



Discovery and Characterization of New Cytotoxic Cyanobacterial Natural Products

Elise S. Cowley

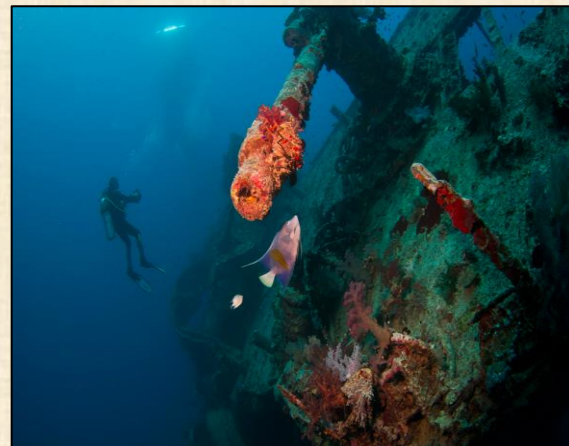
Mentors: Kerry McPhail, PhD, Phil Proteau, PhD,
and Christopher C. Thornburg, PhD

Undergraduate Honors and Bioresource Research
Thesis Seminar Presentation

May 28, 2013

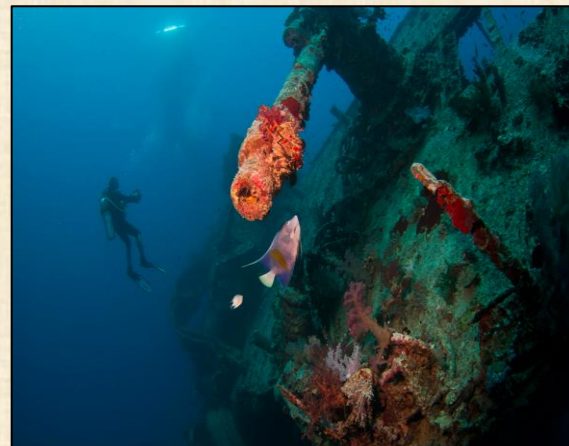
Roadmap

- Background and Relevance of Natural Products
- Sample Collection by McPhail Lab
- Goal of Project
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- Results
 - Known Compounds
 - New Compounds
 - Hydrophobic Fraction
- Discussion
 - Relevance of Results
- Limitations
- Future Work
- Acknowledgements
- Questions



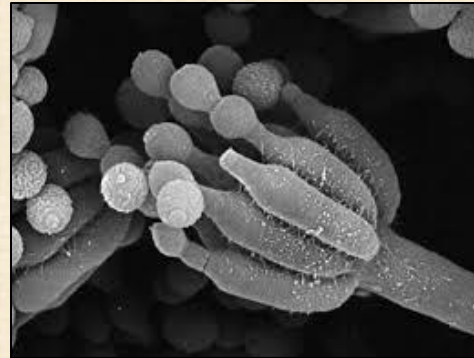
Roadmap

- **Background and Relevance of Natural Products**
- Sample Collection by McPhail Lab
- Goal of Project
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- Results
 - Known Compounds
 - New Compounds
 - Hydrophobic Fraction
- Discussion
 - Relevance of Results
- Limitations
- Future Work
- Acknowledgements
- Questions



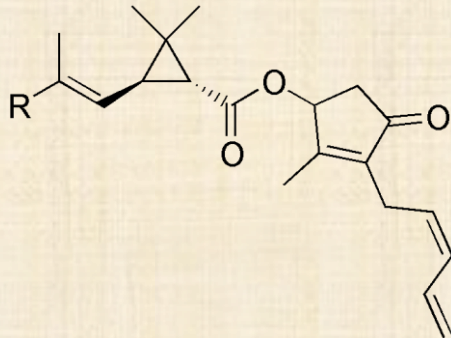
What are Natural Products?

- Secondary Metabolites
- What produces secondary metabolites?
- Why produce secondary metabolites?



Why are Natural Products Important?

Pharmaceutical Applications



www.sciencedirect.co

Agricultural Applications



www.kohna.ucla.edu

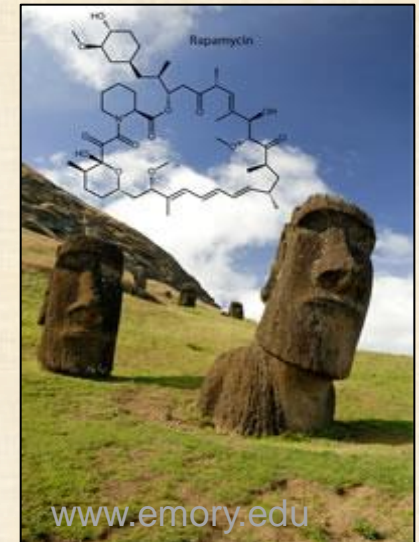


PACIFIC YEW
TAXUS BREVIOLIA

www.elucidationimages.com



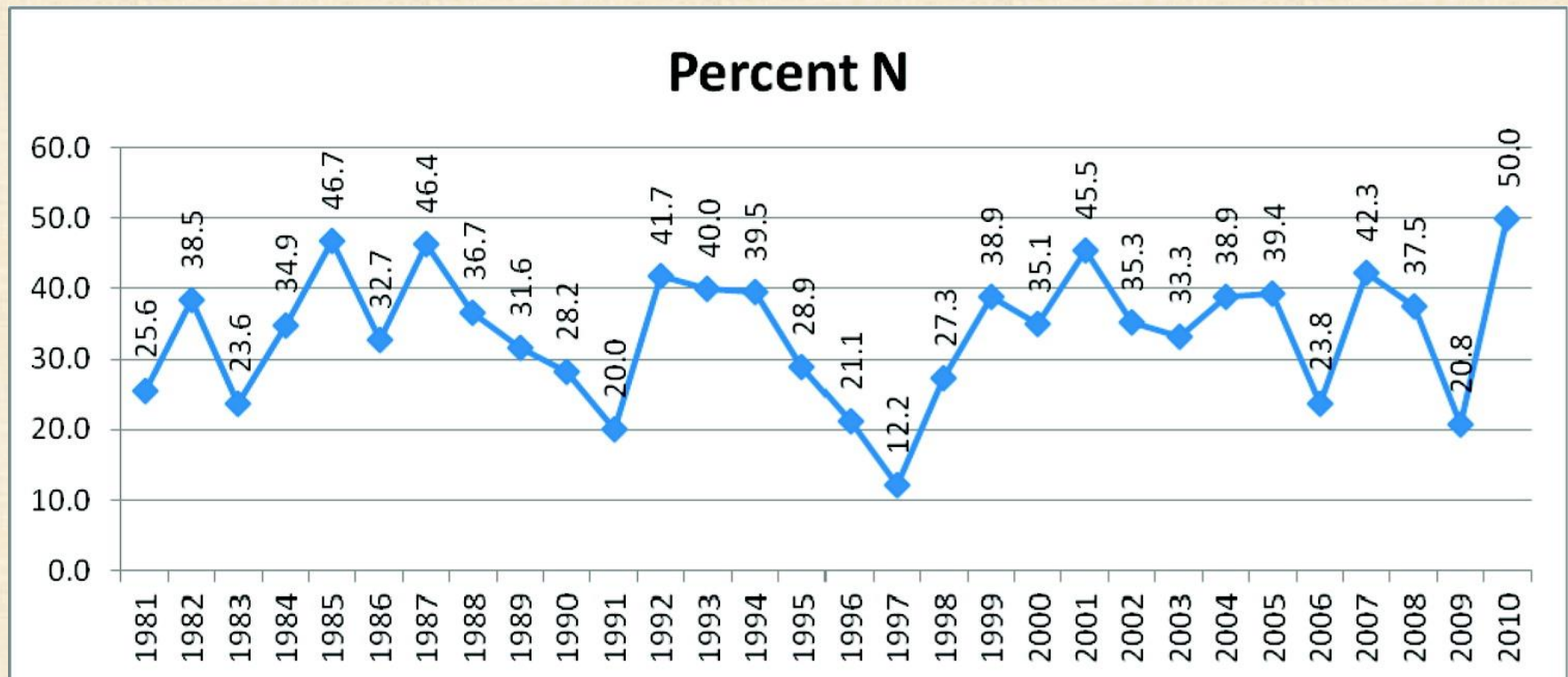
www.lipitor.com



www.emory.edu

Why Search for More Natural Products?

- Pharmaceutical Importance: (Newman, Cragg, 2012)



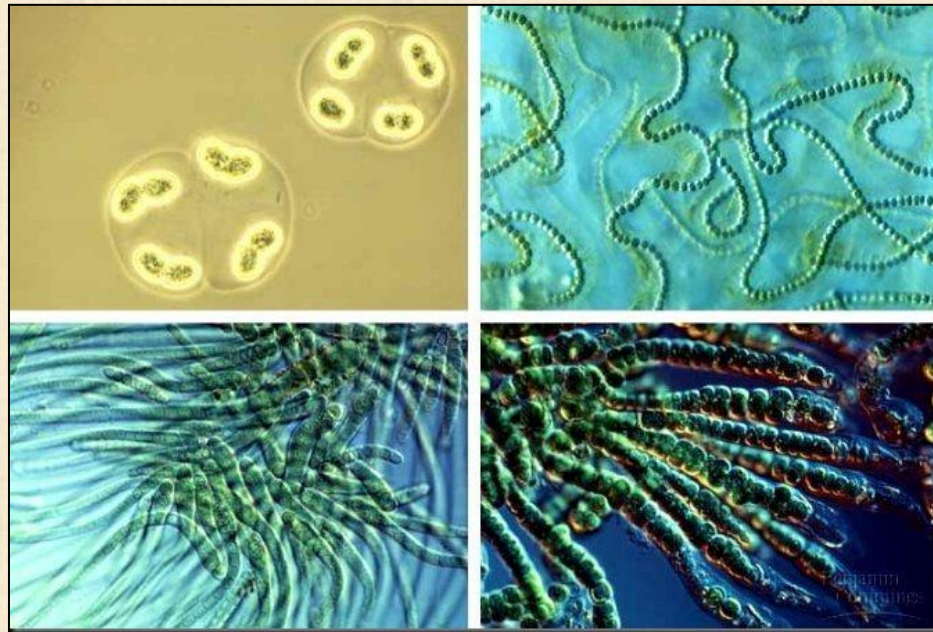
Why Search for More Natural Products?

- Antibiotic Resistance (Silver, Bostian, 1993)
- Anticancer Resistance (Simmons, et al, 2005)
- Constant need for new compounds (Strobel, Daisy, 2003)
- Exotic Environments (Baker, et al, 2007)



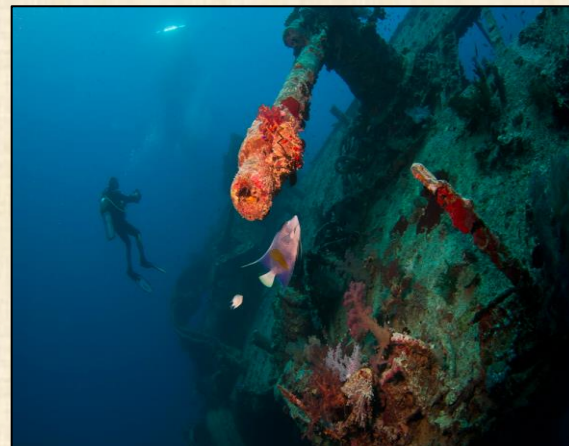
Cyanobacteria as Sources of Natural Products

- Ancient Phylum
- Ubiquitous
- Classified alongside Myxobacteria and *Streptomyces* as sources of pharmaceuticals (Tan, 2010)



Roadmap

- Background and Relevance of Natural Products
- **Sample Collection by McPhail Lab**
- **Goal of Project**
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- Results
 - Known Compounds
 - New Compounds
 - Hydrophobic Fraction
- Discussion
 - Relevance of Results
- Limitations
- Future Work
- Acknowledgements
- Questions



Red Sea Exploratory Expedition



➤ OSU Collections

▪ May 2007

An underwater photograph showing a diver on the left, swimming towards a large, colorful coral reef structure. The water is clear and blue. The text 'Overall Goal: Isolate Novel Secondary Metabolites' is overlaid in large, yellow, outlined letters.

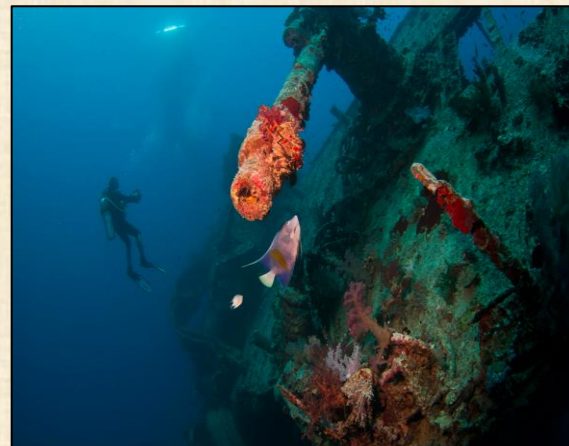
Overall Goal: Isolate
Novel Secondary
Metabolites



▪ Fine sediment deposition

Roadmap

- Background and Relevance of Natural Products
- Sample Collection by McPhail Lab
- Goal of Project
- **Methods**
 - **Culturing**
 - **Chromatography**
 - **Compound Hypothesis**
 - **Compound Characterization**
- Results
 - Known Compounds
 - New Compounds
 - Hydrophobic Fraction
- Discussion
 - Relevance of Results
- Limitations
- Future Work
- Acknowledgements
- Questions



Culturing Process-*Moorea* sp.

➤ Purification Process

- Brown contaminant
- Low light
- Overtook brown diatom

➤ Harvest

- 6 months
- Yield: 0.85 g



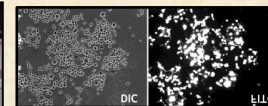
Large-scale cultures of *Moorea* sp. ¹²



Materials and Methods



Brine Shrimp Assay



Neuroblastoma Assay



➤ Extraction and Separation

➤ Further Separation to Pure Compounds

➤ Structure Elucidation

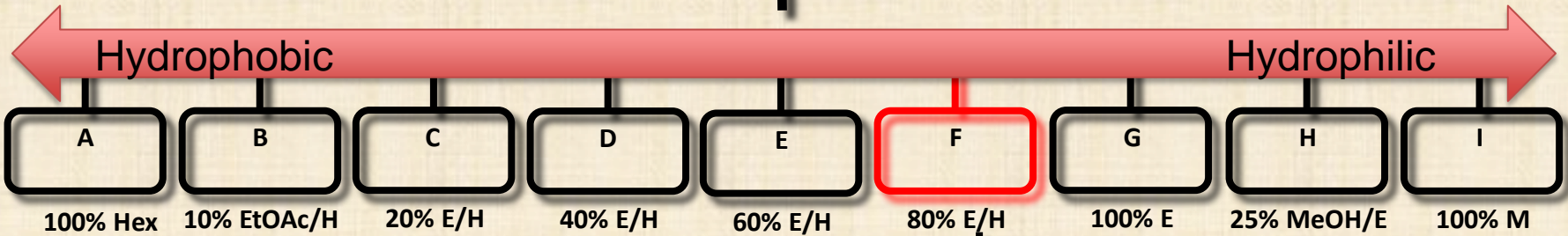
Chromatography Overview



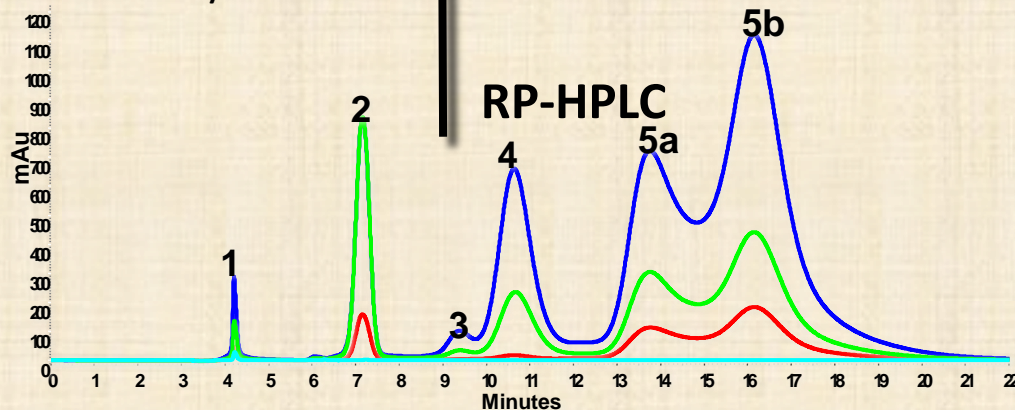
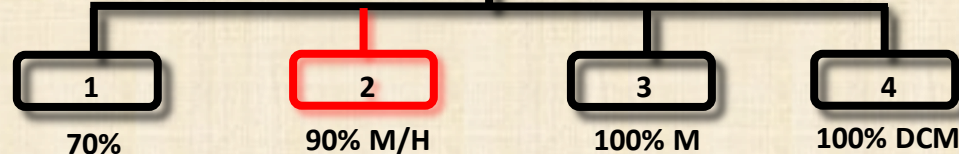
NP-VLC



Chemical Extraction

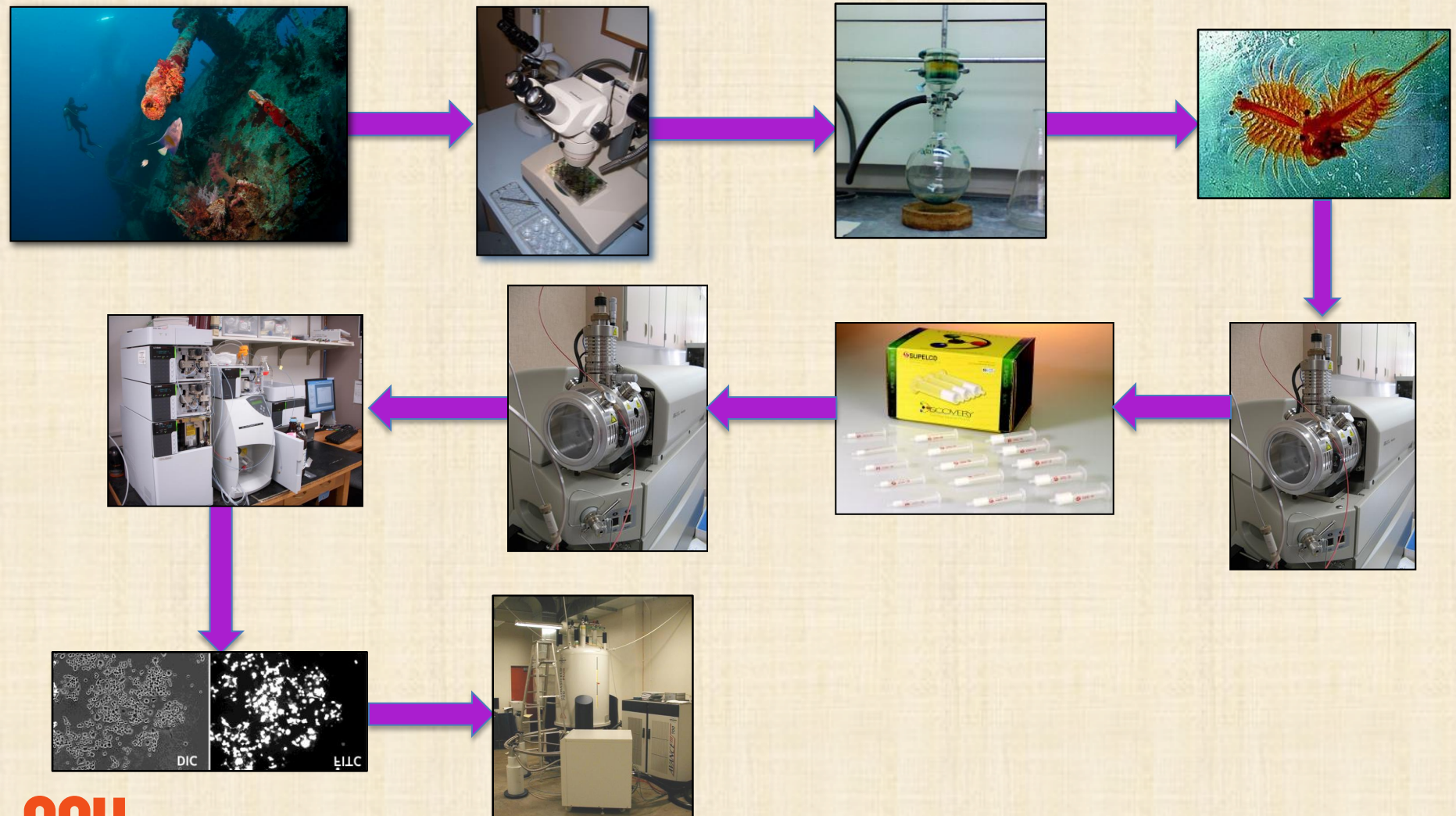


RP-SPE



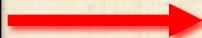
- **NP-VLC:** Normal Phase Vacuum Liquid Chromatography
- **RP-SPE:** Reversed Phase Solid Phase Extraction
- **RP-HPLC:** Reversed Phase High Performance Liquid Chromatography

Materials and Methods




Moorea species

Nabq Mangroves near Sharm el Sheikh, Egypt



- 50 liters collected over 12 months
- 0.85 g Organic Extract

 Active Fractions

NP-VLC

Hydrophobic

Hydrophilic

Fraction	Weight (mg)	Composition
A	37.2	100% Hex
B	62.6	10% EtAc/H
C	108.1	20% E/H
D	144.2	40% E/H
E	12.7	60% E/H
F	131.4	80% E/H
G	165.0	100% E
H	190.7	25% MeOH/E
I	88.6	100% M

Brine Shrimp Assay (BSA)
1mg/ml: 4 %

0% 100% 100% 89% 100% 100% 85% 0%

BSA
0.1mg/ml: 5 %

0% 96% 97% 7% 85% 94% 7% 0%

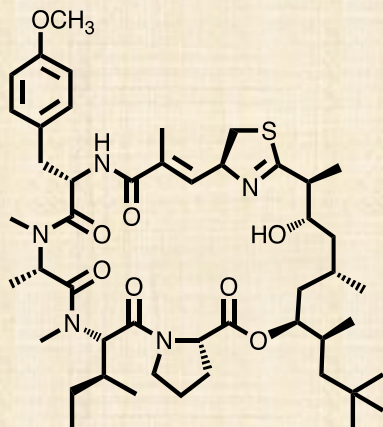
Mass Spectrometry
and RP-SPE



Moorea sp. Hypothesized Compounds

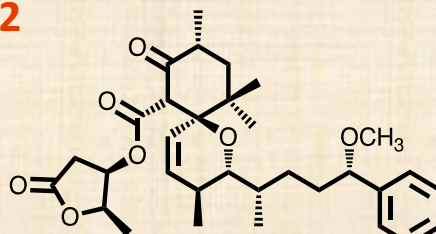
7 known compounds & 2 new compounds

1



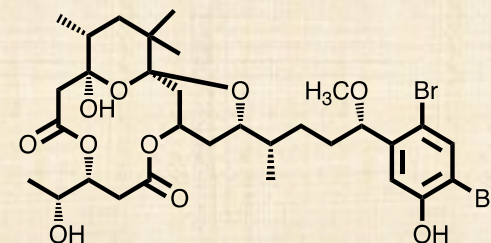
apratoxin D

2



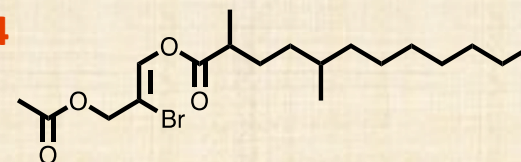
30-methyloscillatoxin

3



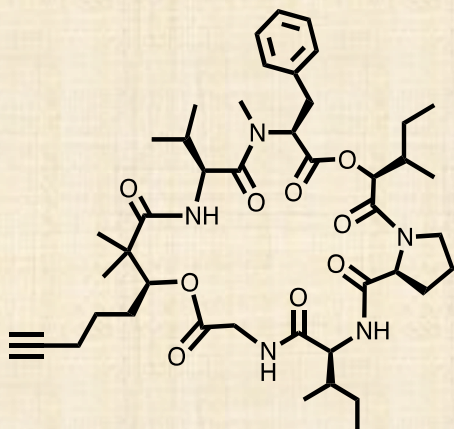
bromoaplysiatoxin

4



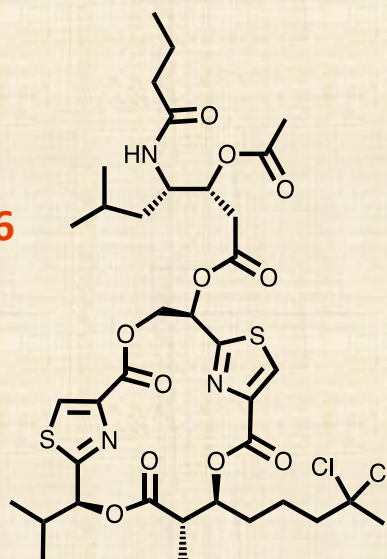
brominated lipid

5



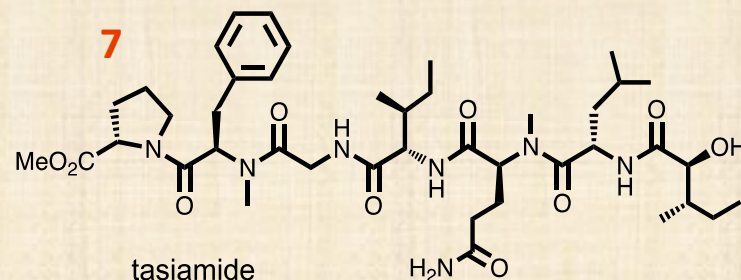
pitipeptolide

6



lyngbyabellin H

7



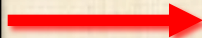
tasiamide

8 = Unknown: m/z 658


9 = Unknown: m/z 854

Moorea species

Nabq Mangroves near Sharm el Sheikh, Egypt



- 50 liters collected over 12 months
- 0.85 g Organic Extract

 Active Fractions

NP-VLC

Hydrophobic

Hydrophilic

	A	B	C	D	E	F	G	H	I
Weight	37.2 mg	62.6 mg	108.1 mg	144.2 mg	12.7 mg	131.4 mg	165.0 mg	190.7 mg	88.6 mg
Extraction	100% Hex	10% EtOAc/H	20% E/H	40% E/H	60% E/H	80% E/H	100% E	25% MeOH/E	100% M
BSA 1mg/ml: 4 %	0%	100%	100%	89%	100%	100%	85%	0%	
BSA 0.1mg/ml: 5 %	0%	96%	97%	7%	85%	94%	7%	0%	

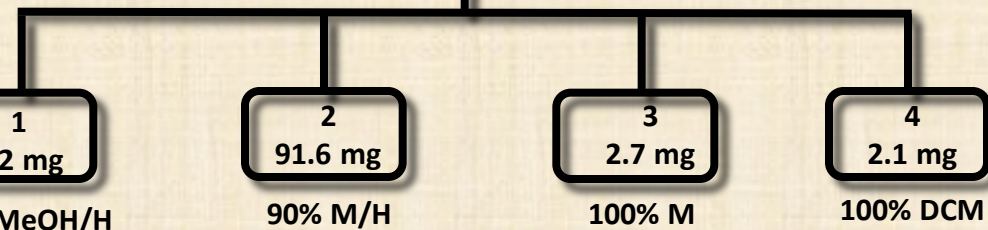


Mass Spectrometry
and Reversed Phase-
Solid Phase
Extraction (RP-SPE)

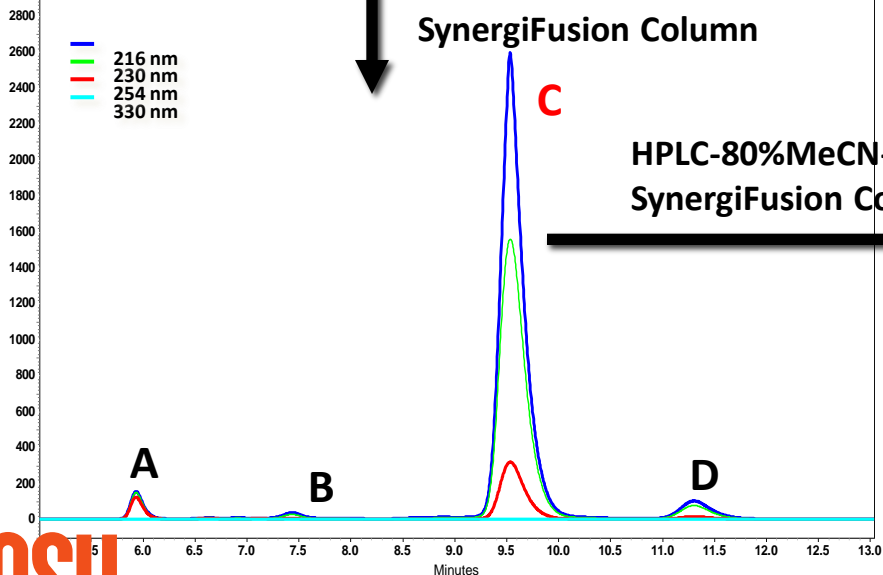
Moorea sp. HPLC Fraction F

F
131.4
mg

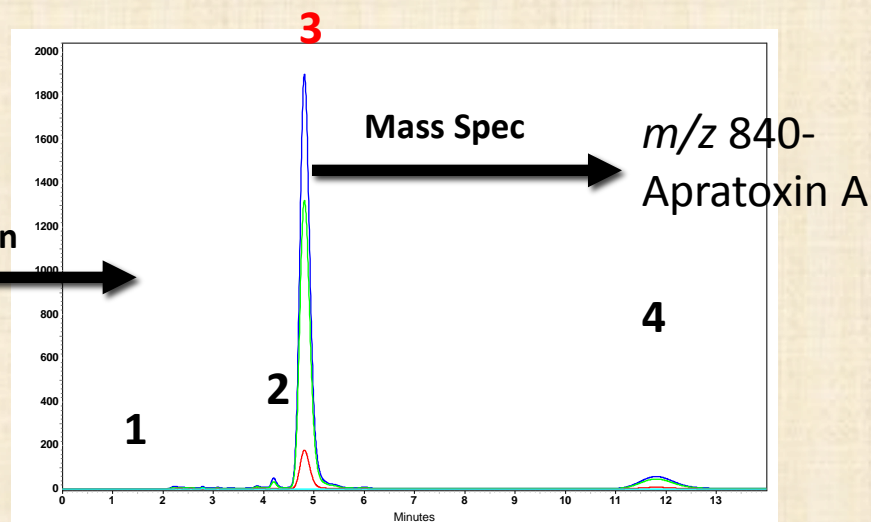
RP-SPE



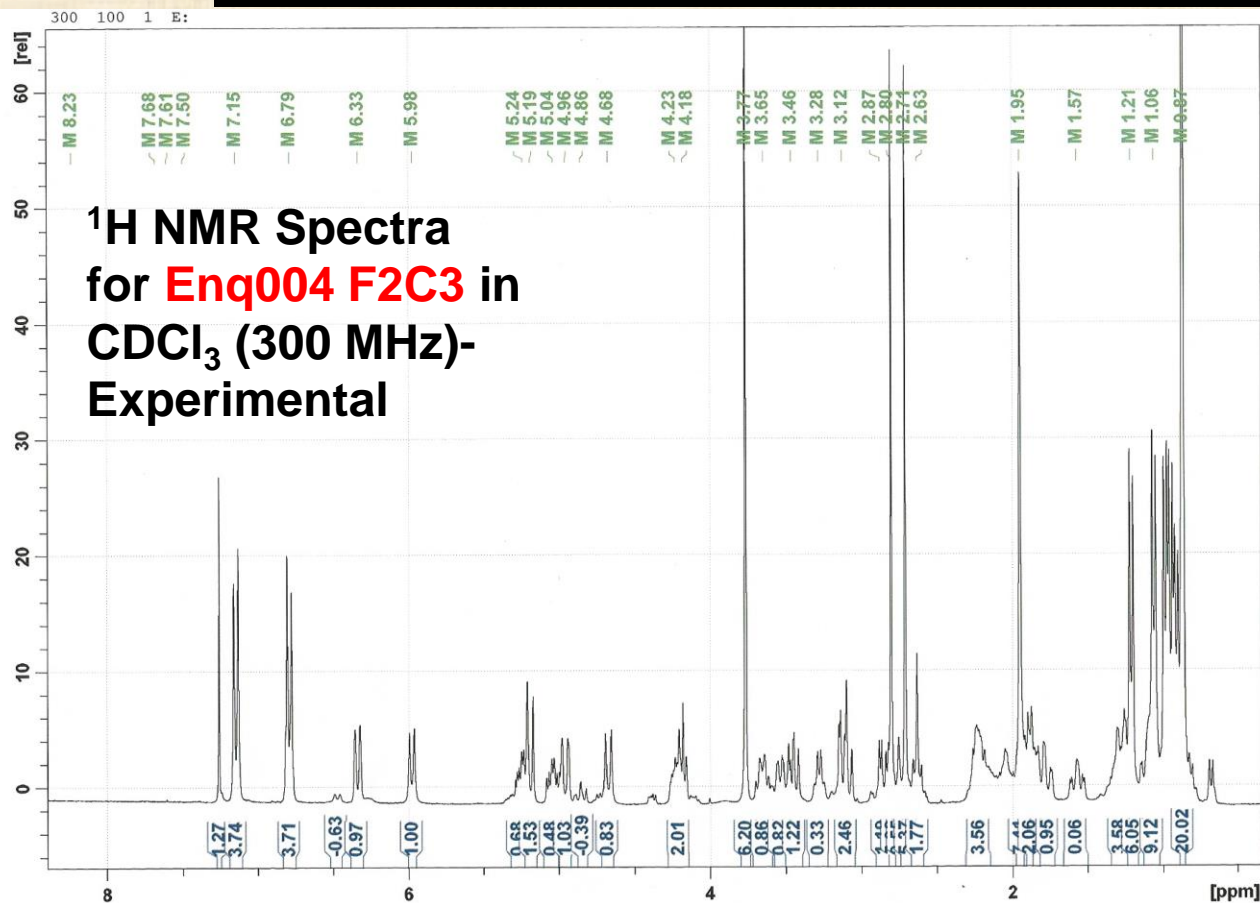
HPLC-90% MeOH-
SynergiFusion Column



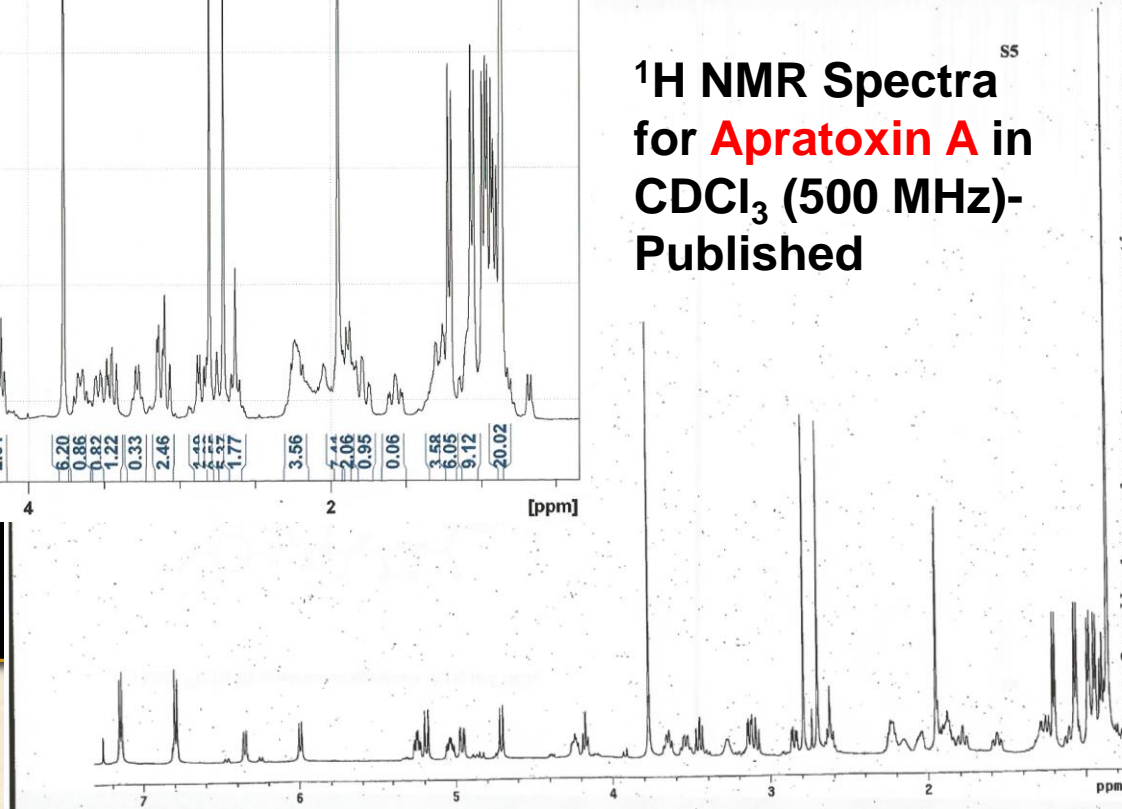
HPLC-80% MeCN-
SynergiFusion Column



Moorea sp. F2C3 Structure Elucidation

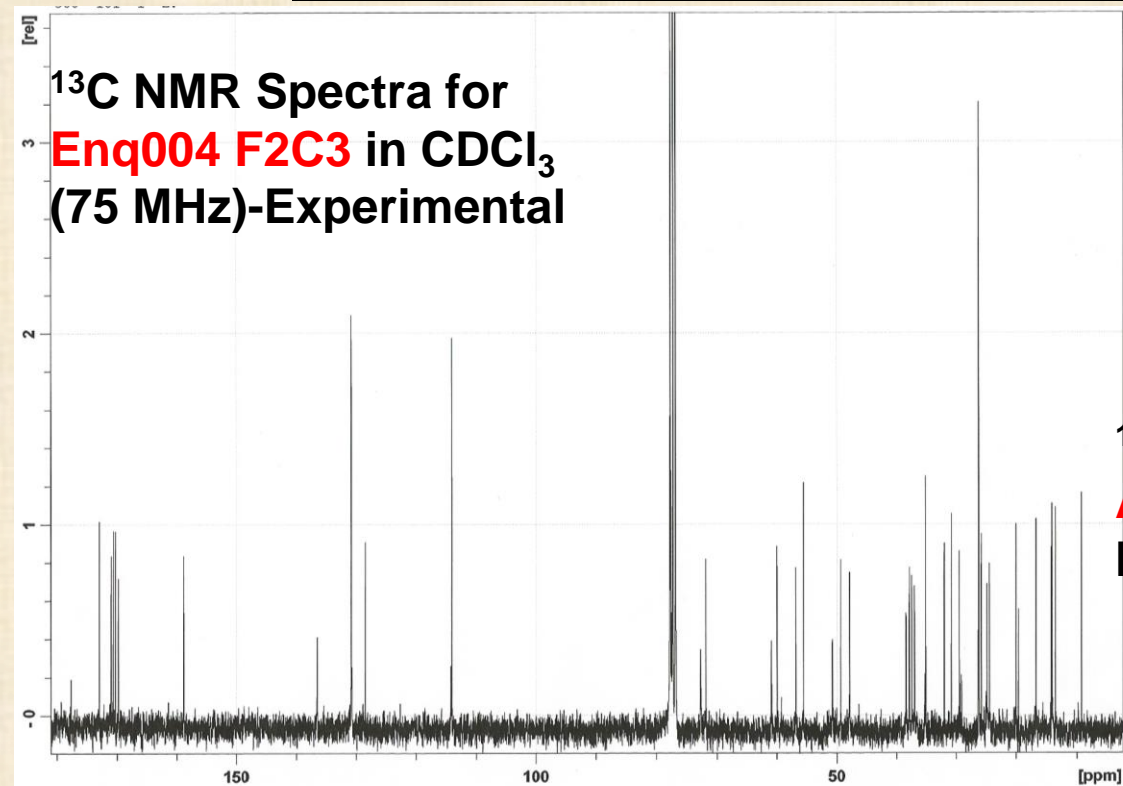


¹H NMR Spectra for Apratoxin A in CDCl₃ (500 MHz)-Published

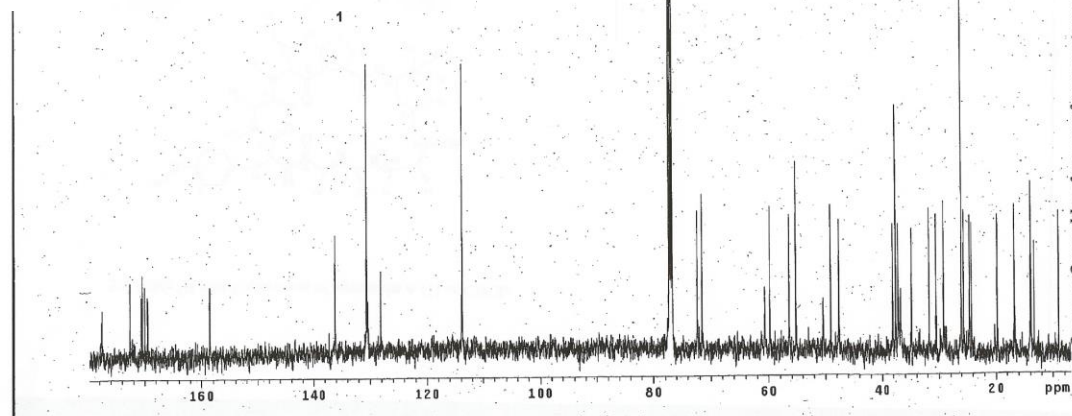


Moorea sp. F2C3 Structure Elucidation

¹³C NMR Spectra for
Enq004 F2C3 in CDCl₃
(75 MHz)-Experimental



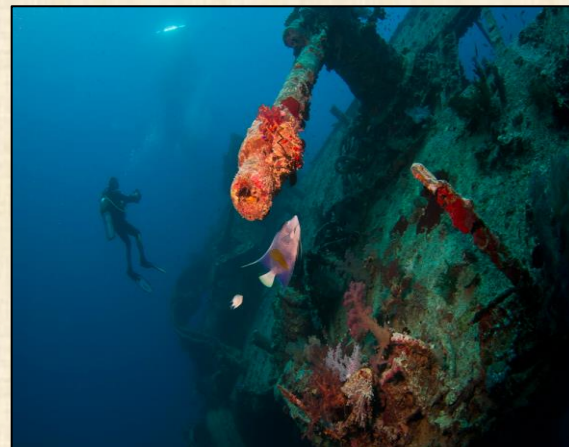
¹³C NMR Spectra for
Apratoxin A in CDCl₃ (75
MHz)-Published



© 2001 American Chemical Society, J. Am. Chem. Soc., Luersch ja010453j Supporting Info Page 6

Roadmap

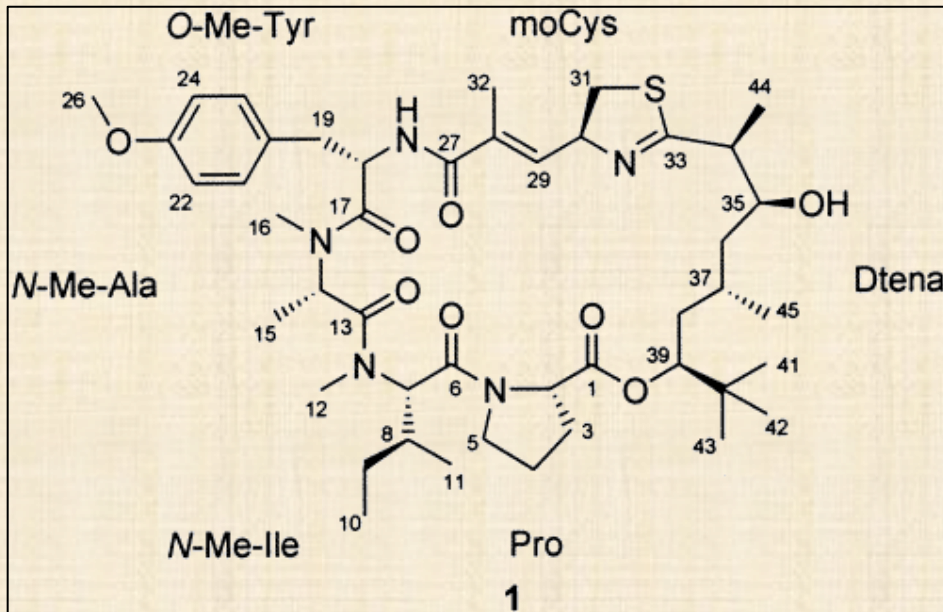
- Background and Relevance of Natural Products
- Sample Collection by McPhail Lab
- Goal of Project
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- **Results**
 - **Known Compounds**
 - **New Compounds**
 - **Hydrophobic Fraction**
- Discussion
 - Relevance of Results
- Limitations
- Future Work
- Acknowledgements
- Questions



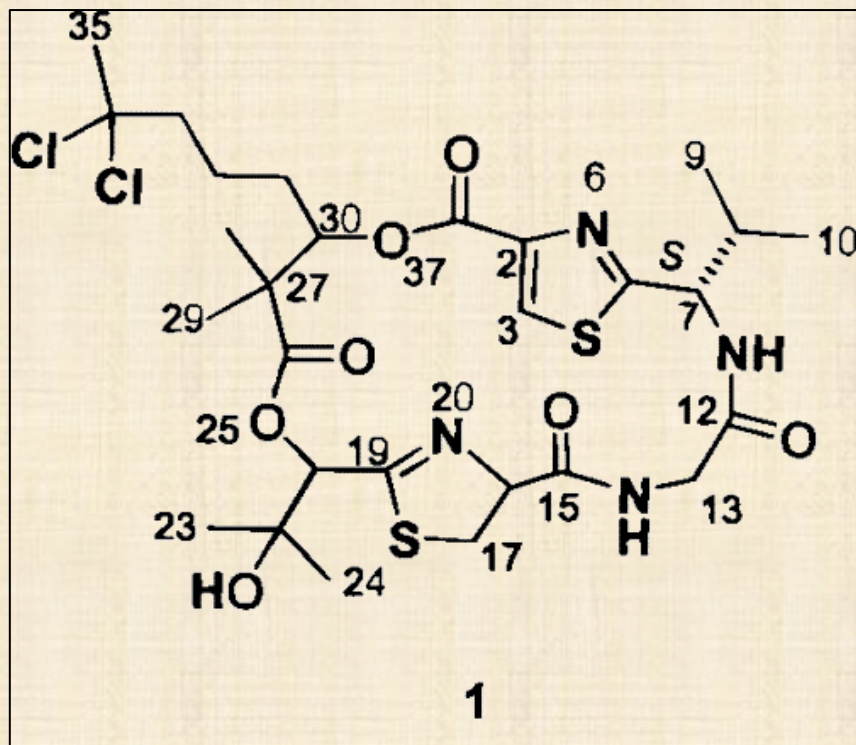
Apratoxin A-Known Compound

Compound Characteristics

- Toxic against oral carcinoma (KB) and human colon adenocarcinoma (LoVo) cells
- Induce G1 phase cycle arrest
- No effect on microfilament network
- No effect on microtubule polymerization or depolymerization
- Lack of selectivity limits potential as antitumor agent, but derivatives are more effective (Luesch, et al, 2001)



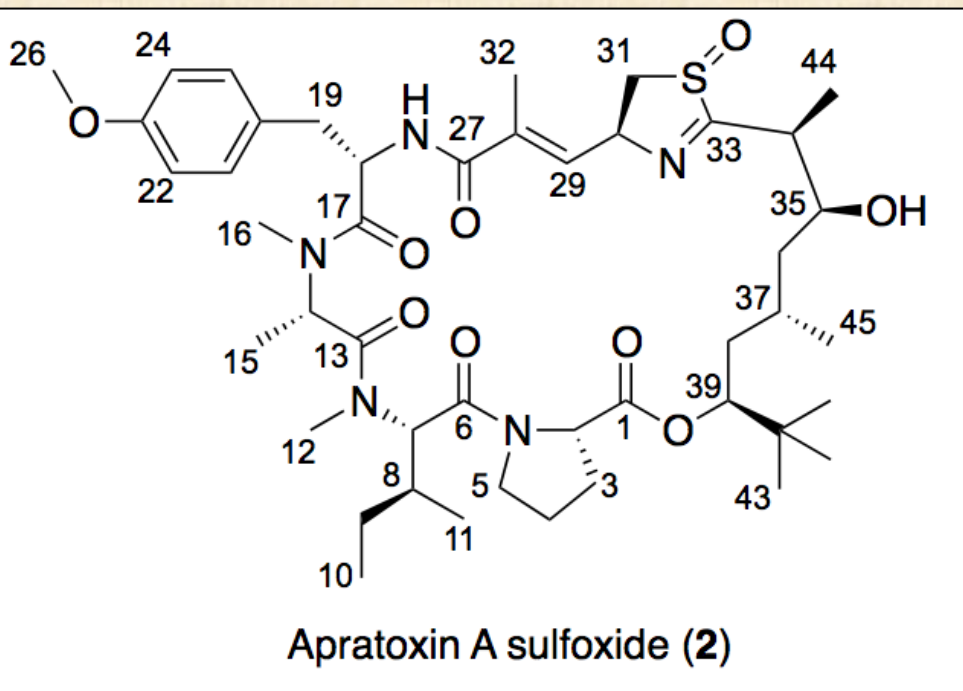
Lyngbyabellin B-Known Compound



Compound Characteristics

- Toxic against oral carcinoma (KB) and human colon adenocarcinoma (LoVo) cells
- Antifungal properties
- Protease inhibitory activity (Luesch, et al, 2000)

Apratoxin A Sulfoxide-New Compound

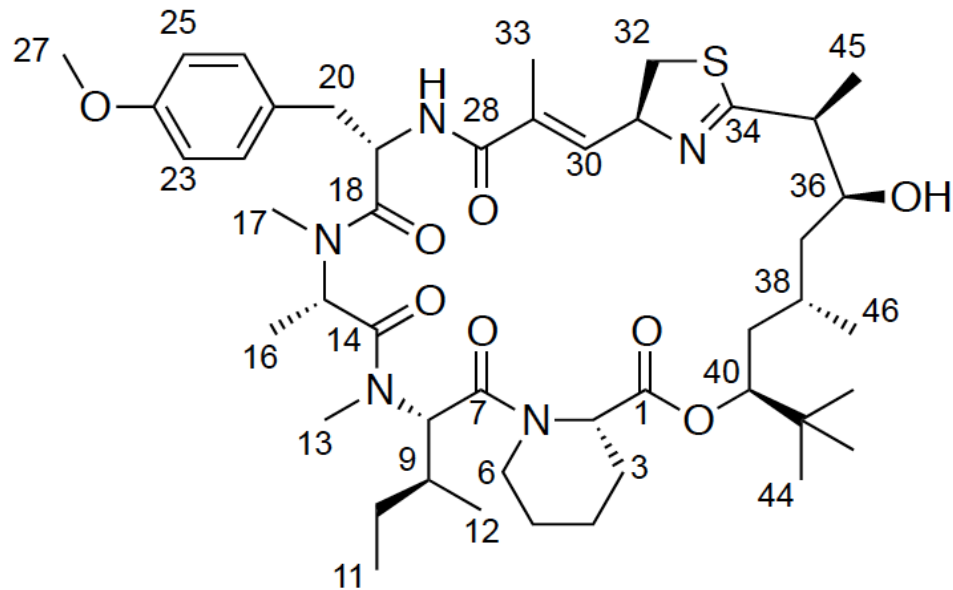


Compound Characteristics

- Similar to Apratoxin A except slower to display toxicity effects
- Toxic against Neuro-2A blastoma cells and NCI-H460 lung cancer cells

Apratoxin H-New Compound

Compound Characteristics

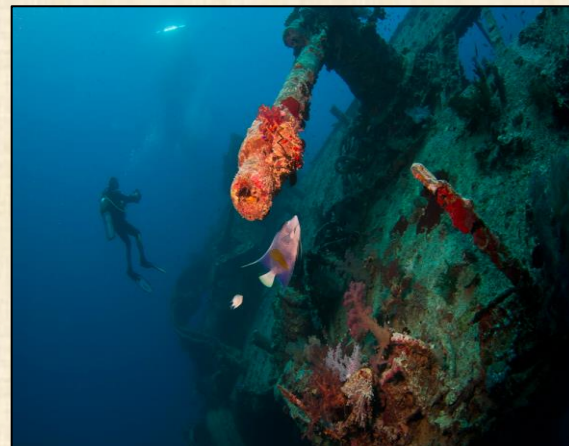


Apratoxin H (1)

- Highly toxic against NCI-H460 lung cancer cells and Neuro-2A neuroblastoma cells

Roadmap

- Background and Relevance of Natural Products
- Sample Collection by McPhail Lab
- Goal of Project
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- **Results**
 - Known Compounds
 - New Compounds
 - **Hydrophobic Fraction**
- Discussion
 - Relevance of Results
- Limitations
- Future Work
- Acknowledgements
- Questions




Moorea species

Nabq Mangroves near Sharm el Sheikh, Egypt



- 50 liters collected over 12 months
- 0.85 g Organic Extract

 Active Fractions

NP-VLC

Hydrophobic

Hydrophilic

A	B	C	D	E	F	G	H	I
37.2 mg	62.6 mg	108.1 mg	144.2 mg	12.7 mg	131.4 mg	165.0 mg	190.7 mg	88.6 mg
100% Hex	10% EtOAc/H	20% E/H	40% E/H	60% E/H	80% E/H	100% E	25% MeOH/E	100% M

Brine Shrimp Assay (BSA)

1mg/ml: 4 % 0% 100% 100% 89% 100% 100% 85% 0%

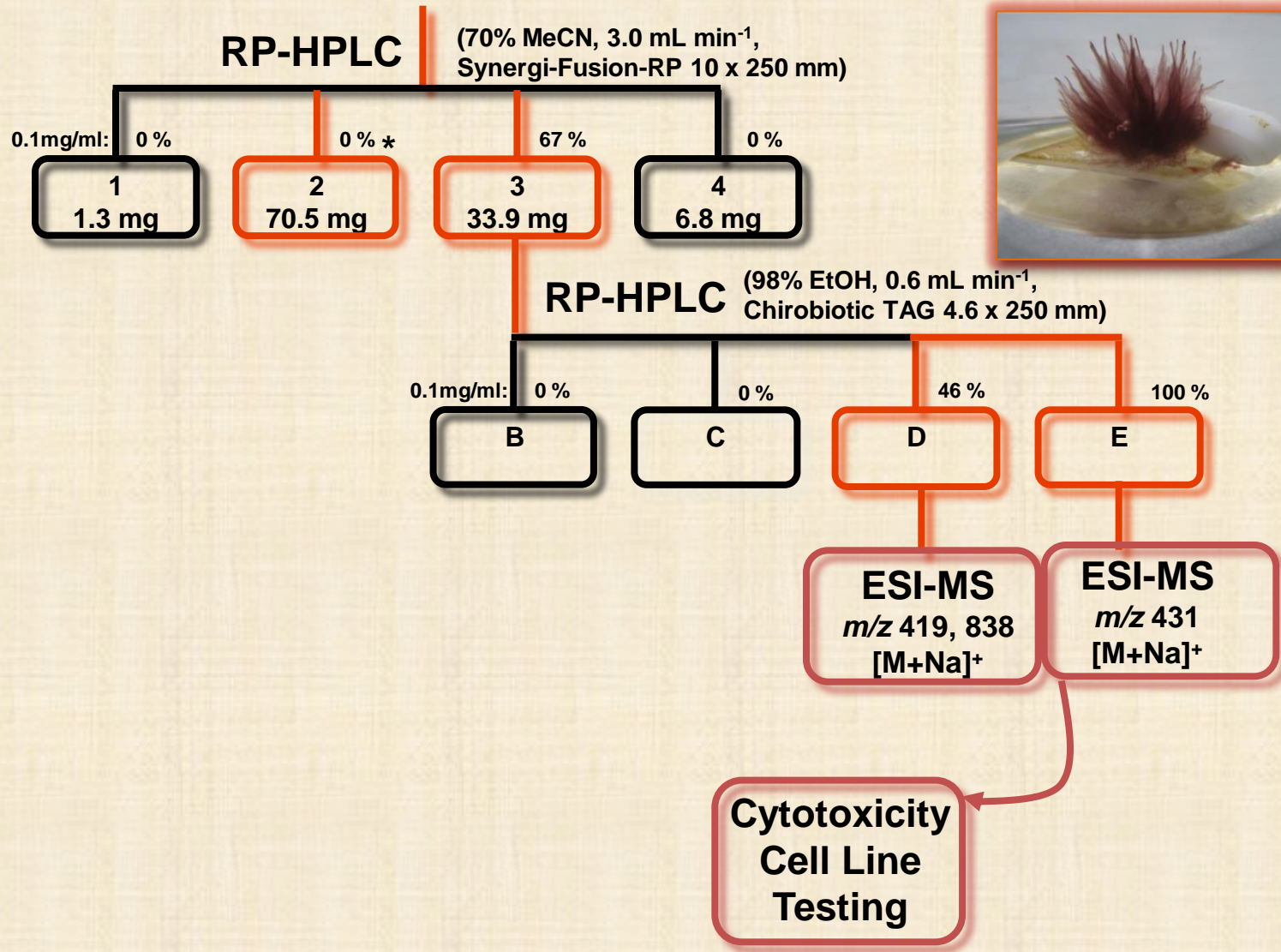
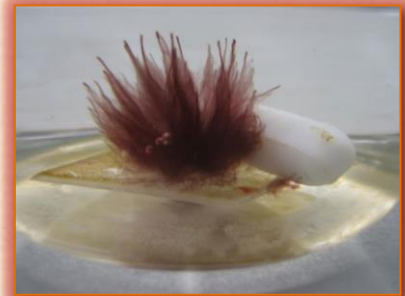
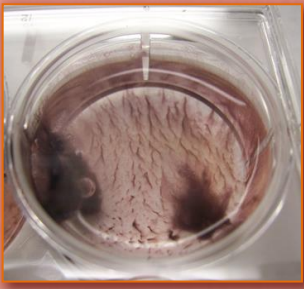
BSA

0.1mg/ml: 5 % 0% 96% 97% 7% 85% 94% 7% 0%

Mass Spectrometry
and RP-SPE

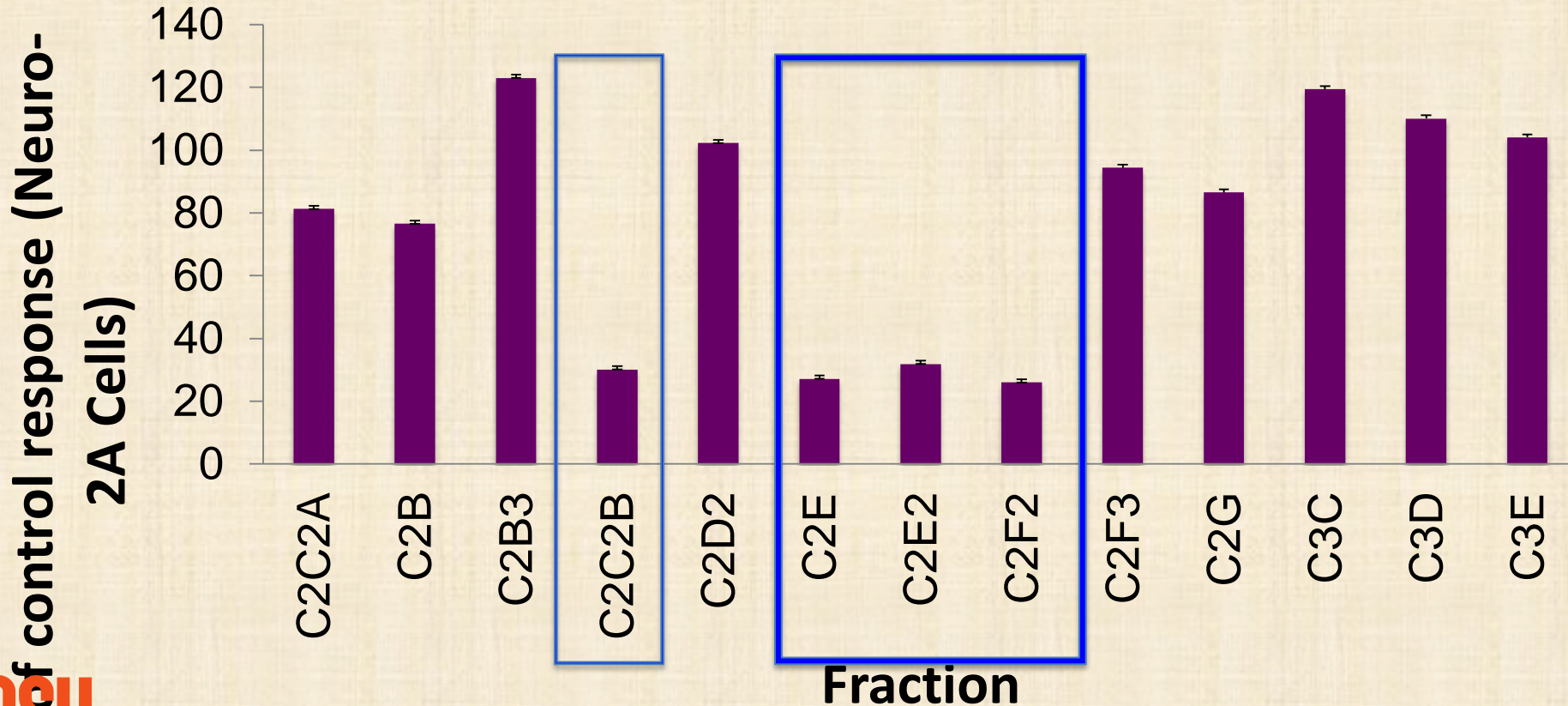
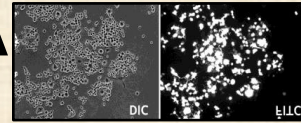
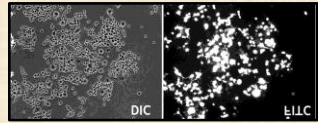


Moorea sp. Fraction C3



Fraction C-Cytotoxicity Assay-Viability

Moorea sp. Fraction C Neuro2A Blastoma Assay-Viable Cells



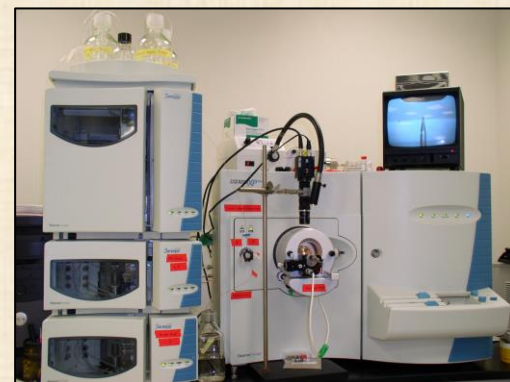
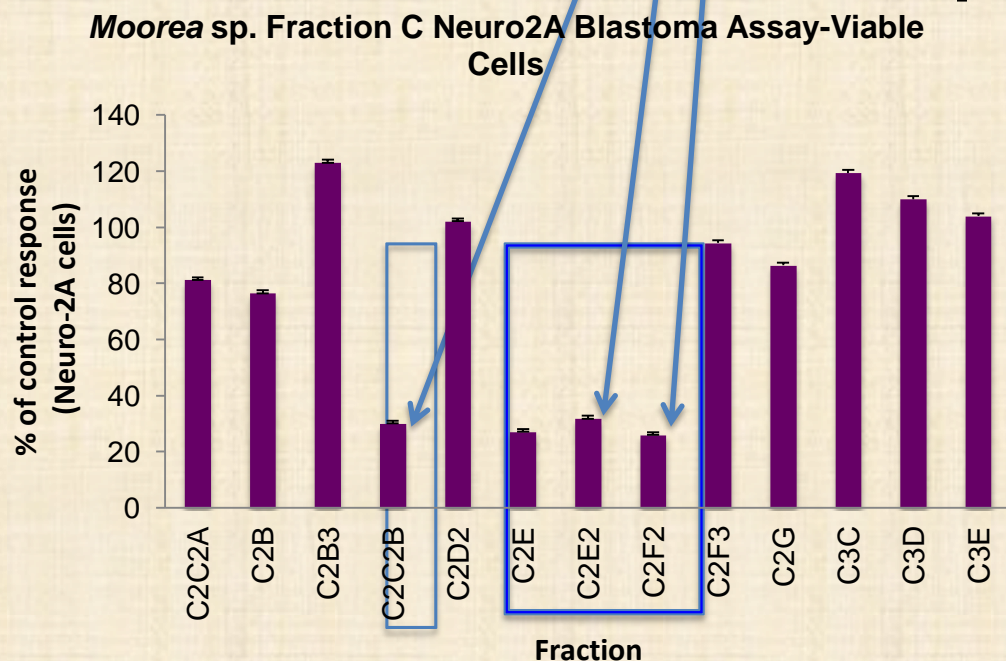
Hypothesized Masses and Applications

➤ Mass Spectrometry Determination

- Structural Components
- No published compounds with same masses
 - MarinLit

➤ ESI-Mass Spectrometry-Direct Inject

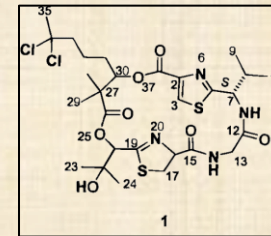
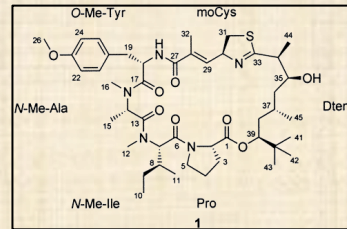
- C2C2B
 - m/z 403, $[M+Na]^+$
- C2E2
 - m/z 431 $[M+Na]^+$
- C2F2
 - m/z 471, $[M+Na]^+$



Results Summary

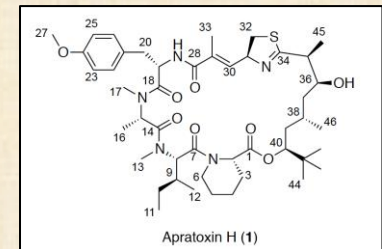
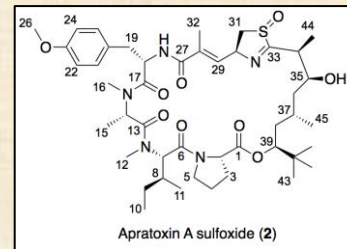
➤ 2 Known Compounds Characterized

- Apratoxin A
- Lyngbyabellin B



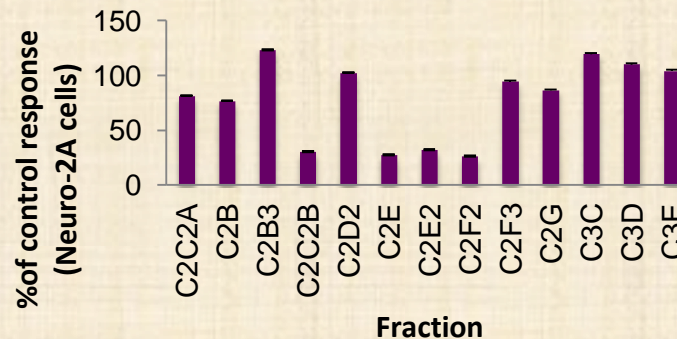
➤ 2 New Compounds Characterized

- Apratoxin A Sulfoxide
- Apratoxin H



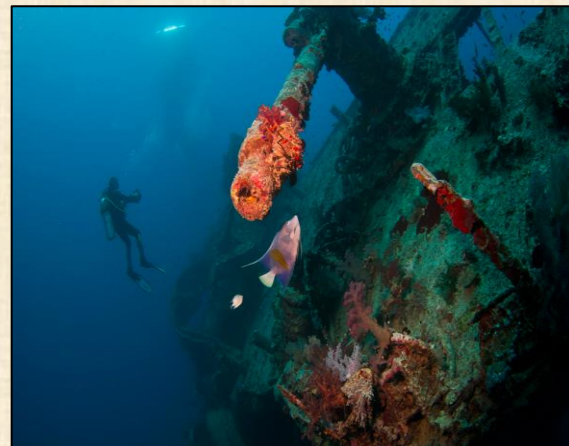
➤ New Hydrophobic Fractions

Moorea sp. Fraction C Neuro2A
Blastoma Assay-Viable Cells

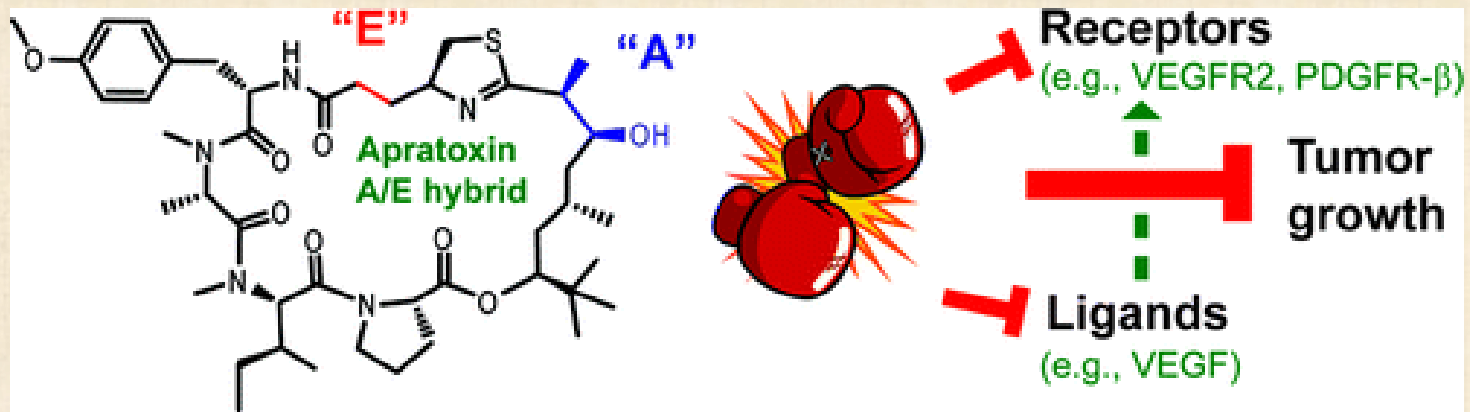


Roadmap

- Background and Relevance of Natural Products
- Sample Collection by McPhail Lab
- Goal of Project
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- Results
 - Known Compounds
 - New Compounds
 - Hydrophobic Fraction
- **Discussion**
 - **Relevance of Results**
- Limitations
- Future Work
- Acknowledgements
- Questions



Conclusions-Known Compounds

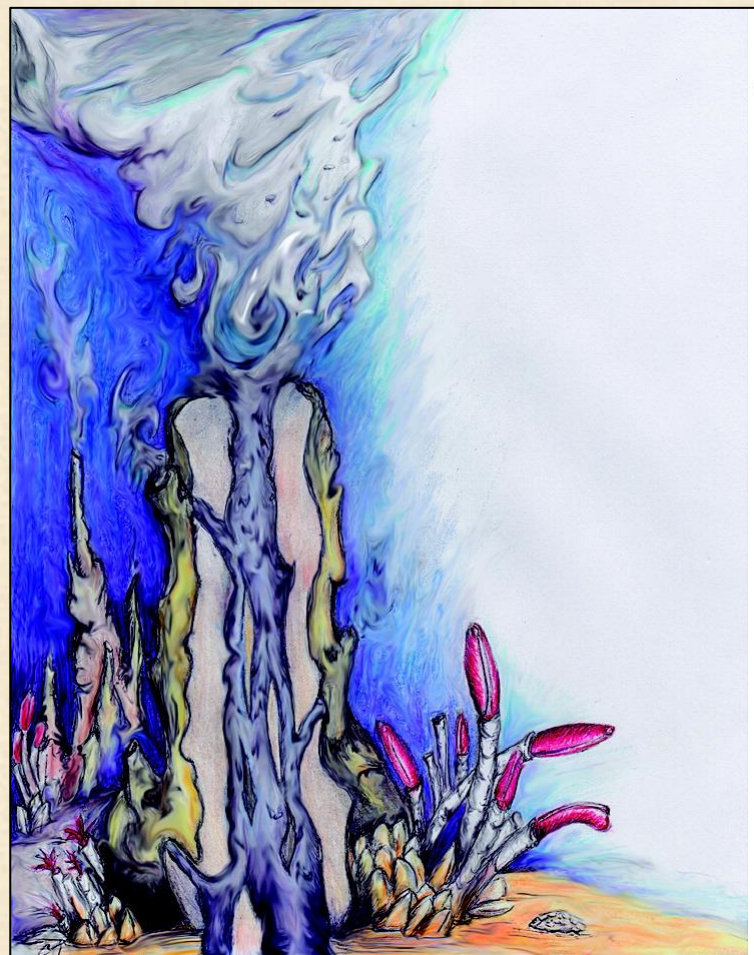


(Chen, Liu, Luesch, 2011)

- Abundance of Apratoxin Production
- Gene Cluster
 - Regulated by Environment
- More Knowns
 - Thornburg classified two more apratoxins and heptachlorin

Conclusions-Unknown Compounds

- New apratoxins
 - Medicinal Applications
- New Chemical Motifs
- More Unknowns?
 - More separation



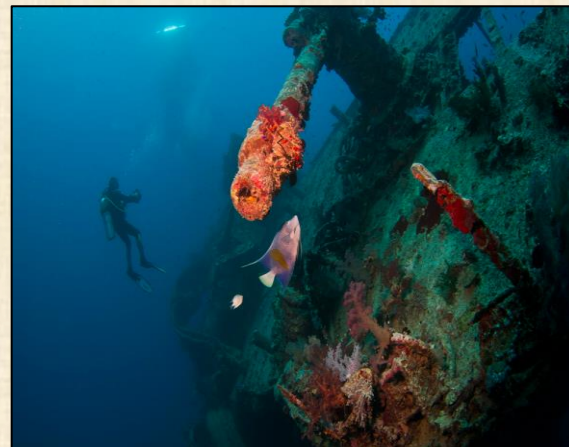
Conclusions-*Moorea* sp. Overall

- Prolific secondary metabolite producer
- Source of potent and selective analogues for synthetic scaffolds
 - Regulated by Environment
- Culturable
- Produces secondary metabolites in absence of environmental stresses
 - More with stress?



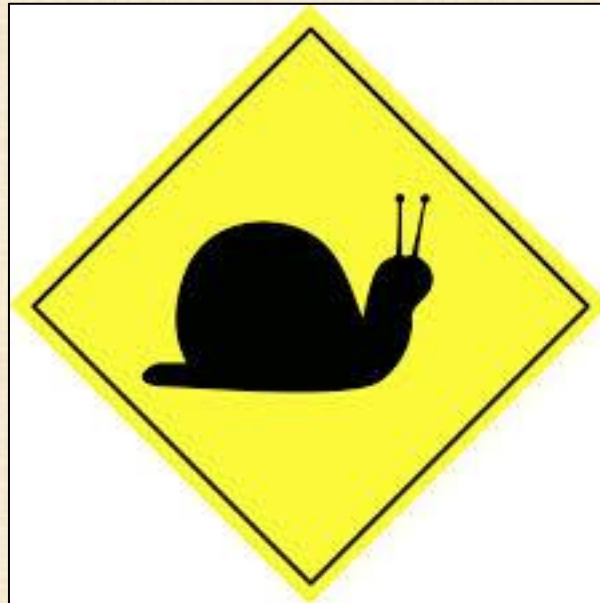
Roadmap

- Background and Relevance of Natural Products
- Sample Collection by McPhail Lab
- Goal of Project
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- Results
 - Known Compounds
 - New Compounds
 - Hydrophobic Fraction
- Discussion
 - Relevance of Results
- **Limitations**
- Future Work
- Acknowledgements
- Questions



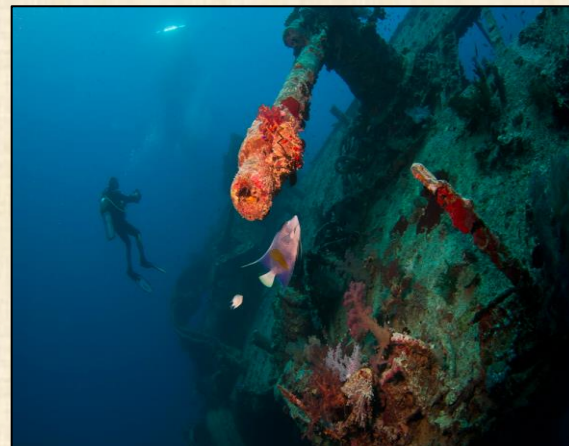
Limitations of the Project

- Relying on slow growing organism
- Sensitive to culture media
- Low resolution LC-MS can't definitively identify compounds
 - Not suitable for untargeted compound screening



Roadmap

- Background and Relevance of Natural Products
- Sample Collection by McPhail Lab
- Goal of Project
- Methods
 - Culturing
 - Chromatography
 - Compound Hypothesis
 - Compound Characterization
- Results
 - Known Compounds
 - New Compounds
 - Hydrophobic Fraction
- Discussion
 - Relevance of Results
- Limitations
- **Future Work**
- **Acknowledgements**
- **Questions**



Future Directions of the Project

- Spectroscopic analysis of pure compounds from HPLC
 - High interest in obtaining enough of the unknown m/z 658 for characterization
- Obtain larger quantities of pure unknowns for biological testing on human cell lines



Acknowledgements



Funding

- Howard Hughes Medical Institute, facilitated by Dr. Kevin Ahern
- Oregon State University Honors College
- Oregon State College of Pharmacy
- Sigma Xi
- E.R. Jackman Internship Grant

Research Lab

- Dr. Kerry McPhail
- Dr. Phil Proteau
- Christopher Thornburg
- Justyna Sikorska
- Oliver Vining
- Mariko Nonogaki



Support

- Wanda Crannell
- Perakis Lab

Questions?