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A Unique Approach to Combating Zebra Mussels

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Abstract

Zebra mussels are an invasive species seen in many countries worldwide. Zebra mussels not only cause a threat to the surrounding wildlife by out-competing other filter feeders resulting in the starvation of native species, but they also impact human activity. To prevent the dangerous effects of zebra mussels, research was done to better understand the properties and structures of their composition and metabolites. With this research being investigative the hypothesis is that by using organic chemistry methods, different components in Zebra mussels will be able to be detected. In addition, the physical structure of the Zebra mussel that is hypothesized to contain a larger variety of metabolites is the innards because they are the structure being protected by the outer shell. Methods used to further separate the compounds and collect data during this experiment include the use of bioassay and structural guided fractionation such as TLC, HPLC, IR, ^{13}C NMR, ^{1}H NMR, filtration, and separation. TLC was used primarily to analyze which samples contained the most components. HPLC was used to separate the components even further by placing the extracts from specific peaks into beakers. IR and NMR were analyzed to look for functional groups. Results, thus far, concluded that the Zebra mussel shells contained more metabolites than the guts and an expected metabolite is to have a structure with four protons attached to a benzene ring. This was determined through TLC separations. Further conclusions are currently under investigation. Additionally, an investigation into the components of Zebra mussels could lead to identifying secondary metabolites such as the polyketide pathway which could play a significant role in their survival.

Introduction

- Zebra Mussels (*Dreissena polymorpha*) are an invasive species that are native to the Caspian Sea in Asia.[3]
- Entered the Great Lakes Region in the 1980's via vessels.
- Colonized the Great Lakes, Mississippi, Tennessee, Hudson, and Ohio river basins within 10 years of entering the Great Lakes.[4]
- Cause damage to native organisms, physical danger to humans and pets, coat and clog pipes and water intakes for residents, powerplants, and cities.
- Zebra Mussels are expected to cost $3.1 billion for maintenance in the next 10 years.[5]

Goals

- The major goal is to collect and analyze data to identify the metabolites responsible for their existence.
- A minor goal is to use organic chemistry methods to develop the most efficient way to separate the compounds in zebra mussels.
- These methods include the use of bioassay and structural guided fractionation such as TLC, HPLC, ^{13}C NMR, ^{1}H NMR, filtration, and separation.

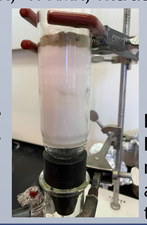

Figure 1: Vacuum Liquid Chromatography. A method used to separate components using silica gel, wool, and numerous solvents of varying polarity.

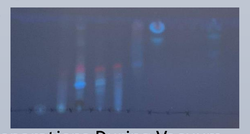

Figure 2: TLC from Separations During Vacuum Liquid Chromatography. The columns with the most components indicated by color changes were analyzed using various other methods throughout the research.

Literature

- Briareum asbestinum[1]
  - Marine invertebrate that was studied to identify the structures of the compounds.
  - The scientists used 2D NMR techniques to establish the structures of the compounds and compare them to the previously studied secondary metabolites of the organism.
- Echium plantagineum[2]
  - Invasive purple to pink flower species native to Mediterranean Basin
  - The scientists used 2D NMR and HPLC techniques to determine that the leaf extracts contained various phenolic compounds.

Methods

- Zebra mussels were obtained from the Mississippi River and froze. They were separated into two different buckets; one shells and the other guts. These buckets were then rinsed with hexane and ethyl acetate separately. The solutions were transferred into separate beakers and evaporated. The products were then analyzed through ^{1}H NMR spectroscopy.
  - According to the 1H NMR, there were more interesting spin systems and chemical shifts that are more characteristic of known biochemical pharmaceuticals. This made it more efficient to continue separating the shells in relation to the guts.
- Shells Vacuum Liquid Chromatography
  - The apparatus shown in Figure 1 was used to separate the molecules in the shell solution based on polarity.
  - A TLC plate was run to determine which separation contained the most activity. The TLC obtained is shown in Figure 2.
- Kirby-Bauer Bioassays
  - Due to the activity in the TLC, a tryptic soy agar was made and swiped with *Staphylococcus aureus*. This expressed the resistance each component was to the bacteria. Data is expressed in Plate 1 and Plate 2 below.
- ^{13}C NMR Spectroscopy
  - An NMR with 14,000 scans was taken of the 5th separation going from left to right on the TLC once it was further separated with hexanes. This spectrum is expressed in Figure 3.

Plate 1: Kirby-Bauer Bioassay Vials 1-4

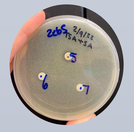

Plate 2: Kirby-Bauer Bioassay Vials 5-7

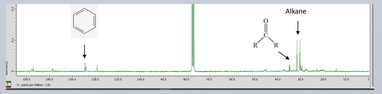

Figure 3: NMR Spectrum of ZebS5.2..1 expressing 2 peaks each representing a benzene ring, another peak farther downfield representing a ketone, and a slightly farther downfield alkane.

- 2D DQF-COZY NMR
  - The double quantum filtered spectrum was run to assign proton connectivity
  - It reduced large singlets, which are generally water or methoxy peaks, to allow for a higher receiver gain to be set.
  - The proton NMR is located in Figure 4, and is used when connecting the cross peaks.
  - The idea is to look for matching cross peaks off of the diagonal. The cross peaks are observed with back lines on Figure 5.

Results

- The methods used thus far have helped separate the components into distinguishable molecules.
- The initial ^{1}H NMR determined that there was more activity in the shells than in the guts.
- The zone of inhibition expressed the most resistance from *S. aureus* in Vials 3, 4, and 5. These vials were obtained after the vacuum liquid chromatogram separated the initial shell components. The precise observations are located in Table 1.
- The ^{13}C NMR expressed large functional groups such as 2 benzene rings, a ketone, and an alkane.
- The 2D DQF-COZY NMR expressed chemical shifts that were coupled and analyzed leading to a structure with a benzene ring attached to 4 protons. An expected structure is shown in Figure 6.

Table 1: Zones of Inhibition using TSA and *S. aureus*

Vial	Observations (diameter)
1	No Inhibition
2	No Inhibition
3	0.8 mm
4	1.1 mm
5	0.8 mm
6	No Inhibition

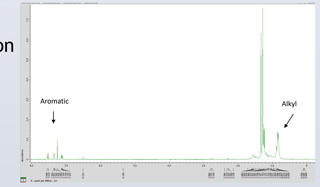

Figure 4: Proton Spectrum that was used with DQF-COZY for analyzation.

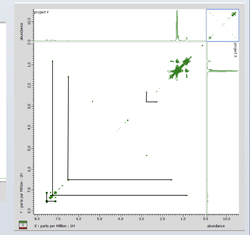

Figure 5: 2D DQF-COZY NMR Spectrum Used to Couple Protons in the Structure

Conclusion

- The 2D COZY NMR was the most efficient organic method used to signify a possible structure of the metabolite within Zebra Mussels.
- The possible structure contains a benzene ring with 4 protons, because of the ^{13}C and the COZY NMR spectra.
- The research is still ongoing, and the expected structure is not confirmed. In the future, a MS will be run and a HSQC NMR spectra will be analyzed.

Figure 6: Structure of metabolite expected.

Acknowledgment

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