Identification of Pheromones from Entomopathogenic Nematodes

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Introduction

Entomopathogenic nematodes of the genus *Steinernema* are lethal parasites of insects that have shown promise as biological control agents [1]. The nematode survives in the soil as a stress-resistant infective juvenile (IJ) that seeks out and infects insect hosts. Once inside the host, the IJ regurgitates a bacterial pathogen (of the genus *Xenorhabdus*) from its gut that ultimately kills the host. According to preliminary data obtained in the Stock lab, *Steinernema* species paired with their native *Xenorhabdus* bacterial species (rather than a non-native species) form a higher percentage of lethal IJ larvae. We are identifying the IJ pheromones from different *Steinernema* species, and we will determine if the chemical composition of these pheromones changes when the *Steinernema* species are paired with native versus non-native *Xenorhabdus* species. The results from this work have the potential to guide the formulation of *Steinernema* nematodes as biocontrol agents (targeting insect pests that attack food crops) and to increase the virulence/effectiveness of these agents.

Experimental

^{1}H, COSY, HSQC, HMBC, and NOESY experiments were performed on the AMRIS Bruker 600 MHz NMR with a 5mm HTS cold probe.

Results and Discussion

Using activity-guided fractionation, we have purified two structurally unusual glucosides with hydroxyls in their side chains from *S. carpocapsae* cultivated on its native bacterial species, *X. nematophila*. Using AMRIS NMR spectrometers, we had previously performed NMR experiments to elucidate the chemical structures of these glucosides, as reported in past annual reports. In the past year, we used a 9-step chemical synthesis to synthesize a candidate glucoside with a defined *R*,*R*-configuration for the diol in its side chain and performed COSY, HSQC, and HMBC experiments on the synthetic compound. These experiments confirmed that our synthetic glucoside has NMR data that match that of the natural glucoside isolated from *S. carpocapsae*. We also performed a proof-of-concept experiment where we took a protected version of the synthetic glucoside with the *R*,*R*-configuration for the diol, derivatized the diol [2], and performed NOESY experiments to confirm the relative configuration of the two hydroxyls. We are now proceeding to perform similar experiments for the natural glucoside to make sure that the NOESY data of the derivatized natural material matches that of the derivatized synthetic glucoside.

Conclusions

We have determined candidate structures for two novel glucosides from *S. carpocapsae* with hydroxyls in the side chains, and we have used chemical synthesis to synthesize one of them with a defined absolute *R*,*R*-configuration for the diol in the side chain. We are now using Mosher's method [3] and Snatzke's method [4] to confirm the absolute configuration of the two hydroxyls in the side chain. With synthetic pheromone in hand, we can now proceed to determine its biological activity, such as whether it affects development of the IJ stage. We can also determine whether it is differentially produced when *S. carpocapsae* is paired with native versus non-native bacterial species.

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