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Chrysanthemyl 2-acetoxy-3-methylbutanoate: the sex pheromone of the citrophilous mealybug, *Pseudococcus calceolariae*

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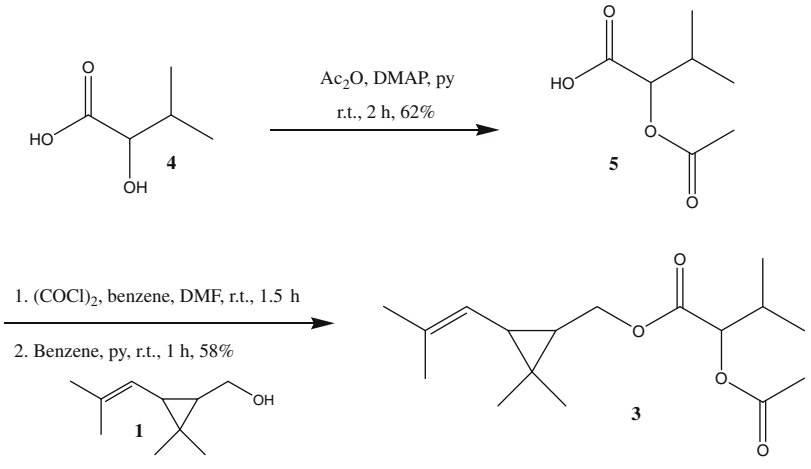
ABSTRACT

Headspace volatiles collected from virgin females of the citrophilous mealybug, *Pseudococcus calceolariae*, contain three compounds not present in the headspace of control samples. The main female-specific compound is identified as [2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropyl]methyl 2-acetoxy-3-methylbutanoate (chrysanthemyl 2-acetoxy-3-methylbutanoate). The other two compounds are identified as [2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropyl]methanol (chrysanthemol) and [2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropyl]methyl 2-hydroxy-3-methylbutanoate (chrysanthemyl 2-hydroxy-3-methylbutanoate). Traps baited with 100 μg and 1000 μg of chrysanthemyl 2-acetoxy-3-methylbutanoate captured 4- and 20-fold more males than traps baited with virgin females.

The citrophilous mealybug, *Pseudococcus calceolariae* (Maskell), is a cosmopolitan species and thought to be native to Australia.[1] This polyphagous species feeds on a wide variety of host plants including citrus, avocado, berries, sugar cane, cocoa, grape and ap-



Scheme 1. Synthesis of chrysanthemyl 2-acetoxy-3-methylbutanoate **3**.

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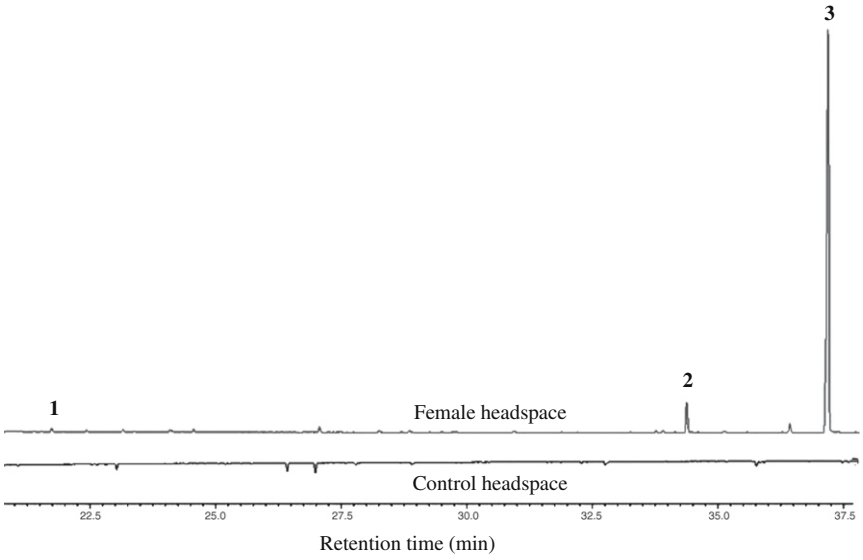

Figure 1. GC traces of extracts of headspace volatile chemicals collected from sexually mature female citrophilus mealybugs on sprouted seed potatoes and of volatile chemicals collected from clean sprouted seed potatoes. Three compounds were exclusively present in the headspace of female mealybugs and were identified as chrysanthemol (**1**), chrysanthemyl 2-hydroxy-3-methylbutanoate (**2**) and chrysanthemyl 2-acetoxy-3-methylbutanoate (**3**).

ple and it has invasively spread from its native habitat and is geographically distributed world-wide, because of trade in plants. In New Zealand, it is a vector of grapevine leafroll-associated virus type 3 (GLRaV-3),[2] which causes significant declines in quantitative and qualitative parameters of vine performance. Due to quarantine restrictions imposed by several countries, the presence of *P. calceolariae* causes significant economic losses to Chilean fruit exporters.

Hitherto, the sex pheromones for eleven mealybug species have been identified.[3] As opposed to lepidopteran species, which use defined blends of a set of common compounds, each mealybug species seems to use a unique pheromone structure for sexual communication, which includes an irregular non-head-to-tail monoterpenoid structure.[4] Evidence for the presence of a sex pheromone in *P. calceolariae* has been previously demonstrated,[5] and obviously, identification of the sex pheromone would enable the development of efficient monitoring systems and new control strategies for this insect. This paper reports the isolation, identification and synthesis of the sex pheromone of *P. calceolariae*. The identification is supported by the results of field trapping experiments using the synthetic sex pheromone.

P. calceolariae collected from vineyards near Hawke's Bay (New Zealand) and from raspberry plantations near Nogales (Chile) were used to establish colonies on sprouted seed potatoes or butternut squash. At the third instar, all males were removed manually from the colony twice weekly to prevent females from mating. Matured virgin females on sprouted seed potatoes were housed in a glass container. A charcoal-filtered air-stream was pulled through the container and the volatiles were collected on an adsorbent trap containing 50 mg of Tenax. Glass chambers containing clean sprouted seed potatoes or squash were used as control. The Tenax traps were extracted with 1 mL of hexane every 4–7 days. Sample volumes were reduced to 10 μL at ambient temperature under a stream of argon and the extracts were analyzed by gas chromatography-mass spectrometry (GC–MS). Female headspace volatiles reproducibly contained three additional compounds compared with the headspace volatiles of control samples (Fig. 1), with retention indices (RI) of 1158 for compound **1**, 1610 for compound **2** and 1744 for compound **3** on a non-polar VF-5ms column. The mass spectra of the three compounds (Fig. 2) indicated that they were structurally related. The mass spectrum of **1** showed a base peak at *m/z* 123 and a weak molecular ion at *m/z* 154. The RI of **1** is in the typical range of monoterpene alcohols and it was tentatively identified as chrysanthemol by a mass spectral database search. The identification was confirmed by comparison of the mass spectrum and retention times on a polar and a non-polar column with those of an authentic standard. An aliquot of female headspace extract was hydrolyzed (KOH, 95% MeOH), resulting in the disappearance of **2** and **3** and an increase in the amount of **1**. This indicated that chrysanthemol was a part of the structure of both unknown compounds **2** and **3** and that they presumably were esters. High resolution GC–MS analysis of the female extract showed that compounds **2** and **3** had exact molecular masses of 254.1781 and 296.1898, respectively, suggesting molecular formulas of $C_{15}H_{26}O_3$ for **2** and $C_{17}H_{28}O_4$ for **3**. Taking into consideration, (1) the presence of additional oxygen atoms in **2** and **3**, (2) the difference of 134 RI units between them and (3) the difference of 42 mass units corresponding to a C_2H_2O fragment, it was hypothesized that **2** bears a hydroxy function, and that **3** is the corresponding acetate. Compounds **2** and **3** were hence proposed to be the esters of chrysanthemol with a five-carbon hydroxy acid and a five-carbon acetoxy acid, respectively. It seemed likely that the acid part of the ester would be an isoprenoid, so reference compounds were synthesized employing commercially available 2-hydroxy-3-methylbutanoic acid, 2-hydroxy-2-methylbutanoic acid and 3-hydroxy-2-methylbutanoic acid. The acids were acetylated and then esterified with a mixture of chrysanthemol isomers (Scheme 1). One of the isomers of chrysanthemyl 2-acetoxy-3-methylbutanoate had an identical mass spectrum and co-eluted on two different columns with insect-produced **3**. The identification of **2** was confirmed by partial hydrolysis of **3**, showing the product to have an identical mass spectrum and to co-elute on two different columns with insect-produced **2**.

An isomeric mixture of synthetic **3** was tested in the field and proved to be highly attractive to male mealybugs in New Zealand and in Chile. In New Zealand, five different doses (0.1, 1, 10, 100 and 1000 μg) were loaded on red rubber septa and placed in red delta traps in vineyards near Hawke's Bay. The amount of 2-acetoxy-3-methylbutanoate significantly affected the number of captured males (Fig. 3). The highest number of males was captured with the 1000 μg loading. There was no difference in the mean numbers of males captured using the 0, 0.1, and 1 μg loading, while

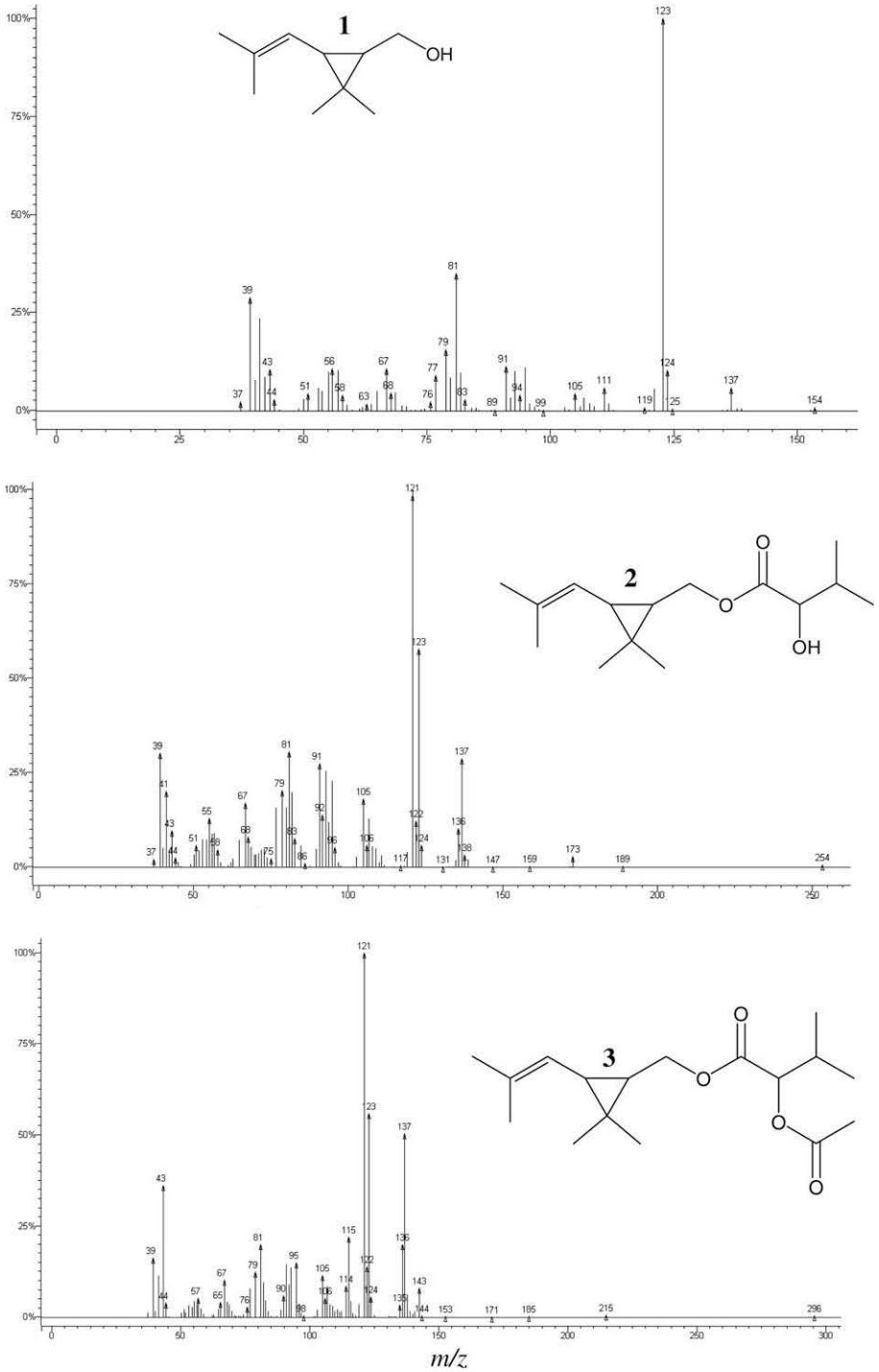

Figure 2. EI mass spectra (70 eV) of the three compounds present in the headspace volatiles of female *P. calceolariae.*

increasing the loading from 1 to 10 μg resulted in a significant increase in the mean number of captures. Traps baited with 100 μg and 1000 μg doses captured 4–20-fold more males than traps baited with virgin females. In Chile, a single dose of 100 μg was assayed in raspberry plantations near Nogales, capturing 1171 ± 270 males (n = 4), while no males were captured in control traps.

In this work, chrysanthemyl 2-acetoxy-3-methylbutanoate **3** was identified as the sex pheromone of the citrophilus mealybug, a world-wide pest of many crops. Male mealybugs were highly attracted to the racemic material; this will greatly facilitate the development of the pheromone for monitoring and control of this pest, because racemic **3** can be readily synthesized from commercially available intermediates. Work is underway to determine the absolute configuration of natural **3**. In addition, studies on optimum doses, trap types, pheromone dispensers, longevity of pheromone lures and control options are in progress.

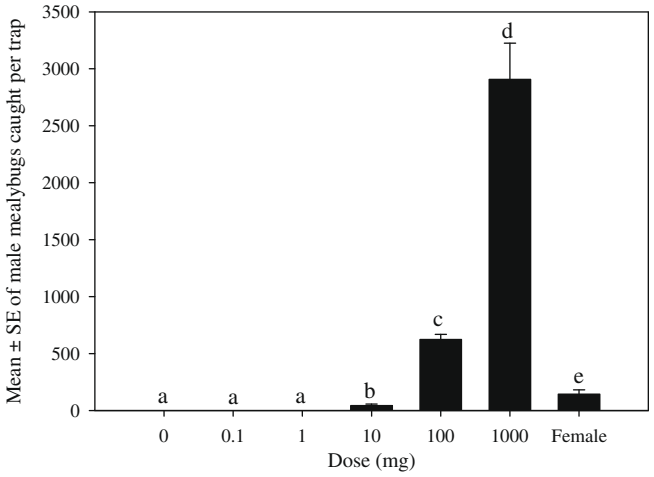

Figure 3. Mean catch ± SE of male citrophilus mealybugs in red delta traps baited with various doses of racemic chrysanthemyl 2-acetoxy-3-methylbutanoate **3** (0.1, 1, 10, 100 and 1000 μg) loaded on red rubber septa. Letters on columns indicate significant differences (FPLS test, $P < 0.05$).

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Supplementary data

Data include experimental procedures on volatile collection, derivatization of female extracts (hydrolysis and acetylation), fractionation and laboratory bioassay, synthesis of chrysanthemyl 2-acetoxy-3-methylbutanoate, field experiment protocols, and a GC trace of the insect-produced compound with a synthetic standard on a chiral GC column. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.106.

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