

Sex pheromone and analogs of the citrus mealybug, *Planococcus citri*: synthesis and biological activity

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Abstract: The citrus mealybug, is a cosmopolitan pest and affects many crops. The female sex pheromone has been identified by Bierl-Leonhardt et al., in 1981 as (+)-(1*R*)-*cis*-2,2-dimethyl-3-isopropenylcyclobutanemethanol acetate **1**. Several groups including our team have synthesized the pheromone. A number of analogs have also been prepared in order to study the structure-activity relationship. We present here a modified synthetic route for the pheromone, preparation of some analogs and the biological activity of these compounds. The starting materials for the pheromone and analogs are *cis*-pinonic acid or *cis*-pinonic aldehyde which can be easily obtained from cheap commercial (+)-*pinene* by cleavage with permanganate or ozonolysis. Conversion of the pinonic derivatives to the pinononic compounds was achieved either by a modified Hundsdiecker reaction (Wolk et al., 1986) or by ozonolysis of the enol acetate of *cis*-pinonic aldehyde (Barton and Fontana, 1996). In the present study, we report our results using the second method, which avoids the use of the unstable pinononyl halides. The key element of the synthesis of **1** is the use of pinononyl aldehyde **2** and its selective reduction to pinononyl alcohol **3**. The latter was submitted to a Wittig reaction and then acetylated; alternatively the sequence was reversed and the Wittig reaction was performed after the acetylation to form the pheromone **1**. The stereochemistry was preserved in all steps and no racemization was observed. Field tests indicated that a number of analogs display considerable biological activity. One of them, a homolog containing an elongated acetate side chain by one carbon **10** has a relatively high activity. This observation has practical importance because the synthesis of the homolog is shorter and more convenient than that of the pheromone. The field tests indicated that the acetate group and the double bond in the pheromone molecule are essential for biological activity.

Key words: Citrus mealybug, *Planococcus citri*, synthetic sex pheromone, analogs, structure-activity relationship, field bioassay.

Introduction

The citrus mealybug, (Risso) is a cosmopolitan pest, affecting subtropical fruits and ornamentals. The female sex pheromone has been identified by Bierl-Leonhardt et al., in 1981 as (+)-(1*R*)-*cis*-2,2-dimethyl- 3-isopropenylcyclobutanemethanol acetate **1**.

Recently, we have initiated a field project to assess the potential of the synthetic pheromone for monitoring and mass trapping of the pest. To this purpose we have developed a modified synthesis of the pheromone and of a number of analogs.

This report describes the general scheme of synthesis of the pheromone termed planococyl acetate (Wolk *et al.*, 1986) and analogs, and evaluates the structure-activity relationship based on outdoor male trapping.

Materials and Methods

Preparation of the P. citri pheromone, planococyl acetate 1. The primary starting material was (+)- α -pinene, purchased from Aldrich containing 95% of the (+) enantiomer according to GC analysis on a chiral 30 m x 0.25 mm column coated with a 0.25 micron film of Cyclodex - B. The (+)- α -pinene was cleaved with ozone to produce pinonic aldehyde **2** which was stored with 2.5% BHT as antioxidant, at low temperature. Aldehyde **2** was converted into the enol acetate **3** as an E/Z mixture. Ozonolysis of acetate **3** gave pinononic aldehyde **4**. This aldehyde is very prone to oxidation by air. It can be stored for a few days with 2.5% BHT under argon at low temperature. The ozonolysis and preparation of the enolacetate were carried out according to the procedure of Barton and Fontana (1996) with the following modifications: The solvent mixture of methanol + dichloromethane was replaced with dichloromethane and NaHCO₃ was added to trap any acid formed. BHT was added after reduction of the intermediate oxonides with dimethyl sulfide to prevent air oxidation of the formed aldehydes. This step was particularly important for pinononic aldehyde **4**, which is very prone to oxidation and conversion to pinononic acid.

The key step of the synthesis involved selective reduction of pinononic aldehyde **4** to pinononic alcohol **5** with Zn(BH₄)₂. The reagent was prepared in situ according to Ranu and Chakraborty (1990), from an ethereal solution of ZnCl₂ and Na(BH₄) in DME without filtering the formed Zn(BH₄)₂ from the by-product NaCl. The reagent solution was added to **4** in THF and the reaction was terminated after 15 min. by careful addition of water. Both steps were performed in an ice-bath. The conversion of **4** to **5** was ca. 95%. The reaction time was critical, less the 15 min reduced the conversion and a longer reduction time resulted in the formation of additional products.

The pinononic alcohol **5** was either submitted to a Wittig reaction with the ylide prepared from triphenylmethyl phosphonium bromide with butyllithium and then acetylated with acetic anhydride and pyridine; alternatively the sequence was reversed and the Wittig reaction was performed after the acetylation of **5**. The first route is shorter and therefore preferable for the preparation of technical grade pheromone (~ 85% purity by GC). Crude intermediate products were used in this sequence, starting from **2**; the pheromone **1** was cleaned at the end of the synthesis by column chromatography on silica with hexane and ethyl acetate as eluent. The

overall yield of **1** from **2** was 20-25%. In the second route, pinononyl acetate **7** was used as intermediate. It was purified by chromatography on silica with hexane plus increasing amounts of ether. Wittig reaction of purified **7** with the ylide, prepared from triphenylmethyl phosphonium bromide with butyllithium, gave a mixture of the pheromone **1** and planococyl alcohol **6** due to partial hydrolysis of acetate during workup. Reacetylation of the mixture and column chromatography gave ~95% pure pheromone **1** in 15-20% yield based on **2**. The general scheme of the synthesis, starting from **2**, is outlined in Figure 1. The pheromone **1** was identical with an authentic sample prepared by a different route (Wolk et al., 1986), as compared by capillary GC and GC-MS in the EI and CI mode.

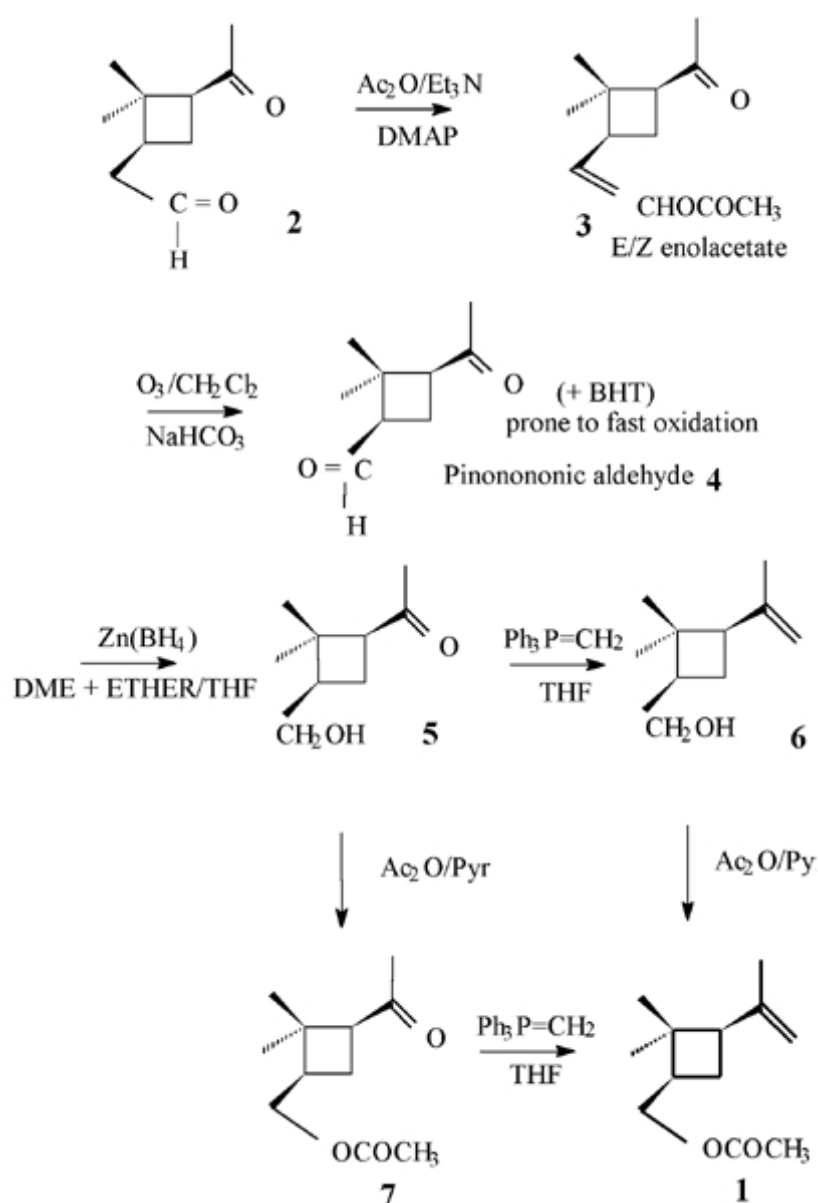


Figure 1

Preparation of the P. citri pheromone analog homoplanococyl acetate 10 (Figure 2). Homoplanococyl acetate **10** was prepared from pinonic alcohol **8** which, in turn, was prepared by selective reduction of **2** with $\text{Zn}(\text{BH}_4)_2$. A Wittig reaction of **8** with and subsequent acetylation yielded **10** in 43% after chromatography on a silica column. This analog was identical to another sample of **10**, prepared previously by a slightly different route (Dunkelblum *et al.*, 1987), as compared by capillary GC and NMR.

Preparation of the P. citri pheromone acid methyl ester analog 13 (Figure 2). This analog was prepared from pinonic acid **11** (Wolk *et al.*, 1986). Methylation of **11** with diazomethane gave the methyl ester **12** and subsequent Wittig reaction with the ylide prepared from triphenylmethyl phosphonium bromide and butyllithium provided **13**. The last step proceeded in low yield and analog **13** was obtained in 20% yield from **11** after chromatography on a silica column.

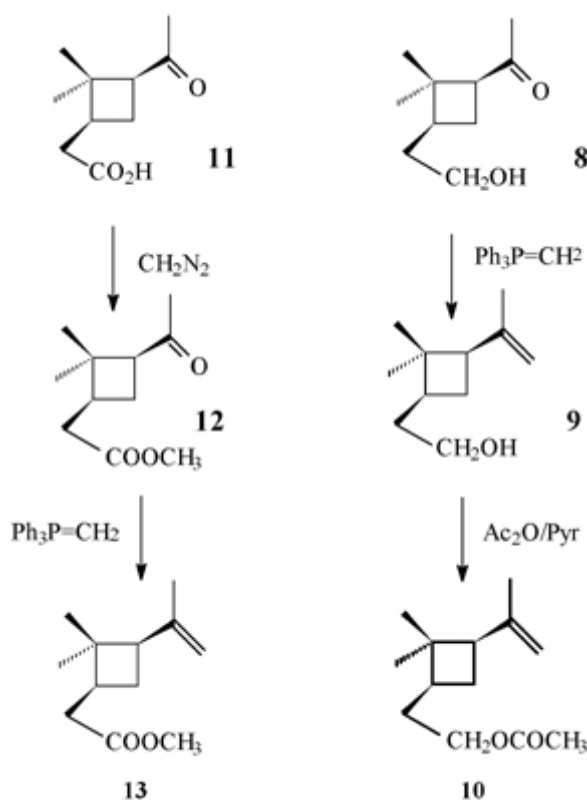


Figure 2

Preparation of the ethylidene analog 14 (Figure 3). The ethylidene analog **14** was obtained from **5** by a Wittig reaction with the ylide prepared from triphenylethyl phosphonium bromide with butyllithium. A *Z/E* mixture of **14** was obtained in 35% yield after column chromatography on a silica column and characterized by GC-MS and NMR. Analog **16** was prepared and tested before (Dunkelblum *et al.*, 1987).



Figure 3

Preparation and analysis of racemic P. citri pheromone 1R. The racemic pheromone was prepared from commercial racemic *cis*-pinonic acid (Aldrich) by our previous procedure (Wolk et al., 1986). The racemic and chiral pheromones were analyzed on a chiral 30 m x 0.25 mm capillary column coated with Cyclodex-B phase of 0.25 μ m film thickness. The column was kept for 2 min. at 60⁰ C and then programmed at 10⁰ C/min to 130⁰ C. The analysis was performed in the split mode with a total flow of 30 ml/min with a split ratio of 20:1. An almost base-line separation was achieved; the (+) chiral pheromone enantiomer eluted at 50,75 min and the (-) enantiomer eluted at 51,10 min.

Field bioassay: Tests were conducted in Sweetie grapefruit, avocado and parsimon plantations. Triangular sticky traps were used, baited with rubber septa impregnated with synthetic pheromone or analogs in 200 μ l hexane. Control traps were baited with dispensers impregnated only with hexane. Traps were suspended in the canopy of the trees at least 25 m apart. The sticky plates were transferred to the laboratory and the trapped *P. citri* males were counted using a stereomicroscope.

The pheromone was tested in a dose response experiment of 25 μ g - 800 μ g. All analogs were tested a number of times and their activity was compared to that of the pheromone **1**. In most tests, a dose of 50 μ g - 200 μ g was used. The analog **10** (homolog) and the ethylidene analog **14** were tested also at a dose of 400 μ g. In all field tests five replicates were used for each treatment.

Statistical Analysis. Trap catch data were transformed to $x + 0.5$ and then subjected to analysis of variance, followed by the Student-Neuman-Keuls multiple range test (at $P < 0.05$), to determine significance between means.

Results and discussion

The citrus mealybug, *P. citri* in Israel is a serious pest of citrus and parsimon plantations. We were interested in the practical use of the pheromone for the management of the pest and in the analogs for the study of structure-activity relationship. The pheromone is not commercial, which is why we devised a new synthesis based on simple reactions (Figure 1). Our previous synthesis (Wolk et al., 1986) was short but involved two complex steps. The key steps in the present method are the isolation of

the air-sensitive pinonic aldehyde **4** and its selective reduction with $\text{Zn}(\text{BH}_4)_2$ to pinononyl alcohol **5**. The aldehyde **4** can be stored with BHT under argon at -18°C . For the synthesis of technical grade pheromone (80-85%), crude intermediates **4**, **5** and **6** were used. The alcohols **5** and **6** were difficult to purify by column chromatography due to strong absorption and loss of material. The pheromone **1**, obtained after acetylation of **6**, was purified by column chromatography. The technical grade pheromone was tested in the field and its activity was statistically comparable to that of 95% pure pheromone. For the preparation of 95% pure pheromone, pinononyl acetate **7** was prepared and purified by column chromatography before the Wittig reaction. This route requires an additional acetylation step due to hydrolysis during the workup of the Wittig reaction.

The synthesis of the homolog **10** is shorter and the yield is higher (~40%) than that of the pheromone (~20%). The yield of the analogs **13** and **14** was relatively low and they were prepared solely for the structure-activity relationship study.

Chiral analysis of the pheromone **1** and its racemate on a Cyclodex-B capillary column indicated that no racemization occurred during all steps of the synthesis. The commercial starting material, (+)- α -pinene contained 95% of the (+) enantiomer, and the final product **1** was approximately of the same chiral purity. The present chiral separation of the (\pm) *P. citri* pheromone represents the first successful separation of the enantiomers of this pheromone. Previously, only the enantiomers of the corresponding planococcyll alcohol **6R** could be separated (Novotny *et al.*, 1989).

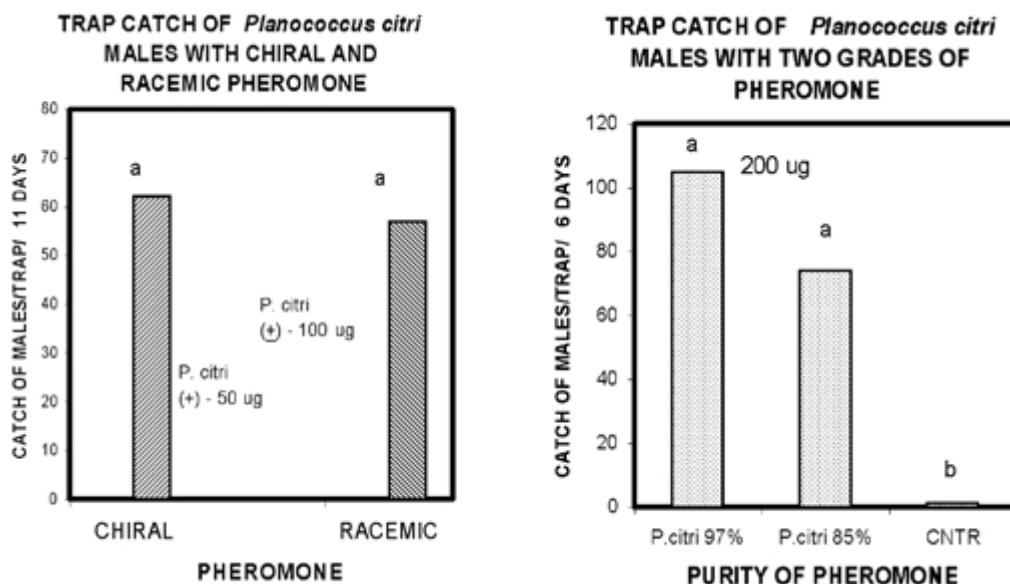


Figure 4

The first two field tests compared the attractiveness of the racemic and technical grade pheromone with high grade chiral pheromone (Figure 4). The racemic

pheromone **1R** was prepared specifically for this purpose by our former method (Wolk et al., 1986), starting from commercial racemic pinonic acid **11R**.

The results indicate clearly that the antipode of the natural pheromone is benign, and therefore the chiral purity of the pheromone is less important. We did not observe any racemization during our synthesis but, even if some occurred, it would not inhibit the activity of the pheromone. The chiral purity of the pheromone was ca. 95%, comparable to that of the chiral commercial (+)- α -pinene. The technical grade pheromone was somewhat less active than 97% pure pheromone but the trap catch of the two pheromone grades did not differ statistically.

A dose response test of the pheromone, using Israeli rubber dispensers, indicated that doses of 100 μ g - 800 μ g attracted statistically the same number of males; lower dosages caught fewer males (Table 1). Therefore, in most tests a dose of 200 μ g of pheromone or analogs was used.

Table 1. Dose response of *Planococcus citri* pheromone.

Pheromone dose 1 (μ g)	Male captures/trap/week
0	2.9c
25	245 b
50	252b
100	337a
200	381a
400	352a
800	397a

The attractiveness of the pheromone analogs **10**, **13** and **14** was assessed in several field tests and their activity was compared to that of the pheromone **1**. Of particular interest was the homolog **10**, which had shown significant activity in previous tests conducted several years ago. The results of a few representative tests are shown in Table 2.

The homolog **10** showed significant activity; about 50% in most tests as compared with that of the pheromone **1**. In Test 2 (Table 2), the activity was the same as that of the pheromone. This result was not be repeated in any other test conducted in sweetie, avocado or parsimon plantations (some tests are not presented). The ethylidene analog **14** has also considerable activity, about 50% as compared with pheromone **1**. However, its synthesis is similar to that of the pheromone, having therefore no advantage for practical application. The analogs **13** and **15** (tested previously) displayed no activity.

Table 2. Trap catch of *Planococcus citri* males (mean/trap/week) with pheromone and analogs. Each treatment was tested in five replicates.

Compound	Amount (μg)	Test 1 August 99 Sweetie	Test 2 October 99 Avocado	Test 3 August 00 Parsimon	Test 4 September 00 Sweetie
Pheromone 1	200	98.7a	182bc	110a	91.6a
Homolog 10	200	56.3b	210ab	59b	
Homolog 10	400		343a		
Analog 13	200	1c			
Analog 14	200		126c		47.4b
Analog 14	400		140c		
Control	Hexane	1c	0.7d	1c	1.8c

The rationale for preparing a series of analogs was: 1) to assess the structure-activity relationship of the pheromone in order to determine which structural functions are essential for biological activity. 2) to obtain an active analog which would be easier to prepare than the pheromone. Both goals were achieved.

The present field bioassays of the analogs, combined with previous results (Dunkelblum *et al.*, 1987) with additional analogs, clearly indicate that the double bond and the acetate group are essential for biological activity. Saturation of the double in analog **15** or replacing the acetate by a carboxy-methyl group analog **13** eliminated the attractiveness. Conversely, elongation of the double bond with a methyl group, ethylene replacing methylene, analog **14**, or addition of one carbon into the acetate side chain in analog **10** has maintained considerable activity, attracting about 50% of males as compared with the attractiveness of the pheromone. The ethylene analog **14** is a 1:1 mixture of *E* and *Z* isomers and we do not know if both or only one geometric isomer is active. The active analogs **10** and **14** maintain the same molecular shape as the pheromone, keeping all essential functional groups of the pheromone.

Conclusions

A practical synthesis of the *P. citri* pheromone has been developed, using two ozonolysis steps and a selective reduction of an aldehydic group in a ketoaldehyde intermediate. Screening all available analogs, showed that analog (homolog) **10** is highly and constantly active. The synthesis of this compound is much more convenient than that of the pheromone or any other analog, therefore, it is of practical importance. It could replace the more expensive pheromone in field work.

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