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Identification and synthesis of the sex pheromone of the vine mealybug, *Planococcus ficus*

Diane M. Hinkens, J. Steven McElfresh and Jocelyn G. Millar*

Departments of Entomology and Chemistry, University of California, Riverside, CA 92521, USA

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Abstract—Sexually mature females of an important agricultural pest, the vine mealybug *Planococcus ficus*, produce the monoterpene (*S*)-lavandulol and the corresponding ester, (*S*)-(+)-lavandulyl senecioate. The racemic ester was highly attractive to mature male mealybugs, whereas lavandulol was not. The naturally produced 2:5 blend of lavandulol and the ester also was no more attractive than the ester alone.

The vine mealybug, *Planococcus ficus*, is a worldwide pest of grapes, figs, apples, and citrus, as well as more tropical crops such as yams, mangos, and avocados.[1] It was recently introduced into California,[2] where it has caused widespread damage to table grapes, both directly, and indirectly through the growth of sooty mold on honeydew produced by the insects as they feed.[2] The insects also transmit viral diseases to grapevines.[3] Control of vine mealybug with insecticides is often ineffectual because the insects produce a protective waxy covering, and because they are frequently hidden under the bark of vines.[4] The mature female is sessile, and emits a sex pheromone to attract flying males.[5] We report here the identification of the sex pheromone, which may prove useful for sampling and control of this pernicious pest insect.

Mealybugs were reared on butternut squash (*Cucurbita moschata*). Squash were infested with newly-hatched mealybug crawlers and after 1 week, the squash were sprayed with a 10 ppm solution of the insect growth regulator pyriproxyfen in 0.1% aqueous Triton-X to selectively eliminate most of the males before they matured.[6] Several treated squash, with primarily mature virgin females, were then aerated in a 1 L glass chamber, passing purified air through the chamber ($\sim$3 L/min) and collecting the volatile chemicals on a 2.5 cm×4 mm ID column of Porapak-Q. Collectors were

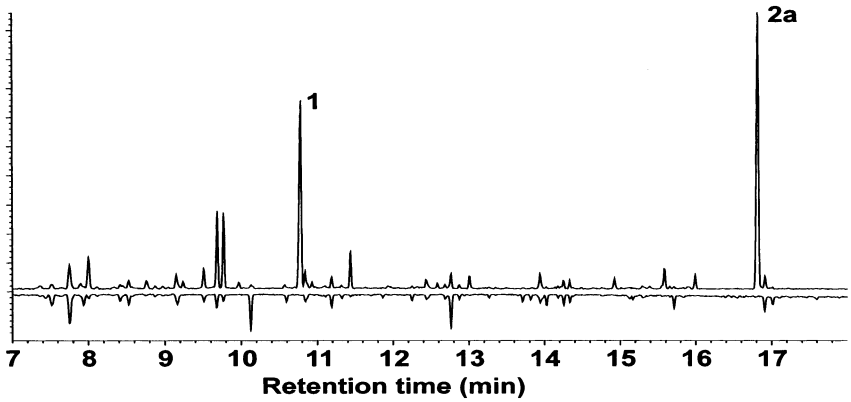

Figure 1. GC traces of extracts of air-entrained volatile chemicals collected from sexually mature female vine mealybugs on butternut squash (upper trace) and volatile chemicals from clean squash (inverted, lower trace). Lavandulol (**1**) and the active pheromone lavandulyl senecioate (**2a**) are indicated.

* Corresponding author. E-mail: jocelyn.millar@ucr.edu

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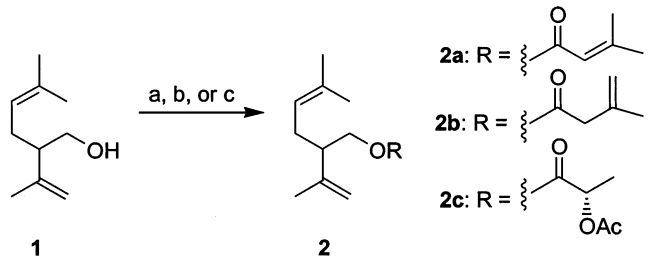

Scheme 1. Reagents and conditions: (a) pyridine, senecioyl chloride, ether, 0°C–room temp.; (b) DMAP, senecioyl chloride, ether, room temp.; (c) acetyl (*S*)-lactoyl chloride, pyridine, ether, 0°C–room temp.

changed every 3–5 days, eluting trapped materials with pentane. The extracts were then analyzed by GC–MS. Control collections were made under identical conditions with uninfested squash.

Comparison of the extracts of volatiles from infested and clean squash revealed two significant insect-produced compounds (Fig. 1). The earlier eluting compound gave a weak molecular ion at m/z 154 (<1%), with significant fragments at m/z 136 (2%, M^+-18), 123 (9%, M^+-CH_2OH), 111 (24%, $M^+-C_3H_7$), 93 (17%, $M^+-H_2O-C_3H_7$), 69 (90%, $C_5H_9^+$), and 41 (100%, $C_3H_5^+$), suggestive of a monoterpene alcohol. The compound was tentatively identified as the known monoterpene alcohol lavandulol (**1**) by comparison with the NBS–NIH MS database, and confirmed by GC retention time matches on 3 GC capillary columns (DB-5, DB-17, and DB-WAX), and a mass spectral match with an authentic standard prepared by hydrolysis of lavandulyl acetate (TCI America).

The second insect-produced compound gave a weak molecular ion at m/z 236 and a base peak of m/z 83, characteristic of a five-carbon monounsaturated ester fragment. Upon base hydrolysis, the compound yielded lavandulol, confirming an ester of a five-carbon monounsaturated carboxylic acid. Furthermore, the difference in retention times between the model compounds geraniol and geranyl tiglate, and between lavandulol and the unknown ester were almost identical, suggesting that the ester was probably conjugated. Because most of the pheromones known from related insects are terpenoids,[7] there were three likely possibilities, (lavandulyl tiglate, senecioate, or angelate), corresponding to the acid portion being a 2,3- or a 3,3-dimethylacrylate structure. Lavandulyl angelate was synthesized by base-catalyzed transesterification of methyl angelate with lavandulol, whereas the other two esters were synthesized by esterification of lavandulol with the corresponding acid chlorides and an amine base in ether. Interestingly, use of 4-dimethylaminopyridine as the base with senecioyl chloride resulted in extensive deconjugation of the acid portion to produce **2b** (Scheme 1), presumably via initial formation of a conjugated ketene from the acyl chloride, followed by nucleophilic attack of the carbonyl carbon by lavandulol.[8] In contrast, reaction of senecioyl chloride and lavandulol at 0°C in ether with pyridine as the base,[9] warming to room temp. overnight, gave exclusively the desired lavandulyl senecioate **2a** (Scheme 1).[10] This compound proved to be an exact match in mass spectrum (Fig. 2) and GC retention times to the insect-produced compound, whereas lavandulyl angelate and tiglate both had different retention times and mass spectra than **2a**; in particular, the latter two compounds gave a base peak at m/z 93 instead of m/z 83 amu. To our knowledge, this is the first report of **2a** as a natural product, although **1** has been previously reported from both plant oils and an insect (the carrion beetle, *Necrodes surinamensis*).[11]

The absolute configurations of insect-produced **1** and **2a** were determined by derivatization of racemic lavandulol, a sample of (*R*)-(−)-lavandulol purified from lavender oil,[12] and a hydrolyzed mealybug aeration extract with acetyl (*S*)-lactic acid chloride and pyridine in ether to form the acetyl lactate ester(s).[13] The diastereomeric derivatives **2c** from racemic lavandulol were resolved almost to baseline on capillary GC (DB-5 column, 100°C/0 min, 5°C/min to 150°C, hold at 150°C), with the derivative from (*R*)-lavandulol corresponding to the later eluting peak. The derivative of insect-produced **1** gave a single clean peak at the retention time of the derivative of (*S*)-(+)-lavandulol, confirmed by sequential coinjections of the derivatized insect-produced **1** with the derivatized (*R*)-(−)- and racemic lavandulol, proving that insect-produced **1** and **2a** must both have the (*S*)-configuration.

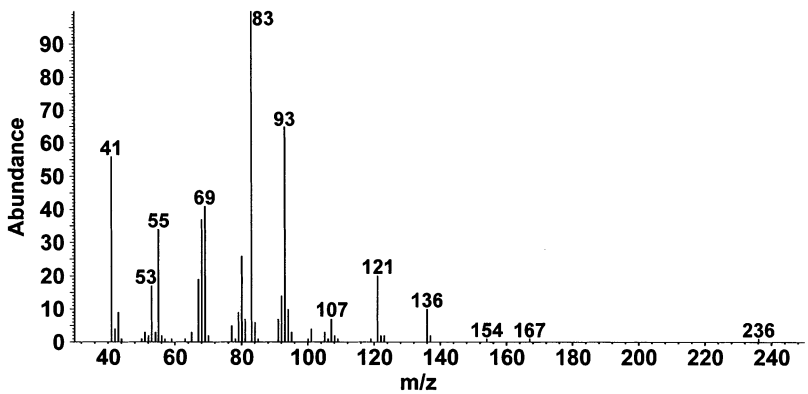

Figure 2. EI mass spectrum (70 eV) of the attractant pheromone component, lavandulyl senecioate **2a**.

Table 1. Numbers of male vine mealybugs attracted to lavandulol **1** and lavandulyl senecioate **2a** as single components or a blend, versus a solvent control*

Treatment	No. of males attracted (mean ± standard error)
Lavandulol	4.5 ± 1.6 a
Lavandulyl senecioate	92.3 ± 20.2 b
Lavandulol + lavandulyl senecioate (2:5)	59.8 ± 21.3 b
Solvent control	6.8 ± 5.7 a

* $n=4$. Data analyzed by two-way ANOVA, followed by a Student–Newman–Keuls test to separate means. Values followed by different letters are significantly different ($P<0.05$).

Laboratory bioassays were run to test the biological activities of **1** and **2a**. For bioassays, male mealybugs were collected by wrapping infested squash (not treated with pyriproxyfen) with double-folded toilet tissue paper.[14] Starting at about ten days after infestation, immature males left the fruit and crawled between the layers of the tissue paper, whereas the females stayed on the squash. The tissues were held until the males completed development, emerging as short-lived flying adults. Thus, tissues with emerging males were placed in the bioassay chamber—a vented, screened, 1.2×0.7×1 m high chamber in a greenhouse. Test compounds in hexane were pipetted onto a filter paper wick standing upright in the center of a 7×7 cm sticky card, with sticky cards and wicks replaced daily, and the positions of treatments rotated among the 4 corners of the chamber daily. Treatments consisted of racemic lavandulol (5 μg), lavandulyl senecioate (5 μg), a 2 μg:5 μg blend of lavandulol:lavandulyl senecioate (the average blend found in insect extracts), and a hexane control. The synthetic lavandulyl senecioate was highly attractive to males (Table 1), whereas the blend was less so, and lavandulol was no more attractive than the hexane control. Further laboratory bioassays with different blends of **1** and **2a** have not revealed a blend that is any more attractive than **2a** alone.

In summary, we have identified a single-component sex pheromone for the vine mealybug, a major pest of numerous crops in the Mediterranean basin, South Africa, and North America. Male mealybugs were highly attracted to racemic **2a**. The fact that the racemic material is highly attractive should greatly facilitate development of the pheromone for insect management purposes, because racemic **2a** can be readily synthesized from commercially available intermediates. Studies on optimum doses, trap types, pheromone dispensers, longevity of pheromone lures, and the biological role of lavandulol are in progress.

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10. Selected physical data for compound **2a**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.67 (s, 1H), 5.08 (br t, 1H, ~7 Hz), 4.83 (br t, 1H, 1.2 Hz), 4.75 (s, 1H), 4.07 (d, 2H, 7.2 Hz), 2.42 (m, 1H), 2.16 (s, 3H), 2.03–2.24 (m, 2H), 1.88 (s, 3H), 1.71 (s, 3H), 1.69 (s, 3H), 1.61 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 166.68, 156.28, 145.10, 132.77, 121.76, 116.12, 112.21, 65.12, 46.18, 28.72, 27.33, 25.71, 20.18, 19.97, 17.79. Elemental analysis calcd for $C_{15}H_{24}O_2$: C 76.23%, H 10.24%. Found: C 76.26%, H 10.24%.
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