****

**Public Oral Examination**

**For the Degree of Doctor of Philosophy**

**Robert M. Hohlman**

***“Harnessing Cascade Biocatalysis for the Chemoenzymatic Synthesis of Unnatural Hapalindole-Type Metabolites and Further Exploration into their Biosynthesis”***

**Friday, April 15, 2022 at 10:00am**

**Palmer Commons Forum Hall**

**Zoom Option:**

[**https://umich.zoom.us/j/92574809643**](https://umich.zoom.us/j/92574809643) **Password: Rhohlman22**

**Committee Members:**

Dr. David Sherman (Chair)

Dr. Roland Kersten

Dr. Alison Narayan

Dr. Andy White

**Abstract:**

In recent years, the rise in resistance by bacteria, viruses, tumor cells, etc. to already approved therapeutics has led to a greater demand for drug production. To meet this demand, a revival of interest in natural products has opened the door for a once dormant pipeline of biologically active compounds. Natural products are secondary metabolites produced by organisms that aid in their survival (but are not critical for the organism’s survival itself). Over the past 40 years, natural products have provided the actual compound (or inspiration) for greater than 50% of all FDA approved drugs. While a diverse library of natural products already exists to be explored as potential therapeutics; many limitations arise. Natural products are generally complex structures that are difficult to produce synthetically in an acceptable amount of steps and yields. Isolation methods from producing strains are also not great enough to meet the demand of drug production. It has long been proposed that the enzymatic machinery from the biosynthetic gene clusters that produce these compounds could be harnessed to not only produce the parent compounds but also derivatives. Today, sequencing technology and protein engineering has made that proposal a reality and opened the door even further for natural product exploration.

This thesis presents results focused on the exploration of the enzymes responsible for the biosynthesis of the hapalindole-type metabolites. Hapalindole-type metabolites are a diverse group of indole alkaloids defined by their polycyclic ring system, various stereoisomers, unique functional groups and promising biological activities. Recently, the biosynthetic gene cluster responsible for their biosynthesis has been elucidated. This revealed a wide range of enzymes that included prenyltransferases, oxygenases, and cyclases. The cyclases, in particular, have drawn interest due to their ability to catalyze a unique three step cyclization cascade: 1) a Cope rearrangement, 2) 6-*exo*-*trig* cyclization, and 3) terminal electrophilic aromatic substitution (EAS) from a common indole C-3 geranylated (3-GC) intermediate. Utilizing cascade biocatalysis with a prenyltransferase and various cyclases, a chemoenzymatic route to produce unnatural hapalindole-type metabolites has been devised on both milligram and *in vitro* scales in reaction vessels and cell-free protein synthesis reactions. Further medicinal testing and semisynthetic modifications to this library could provide a drug candidate to combat increasing drug resistance.

Another member of the hapalindole-type metabolites, the ambiguines, showcases similar biological activity but a greater amount of structural modifications. One of the most interesting is the addition of a fifth (E) ring. To date, it is unknown how this fifth ring is formed but it is proposed to arise from reactions catalyzed by Rieske-type oxygenases. To tackle this question, two routes were explored; one focusing on the Rieske-type oxygenases themselves and another exploring a chemoenzymatic synthesis of a pentacyclic ambiguine. While the Rieske-type oxygenases proved challenging to elucidate, the chemoenzymatic route has shown promise. To date, an optimized, efficient biocatalytic method for generating an unnatural ambiguine derivative, 12-*epi*-ambiguine H nitrile, has been developed. Efficient biosynthesis of the unnatural derivative is crucial to further synthetic chemistry or biocatalysis efforts to generate the E-ring. In addition, during early phases of this work, we uncovered an unexpected new metabolite of a one pot reaction previously unknown in the Stig Cyclases. This finding strengthens the Cope rearrangement hypothesis and provides a potential synthon towards total synthesis efforts of a diverse range of compounds. These works showcase the potential natural product enzymes have to produce complex metabolites and provide a new route to further develop the diverse library of already known natural products.

#### Publications:

“Structural diversification of hapalindole and fischerindole natural products via cascade biocatalysis” **Robert M. Hohlman**, Sean A. Newmister, Jacob N. Sanders, Yogan Khatri, Shasha Li, Nikki R. Keramati, Andrew N. Lowell, K. N. Houk and David H. Sherman *ACS Catal.* 2021, 11, 8 4670-4681 https://doi.org/10.1021/acscatal.0c05656

“Recent advances in Hapalindole-type cyanobacterial alkaloids: biosynthesis, synthesis, and biological activity” (Review) **Robert M. Hohlman** and David H. Sherman *Nat. Prod. Rep.*, 2021, 38, 1567-1588 https://doi.org/10.1039/D1NP00007A (Cover article, September 2021)

“Multi-component microscale biosynthesis of unnatural cyanobacterial indole alkaloids” Yogan Khatri, **Robert M. Hohlman**, Johnny Mendoza, Shasha Li, Andrew N. Lowell, Haruichi Asahara and David H. Sherman (*co-first*) *ACS Synth. Biol.* 2020, 9, 6, 1349–1360 https://doi.org/10.1021/acssynbio.0c00038

“Engineered production of hapalindole alkaloids in the cyanobacterium *Synechococcus* sp. UTEX 2973” Cory J. Knoot, Yogan Khatri, **Robert M. Hohlman**, David H. Sherman and Himadri B. Pakrasi *ACS Synth. Biol.* 2019, 8, 8, 1941-1951 https://doi.org/10.1021/acssynbio.9b00229

“Control of stereoselectivity in diverse hapalindole metabolites is mediated by cofactor induced combinatorial pairing of Stig cyclases” Shasha Li, Sean A. Newmister, Andrew N. Lowell, Jiachen Zi, Callie R. Chappell, Fengan Yu, Robert M. Hohlman, Jimmy Orjala, Robert M. Williams, and David H. Sherman *Angew. Chem. Int. Ed.* 2020, 59,2-9 https://doi.org/10.1002anie.201913686

“The discovery and elucidation of 11-DMAC: Strengthening the Cope rearrangement hypothesis for the biosynthesis of hapalindole metabolites” **Robert M. Hohlman**, Nikki R. Keramati and David H. Sherman, *In preparation (co-first)*

**Select Oral Presentations:**

“Structural diversification of hapalindole and fischerindole natural products via cascade biocatalysis”, ACS Fall National Meeting, Atlanta, GA, 2021

“Structural diversification of hapalindole and fischerindole natural products by versatile Stig cyclases”, NSF-CCHF Annual Conference, Virtual, 2020

“Exploration of *Stig* Cyclases as Biocatalytic Tools for Unnatural Indole Alkaloid Production”, College of Pharmacy Research Day, University of Michigan, 2020

“Exploration of *Stig* Cyclases as Biocatalytic Tools for Unnatural Indole Alkaloid Production”, Department of Medicinal Chemistry Seminar, University of Michigan, 2020

**Select Awards:**

2021 Rackham Pre-doctoral Fellowship

2020 ACS Medicinal Chemistry Pre-doctoral Fellowship

2019 Department of Medicinal Chemistry Graduate Student Instructor of the Year

**Future Plans:**

Robert has accepted a position as a Scientist 1-Enzymatic Synthesis with Cayman Chemical in Ann Arbor.