Isolation of Antibacterial Compounds from *Artemisia californica*

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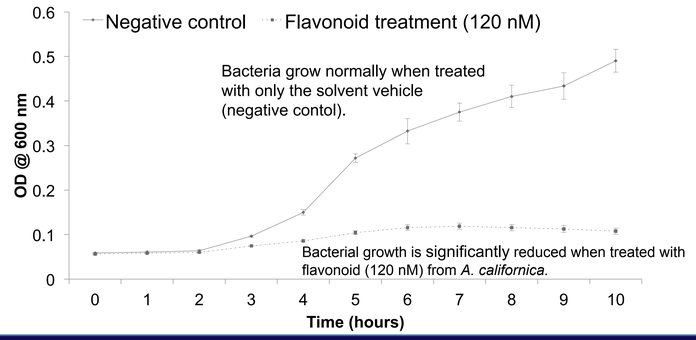
Abstract

The Chumash Native Americans of Southern California have well-documented traditions of using plants for medicinal purposes. If a specific plant has traditionally been used by Chumash for the treatment of cuts, wounds and infections, it may contain chemicals with anti-bacterial properties. One plant that fits these criteria is *Artemisia californica* (coastal sage). Because of the widespread use of antibiotics over the past sixty years bacteria are evolving greater resistance to known antibiotics, but unfortunately the rate of antibiotic discovery has diminished during the past twenty years. Therefore, novel and effective antibiotics are essential for the continued treatment of bacterial infections. A target-specific anti-bacterial assay was used to identify compounds from *A. californica* that inhibited bacterial growth by inhibiting the FabI enzyme. Compounds which demonstrate decreased potency against a bacterial strain over-expressing FabI compared to a control strain have been isolated and characterized. The decreased activity in the over-expressing FabI strain suggests that the mode of action of this flavonoid is FabI inhibition.

Introduction

For thousands of years, natural products have been used as medicinal purposes. Therefore they are great candidates for potential source of new drugs.[1] *Artemisia californica* has been used for medicinal purposes such as treatment of cuts, wounds, infection and coughs by the Chumash Native Americans.[2] Previous work in our lab has shown that a flavonoid compound from costal sage extract has antibacterial activity. Unfortunately, not enough of the compound has been isolated to identify its chemical structure and properties. The objective of this project is to isolate the flavonoid compound from A. *californica* so that its structure and biological activity can be fully characterized.



Results

Flow Chart of Fractionation

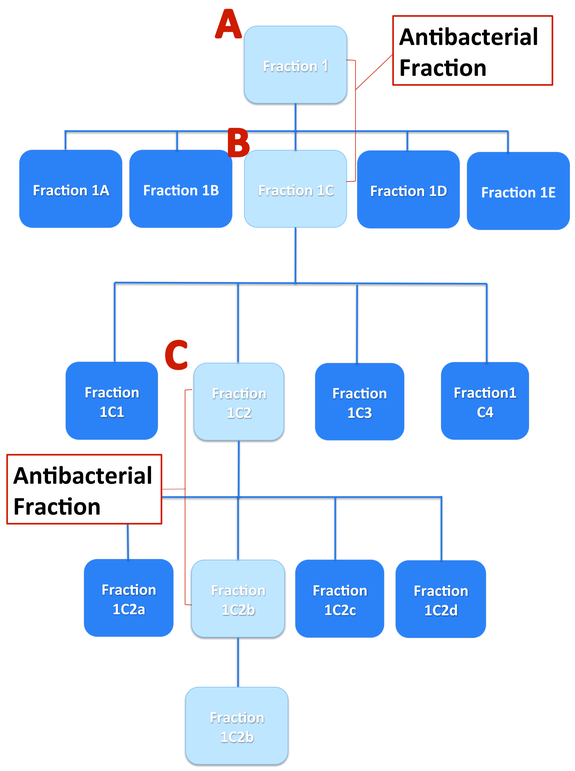


Figure 1. The presence of the compound of interest in this project was confirmed in Fraction 1 using LCMS analysis. A series of chromatographic separations were then performed which generated the fractions shown above. Fractions containing the compound of interest with antibacterial activity (light blue) were selected at each stage for further fractionation. Each fractionation was performed using HPLC with a C18 column as described in the experimental section.

Chromatograms of Active Fractions

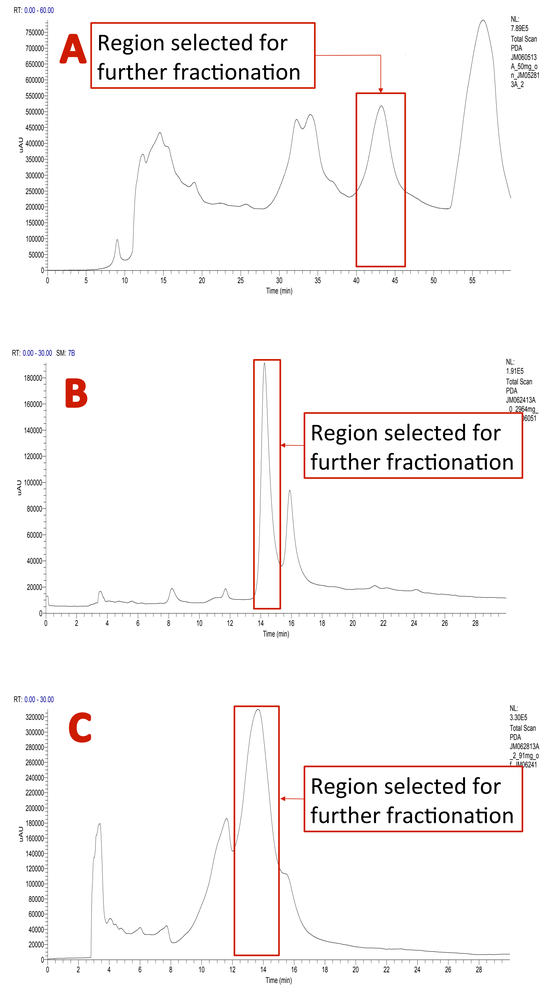


Figure 2. The chromatograms corresponding to each of the fractions with antibacterial activity from Figure 1. A red box is shown around the region selected for further fractionation in each chromatogram.

Mass Spectrum of Boxed Region in Each Chromatogram

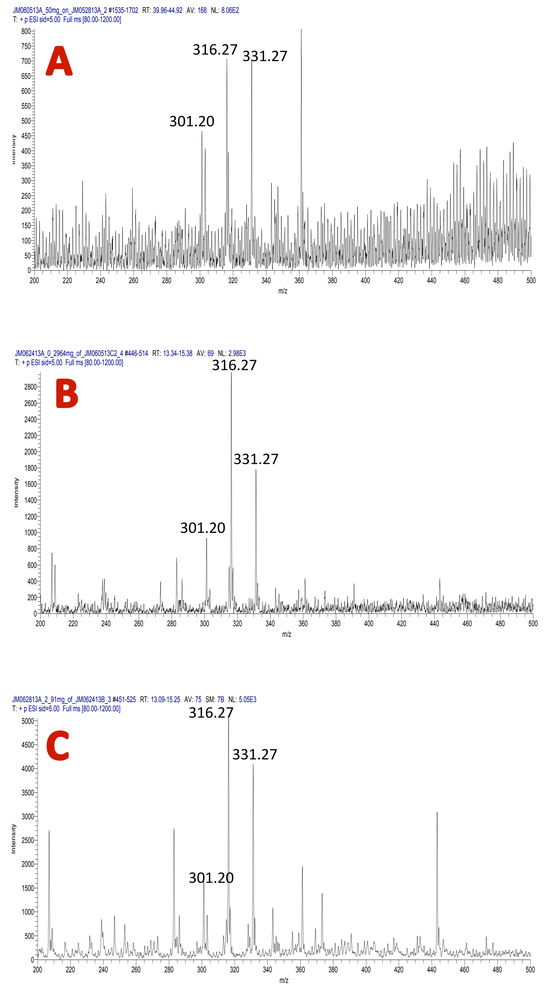


Figure 3. The mass spectrum of the boxed region in each of the chromatograms shown in Figure 2. Each spectrum contains peaks with *m/z* values of 301, 316 and 331, which correspond to compound of interest.

Experimental Methods

The initial *A. californica* sample used in this project (Fraction 1) was taken from the results of a previous project in our lab. Chromatographic separations were performed using a reverse phase HPLC system using a C_{18} column with a methanol-water gradient as the mobile phase. Fraction 1 was fractionated using a preparative scale column with a 55%-100% MeOH gradient over 60 minutes and the eluent was collected into separate vials using a fraction collector. The collected eluent was separated into five fractions and the solvent was removed by using a rotary evaporator. The third fraction (1C) contained the compound of interest and was further separated using a semipreparative scale with a 60%-66% MeOH gradient over 30 minutes. The eluent was again collected using a fraction collector and separated into four fractions and the solvent was removed using a rotary evaporator. The third fraction (1C2) was found to contain the compound of interest and was further fractionated using a semipreparative scale column with a 60%-64% MeOH gradient over 30 minutes.

Conclusions

- The peaks in the mass spectra with *m/z* values of 301, 316 and 331 indicate that the compound of interest was isolated from the parent fractions in each fractionation step.
- Not enough of the compound of interest was purified to allow for complete structural characterization of the compound using NMR.

Future Directions

- Future efforts will attempt isolate a greater amount of the compound of interest so that its structure can be fully determined.
- Once the structure of the antibacterial compound is characterized, further work will seek to more fully characterize its biological properties.

References

(1) Butler, M. S. *Journal of Natural Products* 2004, *67*, 2141-2153.

(2) Timbrook, J. *Chumash Ethnobotany: Plant Knowledge Among the Chumash People of Southern California*; Heyday Books, 2007.

(3) Dale, N. *Flowering Plants: The Santa Monica Mountains, Coastal and Chaparral Regions of Southern California*; 1st ed.; Capra Press, 1986.

Acknowledgements

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