., Guerrero, A., Ducrot, P.-H., Boyer, F.-D., Gieselmann, M. & Roelofs, W. L. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 9867–9872.
25. Bierl-Leonhardt, B. A., Moreno, D. S., Schwarz, M., Fargerlund, J. & Plimmer, J. R. (1981) *Tetrahedron Lett.* 389–392.
26. Arai, T., Sugie, H., Hiradate, S., Kuwahara, S., Itagaki, N. & Nakahata, T. (2003) *J. Chem. Ecol.* **29,** 2213–2223.
27. Nakahata, T., Itagaki, N., Arai, T., Sugie, H. & Kuwahara, S. (2003) *Biosci. Biotechnol. Biochem.* **67,** 2627–2631.
28. Weyerstahl, P., Marschall, H., Thefeld, K. & Rustaiyan, A. (1999) *Flavour Fragrance J.* **14,** 112–120.