Identification of Novel Pheromones from *Caenorhabditis elegans* Using Comparative Metabolomics and Multi-dimensional NMR Spectroscopy

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Introduction

The nematode *C. elegans* secretes ascarosides into its environment and uses them in chemical communication. The ascarosides are derivatives of the 3,6-dideoxysugar ascarylose, modified with different fatty acid-derived side chains (**Figure 1**). *C. elegans* uses specific ascarosides, which are together known as the dauer pheromone, to trigger entry into the stress-resistant dauer larval stage.[1] In addition, *C. elegans* uses specific ascarosides to control certain behaviors, including mating attraction, aggregation, and avoidance.[2, 3] In this project, we will use metabolomics and multi-dimensional NMR spectroscopy to identify novel ascarosides, and then we will characterize the biological activity of these ascarosides in *C. elegans*.

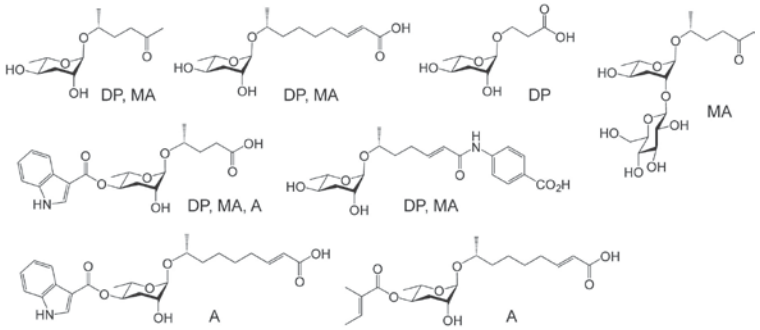

Figure 1. Ascarosides produced by *C. elegans* with different developmental and behavioral effects (DP = dauer pheromone; MA = male attraction; A = aggregation).

Experimental

dqf-COSY, HSQC, and HMBC experiments were performed on a Bruker Advance 600 MHz NMR with a 1 mm microcryoprobe.

Results and Discussion

In order to identify novel pheromones produced by *C. elegans*, we have pursued a metabolomic approach in which we have compared the metabolites produced by wild-type worms and mutant worms (*daf-22*)[4], which do not produce short-chain ascarosides. We have focused on several fractions from wild-type worms containing candidate novel ascarosides, and have obtained dqf-COSY, HSQC, and HMBC experiments on one of these fractions. These NMR data have enabled us to put together several fragments for the molecule, but we have not been able to connect the fragments, in part because the fraction we are characterizing is not pure. We will combine this fraction with several surrounding fractions, and re-fractionate to obtain larger amounts of pure compound, and then we will redo the above described NMR experiments.

Conclusions

We have identified a novel ascaroside, but need to further characterize it by NMR before assigning a structure. Once characterization is complete, we will synthesize the ascaroside and study its biological activity. In addition, we have many additional fractions containing candidate novel ascarosides that we will evaluate by NMR.

Acknowledgements

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References

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[2] Srinivasan, J., *et al.*, Nature, **454**, 1115-1118 (2008).
[3] Srinivasan, J., *et al.*, PLoS Biol, **10**, e1001237 (2012).
[4] Butcher, R. A., *et al.*, Proc Natl Acad Sci U S A, **106**, 1875-1879 (2009).