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

J.K. Nunnery, P. Singh, S. Chinta, Y. Long (UF, Chemistry); E.S. Conner (LSU, Chemistry), X. Zhang, D.H. Powell (UF, Chemistry), J.R. Ragains (LSU, Chemistry); R.A. Butcher (UF, Chemistry)

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. *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 are using LC-MS-based comparative metabolomics and multi-dimensional NMR spectroscopy to identify novel ascarosides. In the future, we will characterize the biological activities of these ascarosides in *C. elegans*.

Experimental

dqf-COSY, HSQC, and HMBC experiments were performed on the AMRIS Bruker Avance 600 MHz NMR with a 5 mm cryoprobe.

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, using LC-MS. We have focused on several fractions from wild-type worms containing candidate novel ascarosides and purified these ascarosides. dqf-COSY, HSQC, and HMBC experiments enabled the chemical structure of one of these ascarosides to be elucidated (**Fig. 1**). We are further purifying additional compounds for characterization by NMR spectroscopy. In addition, we are using LC-MS to compare the metabolome of wild-type worms with those of additional biosynthetic mutants.

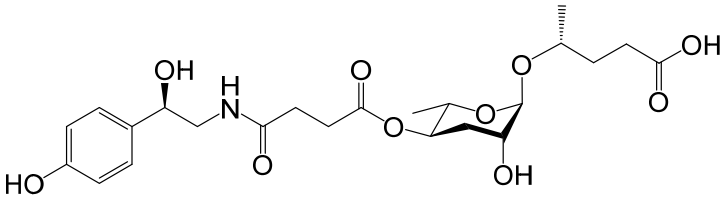

Figure 1. One of the ascaroside pheromones identified using a comparative metabolomics approach.

Conclusions

Our results suggest that LC-MS-based comparative metabolomics, followed by NMR characterization of purified compounds, is a viable approach for the identification of novel secondary metabolites in *C. elegans*. Further studies will be required to identify the biological function of molecules identified using this method.

Acknowledgements

Funding was provided by a subcontract from Harvard University (source agency: Human Frontier Science Program, RGP0042).

References

[1] Butcher, R. A., *et al.*, Nat Chem Biol, **3**, 420-422 (2007).
[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).