Compounds found in Baltic amber and their potential medicinal uses-a URS project

Lauren Warmka

Ambrose Lab, Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55414

Abstract: Amber, the fossilized resin from the extinct family of pine tree, *Sciadopityaceae*, is used extensively in medicinal folklore. It is reported to have analgesic, anti-infective, antifungal, wound healing, anti-inflammatory, and anticancer properties. Amber, and specifically Baltic amber, has medicinal potential, yet a comprehensive analysis of the compounds in Baltic amber and their bioactive properties has yet to be done. The goal of this project is to separate the potentially bioactive small molecules in the amber and test them against different assays. This project is still in progress, but the completed research has produced a class of compounds, abietic acid derivatives, with medicinal potential.

1. Introduction

Amber is the fossilized resin of tree sap from the extinct family of pine trees *Sciadopityaceae* of the Eocene era 44 million years ago.[1-4] Amber is mentioned commonly in medicinal folklore for the treatment of many different ailments and said to have analgesic, anti-infective, anti-fungal, wound healing, anti-inflammatory, and anti-cancer properties.[5] This project focuses on amber from the Baltic region, known as class Ia amber, because it is the only class of amber known to contain succinic acid and its different derivatives.[2,6]

The succinic acid derivatives in Baltic amber have been researched, yet a complete study of all of the compounds in Baltic amber has not been done. This project focuses on the non-succinic acid containing compounds that can be found in Baltic amber and their medicinal properties. This project is still in progress, and specific results may not be published yet. This URS project is a snapshot of the larger Baltic Amber project in progress at the Ambrose lab, and does not include the work done on this project before or after the timeline of the URS project. In short, this is a small piece of a larger project.

2. Materials and Methods

Extractions:

Before this URS project was started, four different extraction processes were optimized using 2 different solvent systems. The two solvents used for the extractions are dichloromethane (DCM) and ethanol (EtOH). EtOH was used for all 4 extraction methods, but DCM was only used for 3 of the extraction methods.

The four extraction methods used were a conservative extraction, a soxhlet extraction, a sonication extraction, and a decoction extraction. The decoction extraction was only done with EtOH as the solvent. This gave 7 different extracts for analysis.

TLC optimization

A TLC solvent system was optimized for TLC analysis of the compounds. The TLC plates were analyzed with UV light, and with a *p*-anisaldehyde stain. The different solvent systems tested in the optimization are summarized in the following table.

Summary of TLC Solvent Systems

Solvent System	EtOAc/ hexanes	MeOH/DCM	EtOAc-MeOH(10% MeOH)/hexanes	Hexane/benzene/ MeOH
Percentage of 1st listed solvent	10%	0%	20%	2 parts/6 parts/1 part
	20%	10%	30%	-
	30%	20%	40%	-
	40%	30%	50%	-
	50%	40%	60%	-
	60%	50%	-	-
	70%	60%	-	-
	80%	70%	-	-
	90%	-	-	-

Table 1: This table outlines the 4 solvent systems that were tested through TLC,and the percentages of each component of the solvent system where the percentage listed is that of the first listed component. The EtOAc-MeOH/Hexanes solvent system was a 10% MeOH 90% EtOAc solvent that was varied with hexanes where the total percentages of each solvent in the system for the 20% solvent system would be (.2*.1)= 2% MeOH, (.2*.9)= 18% EtOAc, and 80% hexanes. Similarily, the hexane/benzene/MeOH solvent system was not varied, but rather combined in a 2:6:1 ratio, respectively, or 22.2% hexane, 66.7% benzene, and 11.1% MeOH.

GC-MS

GC-MS analysis was done with an Agilent QTOF instrument. The optimization of the GC-MS conditions was completed by the graduate student working on this project. All 7 of the extracts plus 8 standard compounds were run through the GC-MS. This instrument is connected with a database to aide in identifying the compounds from their corresponding peaks. The 7 extracts were run through the GC-MS once, and then concentrated and run through the GC-MS again. The 8 standard compounds tested were:

- 1-methylnapthalene
- 2,6-dimethylnapthalene
- Acenaphthene
- Fluorene
- Phenanthrene
- Retene
- Anthracene
- 2-methylanthracene
- 9-methylanthracene

3. Results

It was hoped that use of a TLC-MS instrument could be integrated into the analysis of the different extracts, for this reason, the TLC conditions were optimized. When analysis of the extracts through TLC-MS was attempted, inconclusive, and useless results were obtained and will not be included as there is no discernable meaning to these results.

Analysis through GC-MS provided much more conclusive results. Because this URS project is just a snapshot of the larger Baltic Amber project, the specifics of these results may not be published at this point. Analysis through GC-MS gave a comprehensive view into the compounds contained in Baltic amber. One class of compounds found in the extracts, abietic acid derivatives, is of particular interest. The mere presence of abietic acid derivatives is the only result of the GC-MS analysis that may be published as of right now.

4. Discussion

No meaningful results were obtained through use of TLC-MS for analysis. This was likely due to the low concentrations of each compound in the extracts. Because there are a large number of compounds found in each extract, the concentrations of each individual compound in the extract are very low. This leads to the conclusion, that analysis by TLC-MS is not helpful for this application.

The presence of abietic acid derivatives in the Baltic amber extracts is promising. Several studies have been done on abietic acids as a class of compounds. Abietic acids are reported to have many different medicinal properties. According to some of these reports, certain abietic acids have proved to have anti-inflammatory, potential therapeutic effects in the treatment of asthma, potential anti-obesity effects, wound-healing effects, antibacterial, and anticancer effects.[7-10] Abietic acids have a lot of therapeutic potential, and their presence in Baltic amber is promising for the therapeutic potential of Baltic amber. Future steps for this project include isolation of the abietic acids from the extracts and testing with assays for medicinal properties.

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