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RAPID COMMUNICATION

(*Z*)-Pentacos-12-ene, an Oviposition-deterring Pheromone of *Cheilomenes sexmaculata*

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Abstract Larvae of the coccinellid beetle *Cheilomenes sexmaculata* (F.) produce an oviposition-deterring pheromone that inhibits egg laying of conspecific females on oviposition sites walked over by first-instar larvae. By use of bioassay-guided fractionation of larval extracts, (*Z*)-pentacos-12-ene was identified as an active component of the cuticular hydrocarbons of the larvae. Other compounds that occur in the active fractions, such as the alkaloid coccinelline and saturated hydrocarbons, were individually tested but proved to be inactive. The synthesis of (*Z*)-pentacos-12-ene is reported.

Keywords Coccinellidae · Pentacosene · Oviposition-deterring pheromone · Coccinelline · Alkenes · Alkaloids

Introduction

Various insects release chemicals that deter females from laying eggs on oviposition sites already occupied by conspecific offspring or eggs. This prevents excessive oviposition in a food resource. Competition for nutritional resources can result in cannibalism, which is a serious threat for conspecific eggs, larvae, and pupae. Only a few oviposition-deterring pheromones (ODPs) have been chemically characterized so far (Anderson 2002). The aphidophagous coccinellid *Cheilomenes sexmaculata* (F.) is a frequent predator of aphids in parts of Africa and tropical Asia (Omkar et al. 2006). Růžička (2006) showed that females of *C. sexmaculata* avoided or reduced oviposition on sites where tracks of first-instar conspecific larvae were present, and chloroform extracts of first instar *C. sexmaculata* possessed ODP activity. Hemptinne et al. (2001) prepared a chloroform extract of tracks of

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the larvae of the coccinellid *Adalia bipunctata* (L.) that showed ODP activity. They identified the constituents to be a complex mixture of typical insect cuticular hydrocarbons but did not test for the presence of compounds not detectable by gas chromatography (GC)/mass spectrometry (MS). Furthermore, no bioassays were performed with individual compounds. We report here the identification of an ODP present in extracts of first instars of *C. sexmaculata*, from bioassay-guided fractionation of the extract.

Materials and Methods

Analysis The procedures in GC/MS are as follows: HP 5890 GC coupled with HP 5973 mass selective detector, splitless injection, BPX-5 column (SGE), 60–320°C, 5°C/min. In electrospray ionization (ESI)-MS, analyses were performed on a Finnigan MAT 95 XLT with direct injection by using a microspray interface in positive and negative ionization mode. Samples were dissolved in a 1:1 CH_2Cl_2/CH_3OH mixture. Dimethyldisulfide (DMDS) derivatives were prepared according to Schulz et al. (2000). In nuclear magnetic resonance (NMR) experiments, a DRX-400 (400 MHz, Bruker) was used. Precocinelline and hippocasine were identified by comparison with respective mass spectra in the National Institute of Standards and Technology database. Injection of coccinelline into the injection port of a gas chromatograph at 250°C furnished a chromatogram that contained these two compounds as the only major peaks.

Separation Unfed first instars (5,000, laboratory culture, generations 5–20 originating from the Arab Emirates, 2004) were extracted with 20 ml chloroform for 10 min. The extract was concentrated under a gentle stream of N_2, separated on Sephadex LH-20 (12×1.4 cm) with dichloromethane/methanol (1:1) as eluent into 35 fractions. Fractions that showed an almost identical compound profile by GC/MS were combined, resulting in six fractions, which were analyzed by GC/MS and ESI-MS. After concentration, residues were redissolved in 5 ml of dichloromethane/chloroform (1:4) for bioassays. The final concentration was 135 larval equivalents/ml, assuming that no dilution by the separation process occurred.

Bioassays Oviposition-deterring effects were studied in dual-choice tests as described (Růžička 1997). The test solution (three different concentrations, see Table 1) or chloroform as control was applied to filter papers in dual-choice tests. Ten females of *C. sexmaculata* were placed in the test chamber for 24 h. Water and surfeit of *Aphis fabae* Scopoli were provided as food. Each test had ten replicates. The numbers of eggs laid on treated and

Table 1 Number of eggs laid by *Cheilomenes sexmaculata* females on filter papers treated with a chloroform extract of conspecific first-instar larvae (*) and with different amounts of (*Z*)-pentacos-12-ene[a] vs solvent controls

Concentration μg/cm^2 or larval equivalent/cm^2 (*)	Treatment	Control	Wilcoxon test
0.25*	46±6.7	81.5±4.7	P=0.002
1.25	121.5±12.4	207.5±10.9	P=0.004
0.125	105.0±16.3	166.7±11.0	P=0.006
0.0125	148.7±10.4	135.9±10.4	P=0.625

[a] Mean number of eggs laid in dual-choice tests with ten replicates of ten females. Altogether one hundred individuals were tested in ten replicates in one test. Wilcoxon paired sample test.

control papers were counted and analyzed statistically by using the Wilcoxon paired sample test.

Chemicals (*Z*)-Pentacos-12-ene was synthesized by alkylation of 1-tridecyne with dodecyl triflate, modifying a procedure of Matsuda et al. (2002). After purification by column chromatography, unreacted 1-tridecyne was removed by washing with $AgNO_3$ solution, yielding pure 12-pentacosyne. Hydrogenation with Lindlar's catalyst and H_2 in pentane provided pure (*Z*)-pentacos-12-ene in 18% overall yield, containing less than 2% of the (*E*)-isomer.

Results and Discussion

Chloroform extracts of first-instar larvae showed strong oviposition deterrent activity in bioassays (Table 1). Analysis of the extract by GC/MS (Fig. 1) showed that the major compounds in the chromatogram, constituting up to 95% of the extract, were the alkaloids precocinelline and hippocasine, accompanied by mostly unbranched C_{23}–C_{35} hydrocarbons. However, NMR analysis revealed that the sample actually contained almost pure coccinelline (LeBrun et al. 1999), but this alkaloid proved to be inactive in our bioassay. The free amine alkaloids observed were formed in the injection port of the gas chromatograph from the alkaloid-*N*-oxide coccinelline. It might be possible that compounds not detectable by GC/MS may play a role as ODP. Therefore, we decided to fractionate the extract by chromatography on Sephadex LH-20. Six fractions were obtained, of which one showed a pronounced oviposition-deterrent activity. The investigation of this fraction by

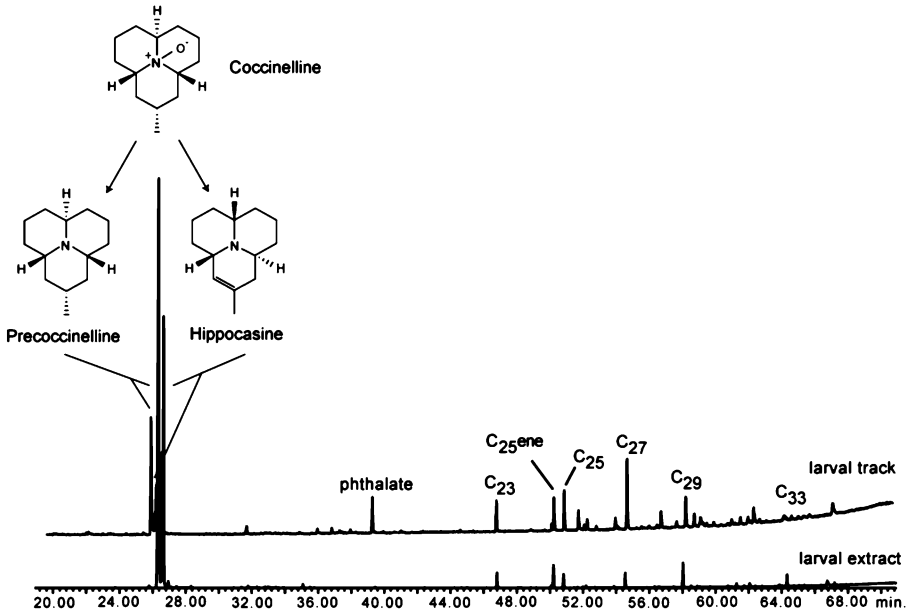

Fig. 1 Gas chromatogram of extracts of *Cheilomenes sexmaculata* first instars and a first-instar larval track

nanospray ESI-MS should reveal the presence of nonvolatile and/or polar compounds not analyzable by GC/MS, but no such compounds were found. Instead, we observed by GC/MS that this fraction contained a complex mixture of cuticular hydrocarbons. Major components in the fraction were pentacosane, heptacosane, and nonacosane, which were bioassayed individually and as a mixture in natural proportions but which were not active. An additional compound present in this fraction was a pentacosene, the only alkene occurring in the mixture in significant amounts. The position of the double bond was determined by using DMDS derivatization (Schulz et al. 2000). The formation of the DMDS adduct showed that the double bond was located at C-12, as evidenced by the strong α-cleavage ions at *m/z* 215 $(C_{12}H_{24}SCH_3)^+$ and *m/z* 229 $(C_{13}H_{26}SCH_3)^+$. Therefore, (*Z*)-pentacos-12-ene was synthesized by alkylation of 1-tridecyne with dodecyl triflate (Matsuda et al. 2002), followed by Lindlar-catalyzed hydrogenation. Gas chromatographic comparison showed that the natural compound did indeed have the (*Z*)-configuration because the two geometric isomers had different retention times on the BPX-5 phase used. In addition, (*Z*)-pentacos-12-ene was also found in extracts of tracks of first-instar larvae, proving the actual presence on the substrate (Fig. 1). This pentacosene showed considerable oviposition-deterrent activity in our bioassay. At high concentrations (1.25 μg/cm^2), the activity was strong, equivalent to the chloroform extract of 20 larvae per substrate. With lower concentrations (0.125 and 0.0125 μg/cm^2), the activity decreased as expected (Table 1). Nevertheless, because the activity of the extract was higher than that of the pentacosene, it is likely that other extract constituents are also active or might act synergistically with (*Z*)-pentacos-12-ene.

Our results prove that (*Z*)-pentacos-12-ene, most likely present on the cuticle and also found in tracks of the larvae, is an ODP of *C. sexmaculata*, the first such pheromone identified from coccinellids.

References

ANDERSON, P. 2002. Oviposition pheromones in herbivorous and carnivorous insects, Chemoecology of Insect Eggs and Egg Deposition. 235–263, in M. Hilker, and T. Meiners (eds.). Blackwell, Berlin.

HEMPTINNE, J. L., LOGNAY, G., DOUMBIA, M., and DIXON, A. F. G. 2001. Chemical nature and persistence of the oviposition deterring pheromone in the tracks of the larvae of the two spot ladybird, *Adalia bipunctata* (Coleoptera: Coccinellidae). *Chemoecology* 11:43–47.

LEBRUN, B., BRAEKMAN, J. C., and DALOZE, D. 1999. Complete assignment of the ^{1}H and ^{13}C NMR spectra of the *Coccinellidae*-defensive alkaloids myrrhine, precoccinelline, and hippodamine, their *N*-oxides and the corresponding hydrochlorides. *Magn. Reson. Chem.* 37:60–64.

MATSUDA, Y., and MORI, K. 2002. Synthesis of the four stereoisomers of 3,12-dimethylheptacosane, (Z)-9-pentacosene and (Z)-9-heptacosene, the cuticular hydrocarbons of the ant, *Diacamma* sp. *Biosci. Biotechnol. Biochem.* 66:1032–1038.

OMKAR, SINGH S. K., and SINGH, K. 2006. Effect of age on reproductive attributes of an aphidophagous ladybird, *Cheilomenes sexmaculata*. *Insect Sci.* 13:301–308.

RŮŽIČKA, Z. 1997. Recognition of oviposition deterring allomones by aphidophagous predators (Neuroptera: Chrysopidae, Coleoptera: Coccinellidae). *Eur. J. Entomol.* 94:431–434.

RŮŽIČKA, Z. 2006. Oviposition-deterring effects of conspecific and heterospecific larval tracks on *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). *Eur. J. Entomol.* 103:757–763.

SCHULZ, S., ARSENE, C., TAUBER, M., and MCNEIL, J. N. 2000. Composition of lipids from sunflower pollen (*Helianthus annuus*). *Phytochemistry* 54:325–336.