Novel nitrogenous heterocycles from the tunicate Clavelina picta
by Michael Franklin Raub

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University

Abstract:
The goal of natural products chemistry is the isolation and identification of novel secondary metabolites from terrestrial or marine organisms. In some cases, nontraditional separation techniques must be developed in the course of the investigation. An equally important and hoped for outcome is that those compounds discovered will express potentially useful biological activity that can be identified and quantified. It was the purpose of this research to investigate the secondary metabolites of the Bermudian tunicate Clavelina picta towards those ends.

In the course of the investigation, thirteen novel compounds were isolated . Two additional compounds were characterized as a mixture. Of the fifteen, twelve were identified as quinolizidines, and three were identified as isomeric mixtures of indolizidines. The isolation of the compounds was achieved via high speed counter current chromatography. Four quinolizidines and the three indolizidine mixtures were found to possess both cytotoxic and antimicrobial activity.

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APPROVAL

of a thesis submitted by

Michael Franklin Raub

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Signature Michael F. Lamb

Date 8/5/90

This thesis is dedicated to my parents, who supported me to the fullest no matter what I did.

"It happened in a season of dreams."
Eudora Welty

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ABSTRACT

The goal of natural products chemistry is the isolation and identification of novel secondary metabolites from terrestrial or marine organisms. In some cases, nontraditional separation techniques must be developed in the course of the investigation. An equally important and hoped for outcome is that those compounds discovered will express potentially useful biological activity that can be identified and quantified. It was the purpose of this research to investigate the secondary metabolites of the Bermudian tunicate *Clavelina picta* towards those ends.

In the course of the investigation, thirteen novel compounds were isolated . Two additional compounds were characterized as a mixture. Of the fifteen, twelve were identified as quinolizidines, and three were identified as isomeric mixtures of indolizidines. The isolation of the compounds was achieved via high speed counter current chromatography. Four quinolizidines and the three indolizidine mixtures were found to possess both cytotoxic and antimicrobial activity.

INTRODUCTION

The investigation of natural products is an ancient phenomenon. Medicinal lore from prehistoric times was surely based on a literal trial and error method. Eating or chewing on a plant was an albeit dangerous, but immediate, method of determining its nutritional, healing or toxic properties. Indeed, Socrates himself was aware of the powerful effect of a constituent of hemlock. The Phoenicians, or one of their many trading partners, developed the use of an exudate from a mollusk as a dye, royal purple. A Polynesian fisherman was probably the first person to observe the killing of fish in a lagoon in the presence of a particular coelenterate, one which harbored palytoxin[1]. Perhaps by dipping his spear in a colony of the organisms, he would insure himself an easier catch!

Based on these observations or simple investigations, a practical application of the natural product has come about. These applications have proven to be, for the most part, of a beneficial nature to mankind. In earlier times, it was enough to use the whole plant or animal responsible for the activity of interest. With the sophisticated methods of modern science, we can no longer satisfy ourselves with merely noting that by drinking tea

made from bitterroot, we are making use of an effective heart tonic[2]. We are finally capable in the twentieth century of isolating and characterizing even very minor metabolites which are responsible for bioactivity, as it now has been named. These methods also allow for the identification of compounds that may not display biological activity but are important nevertheless for their novelty or commercial utility.

In the realm of modern marine natural products, a multitude of novel and biologically active compounds have come to light in the last three decades. J.T. Baker and V. Murphy cite that the upswing of research in marine natural products took place in the mid 1960's[3]. The trend in the 1970's was that of discovering compounds both novel and biologically active. This interest led, for example, to Hoffmann LaRoche establishing a facility in Australia with the expressed purpose of finding marine natural products with potential as drugs[4]. The National Institutes of Health have expressed increased interest in recent years in natural products for cancer treatment. A vast program involving the collection of organisms and testing of abstracts with a wide array of cell lines is underway at present. It was felt that, given the few cell lines used earlier for testing, some biologically active compounds could have been overlooked. The testing

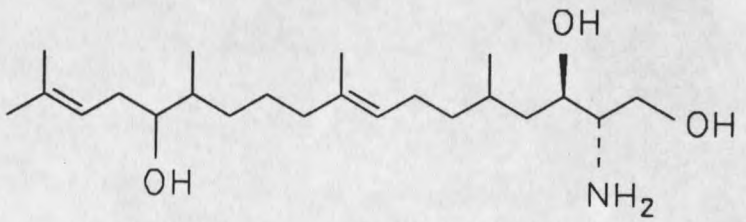
program now includes bioassays with the HIV-1 virus as well.

Not all types of marine organisms seem to have received the same amount of interest from natural products chemists. Very little work was done on tunicates prior to 1979, although there does not seem to be any specific reason for neglecting these invertebrates. It was perhaps the isolation and identification of the biologically active cyclic peptides by Rinehart[5] that gave impetus to an increase in interest in tunicates. Tunicates have been an object of study at the natural products laboratory at Montana State University since the early 1980's. This report will discuss the research into the biologically active metabolites of the Bermudian tunicate *Clavelina picta*.

CHEMISTRY OF THE TUNICATES

Whereas most research interests were devoted traditionally to other marine invertebrates over the course of many decades, no marine natural products chemist can deny that tunicate metabolites deserve attention. The most important reason for this newly achieved notoriety is the substantial numbers of compounds that display a variety of biological activities. The basic structural characteristics are also not limited to one or two types; the only commonality among the compounds is that they are nitrogenous.

From an *Aplidium* species were isolated two compounds, the 2,3-*erythro*-isomer, **1**, and the 2,3-*threo*-isomer, **2**, of aplidiasphingosine. In the original paper by Carter and Rinehart, the compounds were cited as having antimicrobial properties and were also cytotoxic toward KB and L1210 tumor cells in tissue culture[6]. The relative configuration was determined in a later synthesis[7].



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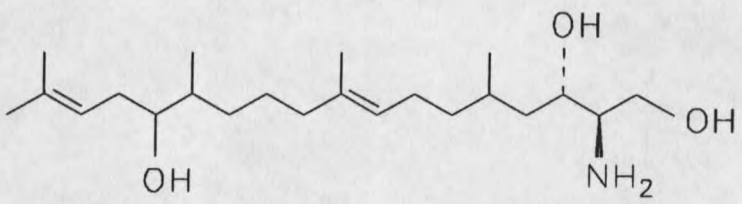
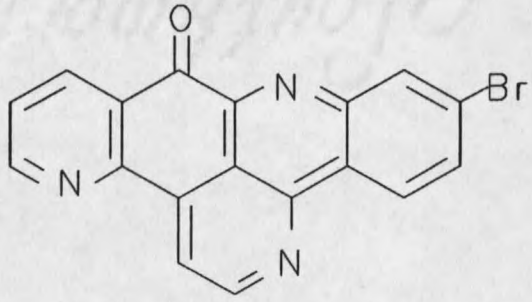


2

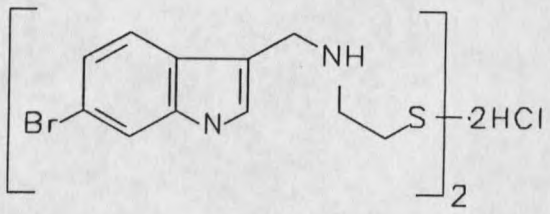
A highly aromatized pentacyclic compound, 2-bromoleptoclinidinone, **3**, was isolated from a *Leptoclinides* species found in the Truk Lagoon in Micronesia. The characterization of the new fused ring skeleton required extensive long range carbon correlation NMR experiments[8]. It was reported to display mild cytotoxicity, ED_{50} (PS=4µg/ml)[9].



3

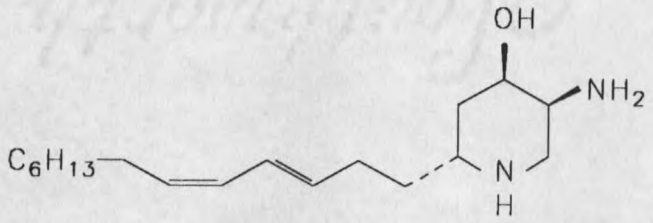
From the tunicate *Polycitorella mariae* was isolated citorellamine, **4**, which possesses insecticidal, cytotoxic and potent antimicrobial properties. The structure

originally proposed by Roll and Ireland[10] was revised and confirmed by total synthesis[11].



4

A Japanese group reported the isolation and characterization of two isomeric compounds from the Okinawan tunicate, *Pseudistoma kanoko*, pseudodistomin A, **5**, and B, **6**. The structure elucidation was done with the triacetate of the molecule, due to the natural product's proclivity for decomposition. The pseudodistomins represent the first piperidines isolated from the marine environment. They exhibit potent antagonistic activity toward calmodulin, a mediator regulating cellular function and a variety of cellular enzyme systems[12].



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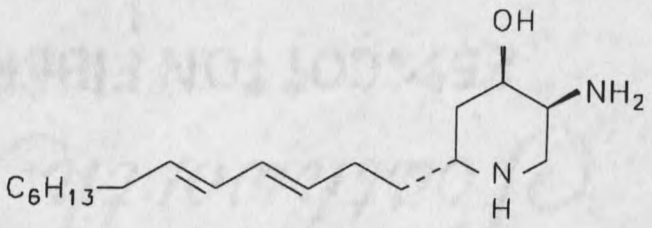

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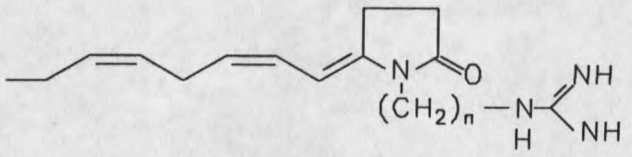
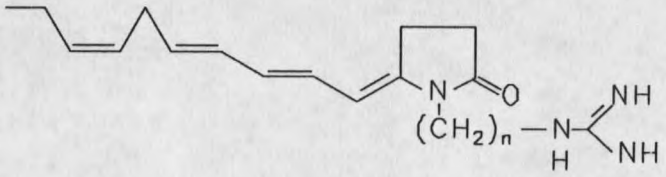
Possessing both antimicrobial and cytotoxic activity are the polyandrocarpidines, A,B, **7,8** and C,D, **9,10** from the genus *Polyandrocarpa*[13,14].



7, n=4
8, n=5



9, n=4
10, n=5

Unique to marine natural products is the 1,2,4-thiadiazole functionality of dendroine, **26**, whose