Extraction of Natural Products from the Brown Alga *Dictyota*

Ashley Nicoll, Megan Rooney, Dr. Evgenia Glukhov, and Dr. William Gerwick

Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, United States

ABSTRACT

Biologically active secondary metabolites have become a new focus in the search for natural product drugs. These metabolites can be isolated from a number of different marine and terrestrial resources. The Gerwick lab works on analyzing marine entities for biologically active secondary metabolites. Many projects begin with algae that have been collected from different parts of the world. In this study, the alga *Dictyota* was collected from American Samoa on July, 16, 2014. *Dictyota* is a genus of brown algae; the main compounds isolated from *Dictyota* in the past have been diterpenes and other non-polar compounds. Recently, several new diterpenes have been isolated from different species of *Dictyota* (Ghada S. E. Abou-El-Wafa et al., 2013).

The process of finding out what compounds could be in the sample of *Dictyota* began with using DCM:MeOH solution to extract the biomass. Next a VLC (vacuum liquid chromatography) column was used to separate the extract into fractions based on polarity. The solvents used were hexanes, ethyl acetate, and methanol in varying proportions. A small portion of each fraction was then run through the LCMS (liquid chromatography mass spectrometry). Next, the data from the LCMS was used in GNPS (Global Natural Product Social) to perform molecular networking. Molecular networking shows what compounds could be in the sample and which compounds are similar to one another. Molecular networking coupled with raw data from LCMS can be used to predict which compounds could be in a sample. If a new compound is found, more tests can be done to further elucidate its structure and test its properties.

EXTRACTING ALGAE

1. A Buchner funnel is used to filter the algae, wrapped in a cheese clothe, after soaking it in DCM:MeOH solution for 45 minutes.

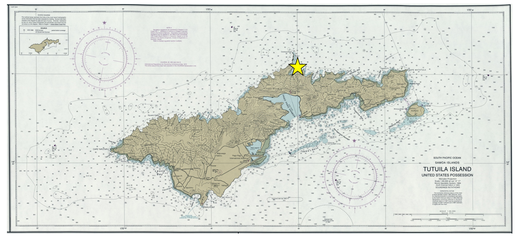
2. A vacuum is applied to the vacuum flask so that the liquid will run through the funnel and into the flask.

3. The extract that has run through the funnel is collected in the flask. Next, it will be put on a RotoVap to evaporate off the DCM and MeOH and just leave the extract from the algae.

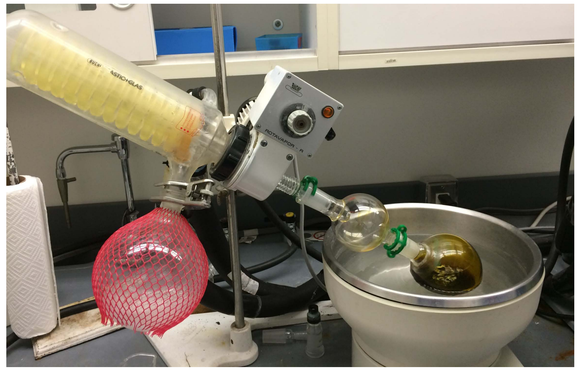
The extraction process was repeated 4 times, until the extract color was very light.

Dictyota

American Samoa



THE ROTOVAP



The RotoVap evaporates off volatile organic solvents from solutions to leave just the organic compounds.

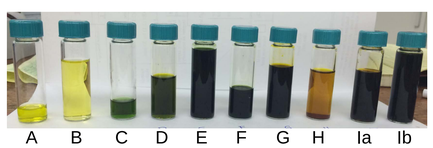
VACUUM LIQUID CHROMATOGRAPHY

The column is made of tightly packed silica so that there are no air bubbles in the column.

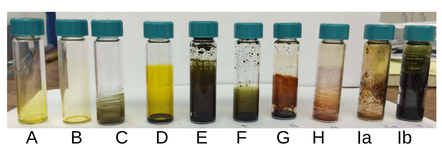
The sample is dissolved in the first solvent, the least polar, and added to the top. Vacuum is then applied and the non-polar parts of the sample move through the column to be collected in the bottom flask as fraction A.

As different solvents are added, the more polar compounds begin to move down the column in bands.

At the end, methanol is run through the column until there are no bands left in the column.

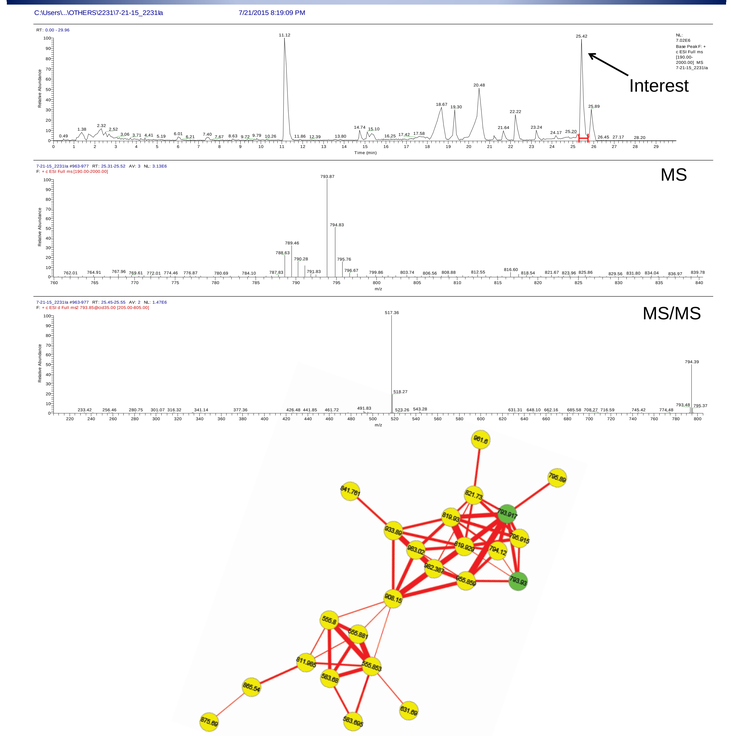


A B C D E F G H Ia Ib



A B C D E F G H Ia Ib

LCMS PROFILE AND MOLECULAR NETWORKING



Above: A picture of the MS and MS/MS data for a peak occurring at 25.42 minutes.
Below: A picture of the cluster where the compound that the peak represents is found. The compound appears in green.

CONCLUSION

From the sample of *Dictyota*, 10 fractions were collected. The fractions contained many different unknown compounds that could be seen using TLC (thin layer chromatography) and LCMS. The unknown compounds in the fractions could be further elucidated and investigated in the future.

ACKNOWLEDGEMENTS

Thank you to Nathan Moss and Bailey Miller for collecting the *Dictyota* sample used and to everyone in the lab for their teaching.

REFERENCES

Abou-El-Wafa, Ghada S. E., Mohamed Shaaban, Khaled A. Shaaban, Mohamed E. E. El-Naggar, Armin Maier, Heinz H. Fiebig, and Hartmut Laatsch. "Pachydictyols B and C: New Diterpenes from *Dictyota dichotoma* Hudson." *Marine Drugs*. MDPI, 22 Aug. 2013.