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Synthesis of citrophilus mealybug sex pheromone using chrysanthemol extracted from Pyrethrum (*Tanacetum cinerariifolium*)

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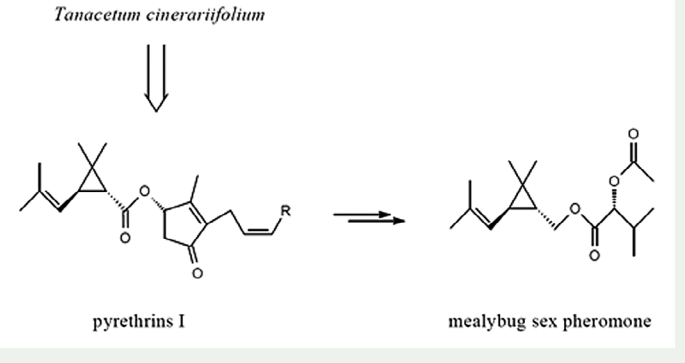
ABSTRACT

A commercial pyrethrum extract was used as a source of chrysanthemol for the synthesis of the citrophilus mealybug (*Pseudococcus calceolariae*) sex pheromone. The chrysanthemic acid esters (pyrethrins I) were isolated and subsequently reduced to obtain chrysanthemol, which was used for ester pheromone synthesis. Field tests showed that the pheromone synthesized using plant-derived chrysanthemol was as attractive to male *P. calceolariae* as the pheromone obtained using a commercial isomeric chrysanthemol mixture.



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Chrysanthemol; pyrethrins; mealybug pheromone

1. Introduction

The citrophilus mealybug, *Pseudococcus calceolariae* (Maskell) (Hemiptera: Pseudococcidae) is a cosmopolitan pest insect found in many regions of the world, attacking various plant species, and causing economic losses in fruit crops (García Morales et al. 2016). Control is usually based on conventional chemical control methods, which are not always efficient due

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to the cryptic habits of the mealybugs. On the other hand, monitoring mealybug populations is done by visual inspections, which is a very time-intensive labour and requires trained personnel. The *P. calceolariae* sex pheromone produced by the females has been identified as (1*R*,3*R*)-chrysanthemyl (*R*)-2-acetoxy-3-methylbutanoate (El-Sayed et al. 2010; Unelius et al. 2011). The potential use of the pheromone for detection, monitoring, and control has been studied in New Zealand and Chile with promising results (Flores et al. 2015; Suckling et al. 2015), so it is desirable to explore possible sources of chrysanthemol for synthesis of the pheromone to be used in field applications.

Pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch.Bip.) (Asteraceae) is a perennial herb that has been used for a long time as a natural insecticide. The active ingredients are six compounds, which are usually grouped according to their chemical structure into chrysanthemic acid esters (pyrethrins I) and pyrethric acid esters (pyrethrins II). The typical pyrethrin I + II content of dried *T. cinerariifolium* flowers is about 1–2%, and in view of the demand for this botanical insecticide, breeding methods for the improvement of pyrethrin content (Li et al. 2014), and extraction methods for the isolation of pyrethrins have been studied extensively. For the latter, extraction using different solvents such as hexane, acetone, and ethanol for example (Nazari and Kambarani 2008; Ban et al. 2010; Nagar et al. 2015), ultrasound-assisted extraction (Babić et al. 2013), and extraction with supercritical CO_2 (Kiriamiti et al. 2003; Martín et al. 2012), among other methods, have been studied regarding their efficiency. Furthermore, numerous methods have been developed for the quantification of pyrethrins (Essig and Zhao 2001; Henry et al. 2001; Ban et al. 2010).

Chrysanthemol can be obtained from pyrethrins I by chemical reduction. Furthermore, pyrethrum extract is commercially available and should yield pure (1*R*,3*R*)-chrysanthemol, as opposed to commercially available chrysanthemol, which is usually sold as a stereoisomeric mixture. Considering the possible advantages (price and stereoisomeric purity), the present work studies if pyrethrum extract can be used as a source of chrysanthemol for citrophilus mealybug sex pheromone synthesis.

2. Results and discussion

2.1. Pyrethrin extraction, purification, and reduction and synthesis of sex pheromone

Ten grams *T. cinerariifolium* extract was treated with methanol at low temperature (experimental details provided in supplementary material). This procedure was performed because the pyrethrins are soluble in this solvent, while other plant extract components, such as waxes and pigments, are not extracted at low temperatures, thus obtaining a refined extract which is more suitable for flash chromatography. Three variables which were considered to most likely have an impact on extraction yield were selected for a simple optimization procedure. Their contribution to the mass obtained from the initial extract was evaluated using a two-level full factorial design in a reduced number of runs. Variables examined, levels considered, and results obtained are presented in Table 1. The normal probability plot obtained for these experiments revealed a significant positive effect for the solvent volume (Figure 1), while the factors time and temperature presented non-significant effects. This means that for the latter two factors, the most convenient experimental values (i.e. shorter times, temperatures closer to room temperature) can be chosen. The mean mass obtained

Table 1. Experimental variables and results obtained for the optimization of the low-temperature extraction of pyrethrum extract.

Experiment	Volume (mL)	Time (min)	Temperature (°C)	Mass (g)
1	75	30	−10	6.73
2	75	30	−70	6.65
3	75	60	−10	6.78
4	75	60	−70	7.32
5	150	30	−10	8.22
6	150	30	−70	8.10
7	150	60	−10	7.96
8	150	60	−70	7.46
9	112	45	−40	8.76
10	112	45	−40	8.33
11	112	45	−40	8.47

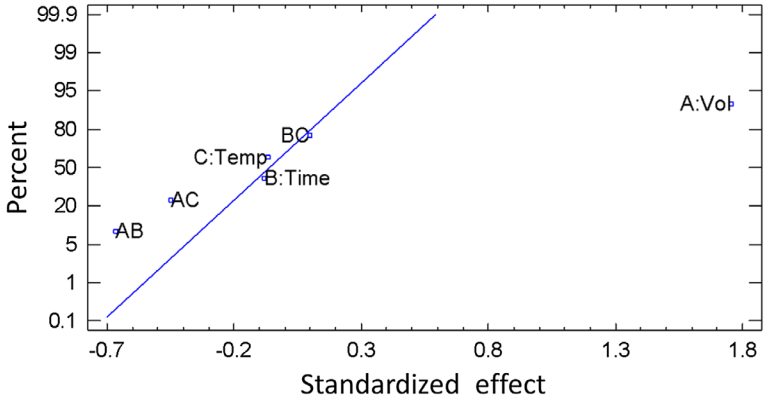

Figure 1. Normal probability plot for standardized effects obtained for the optimization of the extraction process.

in three experiments at the intermediate '0' levels was 8.52 g, while the predicted value was 7.70 g. This suggests a response curvature due to a maximum in the experimental domain. Generally, this situation is resolved conducting additional experiments and modelling the results with a second experimental design order. However, in order to decrease the number of experiments, the experiment which presented the maximum response in the factorial design, in this case the '0' level, was chosen as the optimum extraction condition.

Pyrethrins I were isolated from the refined extract in a 13–18% yield based on the mass of the refined extract submitted to chromatography (Table S1). The identity of pyrethrins I was confirmed by comparing gas chromatographic retention times and mass spectra of isolated compounds with those of an authentic standard. The commercially available plant extract contained 25% pyrethrins. According to GC analysis, it was estimated that approximately two thirds of the total pyrethrin content of the plant extract corresponded to pyrethrins I and one third to pyrethrins II, i.e. 17% and 8% ca., respectively. Considering the 13–18% yield of pyrethrins I from the refined extract, and the 85% yield of refined extract, this translates to an approximate 66–92% recovery of pyrethrins I from the commercial extract. This simple calculation, however, does not take into account a possible degradation

which pyrethrins might suffer over time and which may account for substantial losses (Atkinson et al. 2004).

The conversion of pyrethrins I to the mealybug sex pheromone was achieved by reduction and subsequent esterification of the resulting chrysanthemol with 2-acetoxy-3-methylbutanoic acid (Figure 2). Treatment of purified pyrethrins I with lithium aluminium hydride resulted in the formation of chrysanthemol, which was isolated and purified by column chromatography in 80% yields. The relative configuration of chrysanthemol was determined to be *trans* by comparing gas chromatographic retention times and NMR spectra with those of an isomeric mixture (Figure S1) (Unelius et al. 2011). Furthermore, upon gas chromatography using a chiral stationary phase, the chrysanthemol obtained presented the same retention time as synthetic (1*R*,3*R*)-chrysanthemol (Figure S2). Gas chromatographic analysis of an isomeric mixture of chrysanthemol on a chiral stationary phase showed three peaks with ratios of 50:32:18 ca. (Figure S2(a)). Considering the *cis*-*trans* ratio of the chrysanthemol isomeric mixture (ca. 35:65), the first peak must correspond to a *trans*-isomer, co-eluting with a *cis*-isomer, the second peak to a *trans*-isomer, and the third peak to a *cis*-isomer. Furthermore, the second peak showed the same retention time as the pure *trans*-(1*R*,3*R*)-isomer (Figure S2(b)), as well as the chrysanthemol obtained from the *Tanacetum* extract (Figure S2(c)). In conclusion, although a complete resolution of all four stereoisomers was not achieved, the absolute configuration of the natural chrysanthemol could be assigned, as expected, as (1*R*,3*R*), matching the configuration of *P. calceolariae* pheromone. Finally, the conversion of (*R*)-2-acetoxy-3-methylbutanoic acid into the corresponding acid chloride by treatment with oxalyl chloride, and subsequent esterification with the plant-derived chrysanthemol yielded (1*R*,3*R*)-chrysanthemyl (*R*)-2-acetoxy-3-methylbutanoate in 60–70% overall yield (Figures 2, S3, S4) (El-Sayed et al. 2010).

We have shown that by using a simple optimization procedure, a recovery of up to 90% of pyrethrins I from the plant extract can be achieved, while the reduction of pyrethrins I to chrysanthemol proceeded with a yield of 80%, without any optimization. These are promising results in view of a possible scale-up of the methodology, particularly considering that there should be room for improvement, for example by further optimizing the amounts of solvents and reagents. In a similar approach, Tabata et al. (2015) reported the use of lavender essential oils in the synthesis of isolavandulyl butyrate, the sex pheromone of the mealybug *Planococcus kraunhiae*, without the need of isolating or purifying lavandulol, and showed

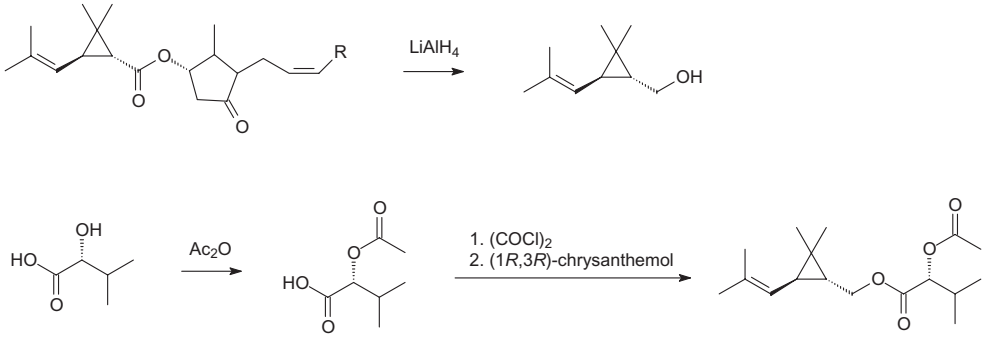

Figure 2. Transformation of pyrethrins I to chrysanthemol and synthesis of chrysanthemyl 2-acetoxy-3-methylbutanoate.

the potential for the use of the lavender oil derived pheromone in mating disruption. In our case, an additional reduction step is required to obtain the monoterpene alcohol needed for the sex pheromone synthesis. Considering the (1*R*,3*R*)-stereochemistry of the chrysanthemol obtained from pyrethrum, it can also be used for the synthesis of (1*R*,3*R*)-chrysanthemyl (*R*)-2-methylbutanoate, the sex pheromone of the Madeira mealybug, *Phenacoccus madeirensis* (Ho et al. 2009), but not for the synthesis of the sex pheromone of the striped mealybug, *Ferrisia virgata*, (1*S*,3*R*)-chrysanthemyl tiglate (Tabata and Ichiki 2017).

2.2. Field tests

Traps baited with pheromone prepared from chrysanthemol obtained from the *T. cinerariifolium* extract ('extract pheromone') and those prepared with commercially available chrysanthemol ('synthetic pheromone') captured the same amount of males, 347 ± 122 vs. 347 ± 94 males (mean per trap in 7 days ± standard deviation), respectively, while no males were captured in traps treated with solvent only. Pheromone molecules have three chiral centres and females produce the (*R*,*R*,*R*)-isomer exclusively (Unelius et al. 2011). Furthermore, stereoisomers with (*S*)-configuration in the acid part are not attractive to males (Unelius et al. 2011), and the unnatural isomers do not interfere with attracting males to the traps in the field (Flores et al. 2015). The 'synthetic pheromone' used in the field test is a mixture of 8 stereoisomers, containing ca. 16% active (*R*,*R*,*R*)-isomer (i.e. ca. 5 µg in 30 µg), while the 'extract pheromone' corresponded to the pure (*R*,*R*,*R*)-isomer.

The advantage of using the pheromone obtained by the method described here is the possibility to use much lower doses as compared to the isomeric mixture without any loss of activity (Flores et al. 2015), and this advantage may outweigh a possible higher cost for its production.

3. Conclusions

Pyrethrum can be used as an alternative source for chrysanthemol in the synthesis of the citrophilus mealybug sex pheromone. The economic feasibility of our method remains to be analysed in more detail. Future studies should be directed at simplifying the procedure presented in this work in order to facilitate a scale-up.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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