Edward Feeney

Rider University, Class of 2021
Major: **Chemistry**

Faculty Advisor: **Danielle Jacobs, Ph.D.,** Associate Professor
Department of Chemistry and Biochemistry

Optimization of Choline Geranate Derivatives as Antibacterial Agents

With the aid of funding from ICFNJ, I have worked toward synthesizing derivatives of the deep eutectic solvent choline geranate. Deep eutectic solvents (DESs) are an interesting set of molecules that are similar to ionic liquids in that they are ionic salts with low melting points. However, they are chemically quite different as they contain a cation, an anion, and a hydrogen bond donor. The resulting indiscrete ionic network leads to interesting chemical properties that can be quite different from traditional ionic liquids. The subset using choline (trimethylaminoethanol) as the cation and various hydrogen bond donors has especially demonstrated exceptional versatility. Choline-based DESs can be simple to prepare, are often biodegradable, and readily solvate many different materials. Furthermore, the specific properties can be modified based on the hydrogen bond donor. Specifically, the DES choline geranate has been reported to have great antibacterial properties against gram-positive bacteria, but former Rider University undergraduate researchers have shown that this is not true for gram-negative bacteria. In an attempt to increase the antibiotic potency against gram-negative bacterial strains, these researchers began synthesizing DES derivatives with variable hydrocarbon chains on the cation. However, it was recently discovered that the synthesis and characterization of these derivatives was not as trivial as originally believed, and that the structure of the proposed DES molecules could not be confirmed. Furthermore, the requisite geranate/geranic acid component is extremely difficult to purify (from commercially available geranic acid) prior to pairing. My research focuses on overcoming these problems by (1) synthesizing pure geranic acid, and (2) comparing the physical and spectroscopic properties of various choline geranate salts and deep eutectic solvents prepared via different techniques. The optimized synthesis of these DES derivatives will pave the way for future research into the antibacterial and biofilm-eradicating properties of choline geranate-derived deep eutectic solvents.

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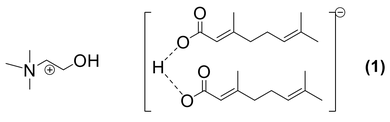
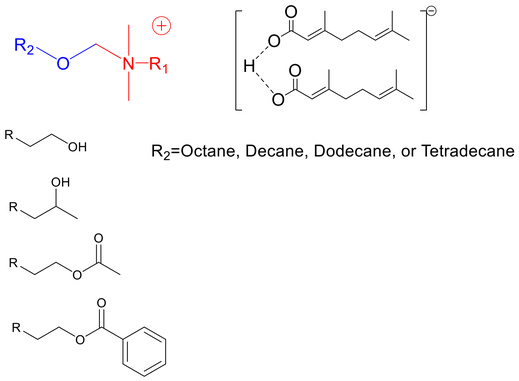
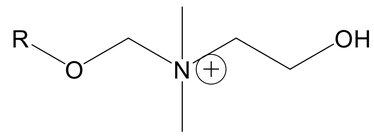
Optimization of Choline Geraneate Derivatives as Anti-bacterial Agents

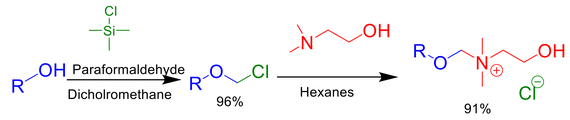


Choline Geranate (1)





R=Dodecane, Tetradecane, Hexadecane, or Octadecane



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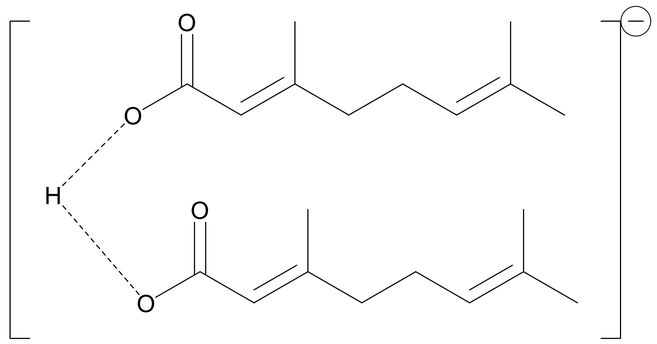
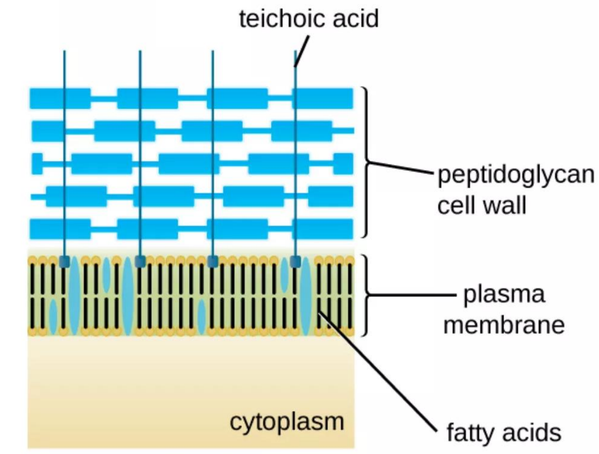


Current Research--First Steps

- In order to synthesize the choline derivative cation the alcohol of desired alkyl chain length is reacted with trimethylsilylchloride and paraformaldehyde in dichlormethane to give a chloromethylether[8]
- The prodcudt is then treated with dimethyl amino ethanol(DMAE) in hexanes to give the choline chloride derivative[9]



Structure of gram positive bacterial walls[4]

Previous research

- Zakrewsky and coworkers discovered that choline geranate(1) and other deep eutectic solvents (DES)are effective in the treatment of biofilms, especially the destruction of persistor cells[3].
- A previous researcher at Rider University, AJ Jemas, noted that they had only tested each DES against gram-negative bacteria, and not gram-positive bacteria. He then synthesized choline greanate and discovered that it was not effective against gram-positive bacteria, specifically *Staphylococcus Aureus* via a disk assay[5]
- AJ then synthesized a number of derivatives centered around the choline cation of choline geranate, but due to difficulties in characterization was unable to confirm their structures[5]
- His work was picked up by Samantha Ottavi who resynthesized all of his derivatives and conducted minimum inhibitory concentration(MIC) testing, who discovered that the original ethanol R_1 with the longer alkyl chain R_2 were more effective[7]
- Despite these results, characterization still resulted in some inconsistencies over whether the synthesized compoundes were actually deep eutectic solvents or not
- This characterization was difficult because of the nature of deep eutectic solvents.
- Deep eutectic solvents are a class of ionic liquid that is not only discrete ions. Instead, they typically consist of an anion, a cation, and a hydrogen bond donor forming a liquid material.[10]
- In the case of choline geranate specifically, characterization becomes especially difficult because the traditional organic mediums of NMR and IR do not easily show the loss of a single carboxylic acid proton and standard hydrogen and carbon NMR's cannot show the presence or absence of a chloride ion

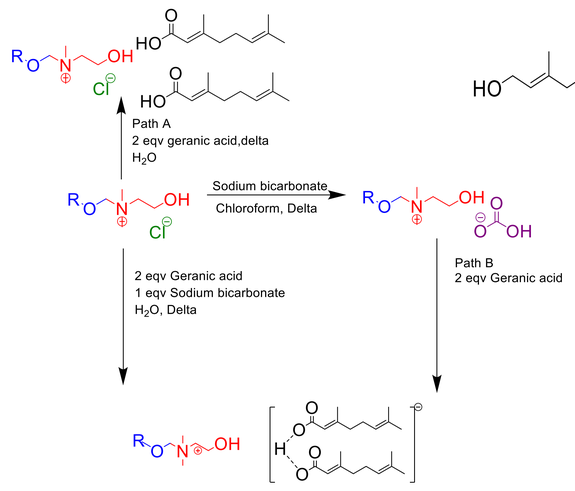
MIC testing Data of previous researchers[7]

Pseudomonas aeruginosa, gram-negative

	Side chain group			
Alkyl length	DMAE	DMAP	Acetic Acid Ester	Benzoyl Ester
14-carbon	>10 mg/mL	>10 mg/mL	>10 mg/mL	10 mg/mL
12-carbon	5-7 mg/mL	>10 mg/mL	10 mg/mL	10 mg/mL
10-carbon	5-10 mg/mL	8-10 mg/mL	3.5-5 mg/mL	5-10 mg/mL
8-carbon	5-10 mg/mL	7-8 mg/mL	> 10 mg/mL	3.5-5 mg/mL

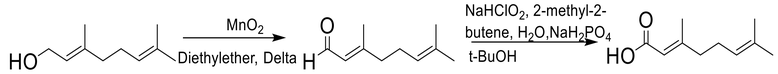
Staphylococcus aureus, gram-positive

	Side chain group			
Alkyl length	DMAE	DMAP	Acetic Acid Ester	Benzoyl Ester
14-carbon	**0.75-1 µg/mL**	3.5-5 mg/mL	3.5-5 mg/mL	1-5 mg/mL
12-carbon	**1-5 µg/mL**	3.5-5 mg/mL	3.5-5 mg/mL	3.5-5 mg/mL
10-carbon	2.5-3.5 mg/mL	3.5-5 mg/mL	2.5-3.5 mg/mL	1-5 mg/mL
8-carbon	3.5-5 mg/mL	3.5-5 mg/mL	5-10 mg/mL	3.5-5 mg/mL



Current Research—continued steps

- The synthesis of the actual deep eutectic solvent have been proposed
- In path a, one equivalent of the choline chloride derivative and two equivalents of geranic acid were mixed in a minimal amount of water with heating
  - This was the original path followed by Samantha and AJ, but we currently believe that it was not effective, as current resynthesis via this route produces what appears to only be a mixture of the choline chloride and geranic acid
- In path b the choline chloride derivative is first mixed with sodium bicarbonate in chloroform under reflux conditions[11], and the product can then be mixed with two equivalents of geranic acid to produce the deep eutectic solvent[3].
- In path c, the choline chloride derivative is added to two equivalents geranic acid and one equivalent sodium bicarbonate to yield the deep eutectic solvent
- Unfortunately, neither path b or path c have been explored due to issues with obtaining the necessary pure geranic acid



The Difficulties of Geranic acid

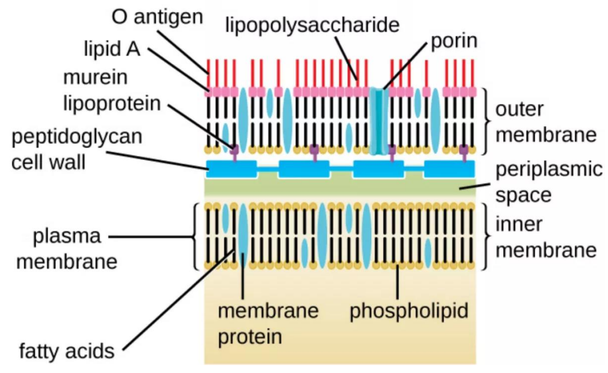
- Geranic acid is available commercially, but it is only available at an 85% purity and the other molecules in the container are not clearly identified.
- We first attempted to recrystallize from acetone at -780 C as did Zakrewsky and collegues,[3] but It resulted in a severe reduction in amount of material, and the material that was retrieved was still not pure.
- We then attempted column chromatography(Et/hex) and this did purify the geranic acid, but a large amount of it coeluted with the impurities
- Due to the wasteful and impractical nature of the purification processes we decided to attempt to synthesize geranic acid from its alcohol, geraniol.
- This product will be obtained via the oxidation of the alcohol with MnO2 to the aldehyde in diethyl ether under reflux conditions, which will then be further oxidized to the carboxylic acid with sodium perchlorate, 2-methyl-2-butene, water and sodium biphosphate in tertbutanol.[12]

Next steps

- We will continue synthesis of geranic acid, as we have not completed synthesis due to dificullties in adapting this synthesis to our lab and goals
- Once the geranic acid has been synthesized we will follow paths b and c in an attempt to synthesize the desired deep eutectic solvent
- The products of these reactions will be characterized via a variety of tests, includingbut not limited to IR and NMR, as well as conductivity and density testing. These can be compared to the starting materials, the products of path a, and the values for choline geranate as previously reported.

Gram positive vs gram Negative Bacteria

- Bacteria can be split into the broad categories of gram-positive and gram-negative[4]
- These groups were first delineated by Hans Christian Gram and positive and negative referred to their reaction to certain stains[4]
- Gram-positive bacteria are stained in their thicker pepditoglycan cell walls[4]
- Gram-negative bacteria have thinner cell walls that are not stained, as well as a cell membrane on both sides of the wall[4]



Structure of gram negative bacterial walls[4]

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