

ISOLATION AND CHARACTERIZATION OF BIOACTIVE  
PSEUDOGUAIANOLIDES FROM *HELENIUM*  
*FLEXUOSUM* SEEDS

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Gyungyoun Baek, Venugopal Mukku



UNIVERSITY OF MINNESOTA  
CROOKSTON

# Background

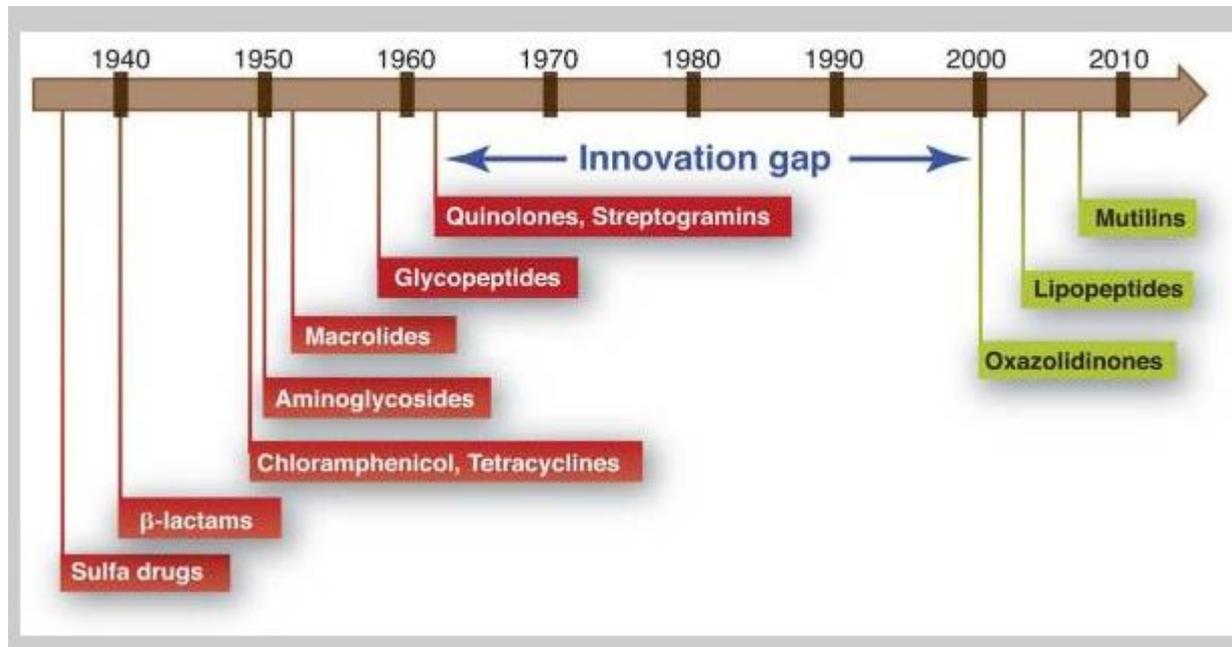
- In 2010, we have initiated a systematic screening of plant seeds for their antibacterial potential.
  - Natural product based drug discovery played a crucial role in the development of modern medicine<sup>1</sup>.
  - Almost 70% of antibacterial drugs are either natural products or are derived from natural products<sup>2</sup>.
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- 1. Li, J. W. H., Vederas, J. C. *Science*, **2009**, 325, 161.
  - 2. Newman, D. J., Cragg, G. M. *J. Nat. Prod.*, **2007**, 70, 461.

# Why screen for new antibacterials?

- Because...
- Some pathogens stopped accepting VISA.
- VISA stands for vancomycin intermediate staphylococcus aureus.
- A veritable list of acronyms (MRSA, VISA, VRSA)
- The list is so long it ESKAPEs my memory.

# Discovery gap

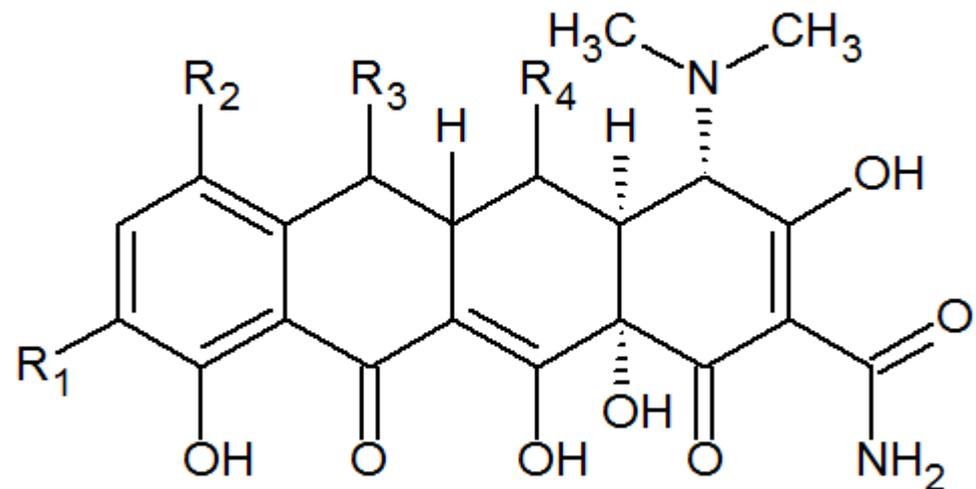
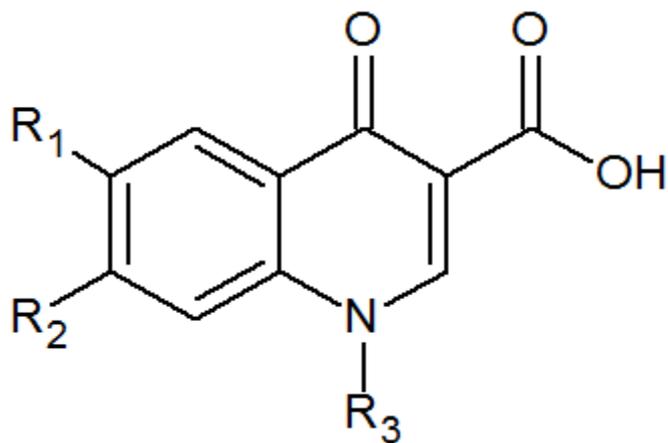
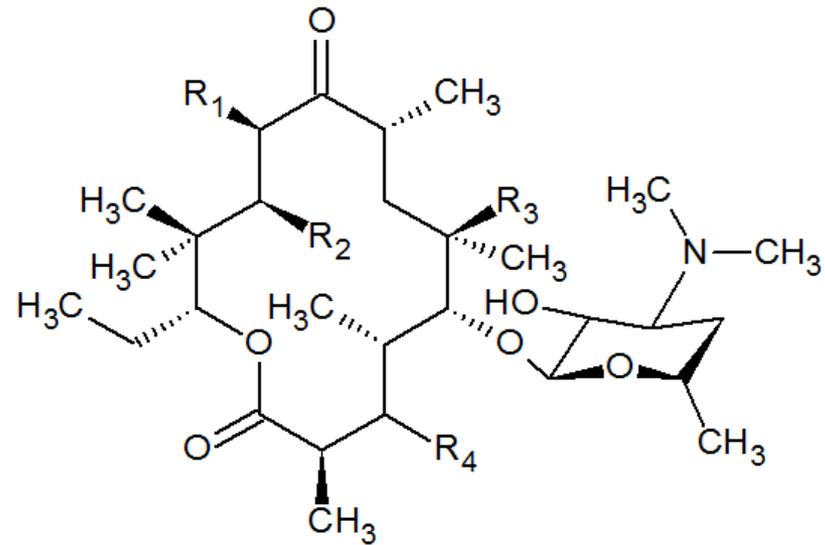
- The early excitement in antibiotic drug development tapered off by the 60s<sup>3</sup>.



- 3. Fischbach, M. A., Walsh, C. T., Science, **2009**, 325, 1089.

## A few structural classes

- Quinolones
- Macrolides
- Tetracyclines



# Back to present

- Our screening of plant seed extracts led to the prioritization of two plants: *Helenium flexuosum* and *Helenium autumnale* (Family: Asteraceae).
- Chemical examination of *H. autumnale* resulted in the characterization of two known flavonoids.

*Helenium  
flexuosum*

A species of annual herb  
in the daisy family  
known by the common  
name purple  
sneezeweed. It is native  
to the eastern half of  
North America



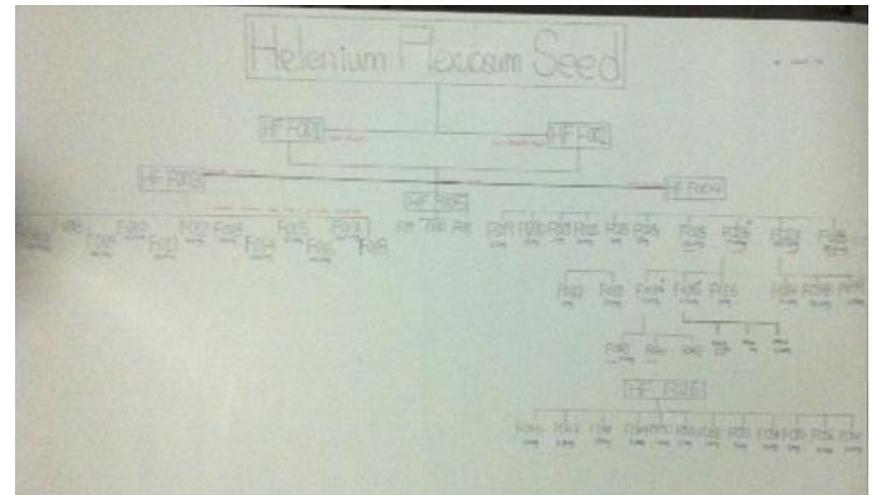
# Method

- *H. flexuosum* seeds (454 g) were powdered and extracted repeatedly with methanol and the combined methanol was evaporated on a rotavapor.
- The crude extract showed activity against *S. aureus* in the disc diffusion assay.



# Kupchan partition

- The residue from the methanol extract was subjected to Kupchan partition. The dichloromethane fraction inhibited *S. aureus* moderately in disc diffusion assay.



## Chromatography

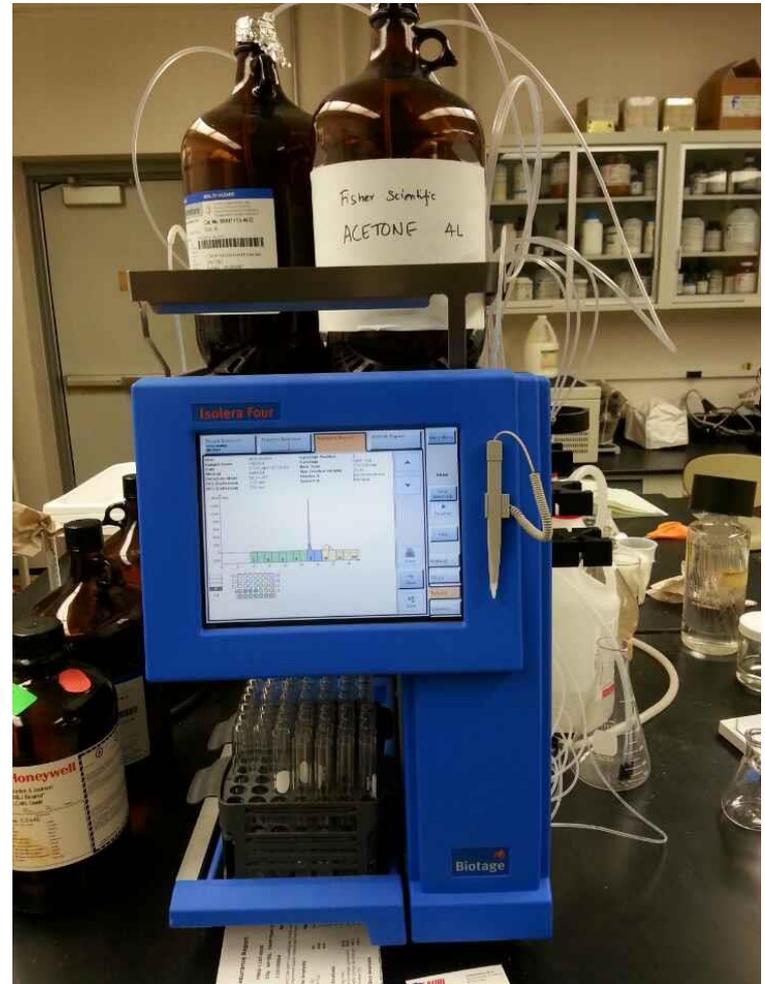
Larger fractions were separated on glass columns using gravity to drive the solvent flow.

As fractions get smaller, Biotage system was used.

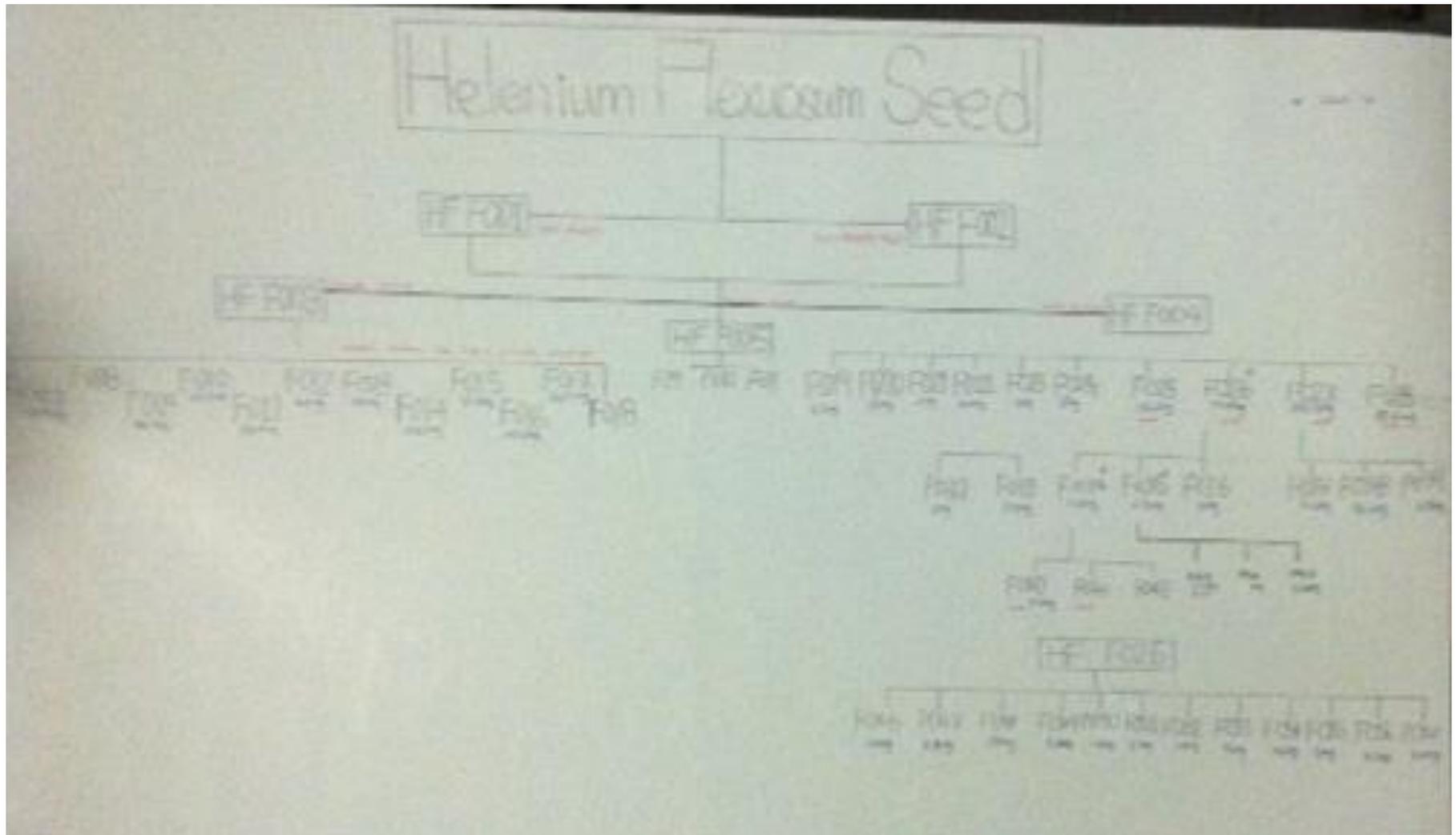


# Chromatography

- Repeated chromatography of the dichloromethane fraction on normal and reverse phase columns using a Biotage flash chromatography system resulted in a number of fractions.



# Fractionation Chart



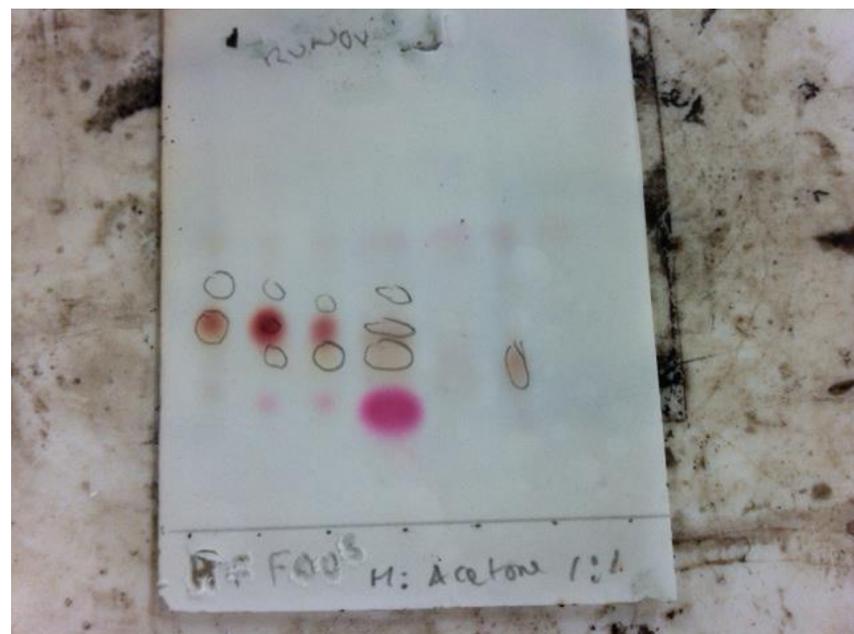
# Purification of selected fractions

- F026 was initially purified using DCM:Methanol (93:7) on a burette column.
- F035 from F026 was further purified using DCM:Methanol (98:2). Purity of the fractions was checked by TLC using hexane:acetone (1:1)
- The final purification was done using toluene: acetone (7:3) to obtain 4 mg (0.0001 % dry weight) of F041 (isohelenol). UV light was used to visualize compounds on TLC followed by heating the TLC plate after dipping it in Methanol:H<sub>2</sub>SO<sub>4</sub> (9:1)

# Fractions visualized under UV light



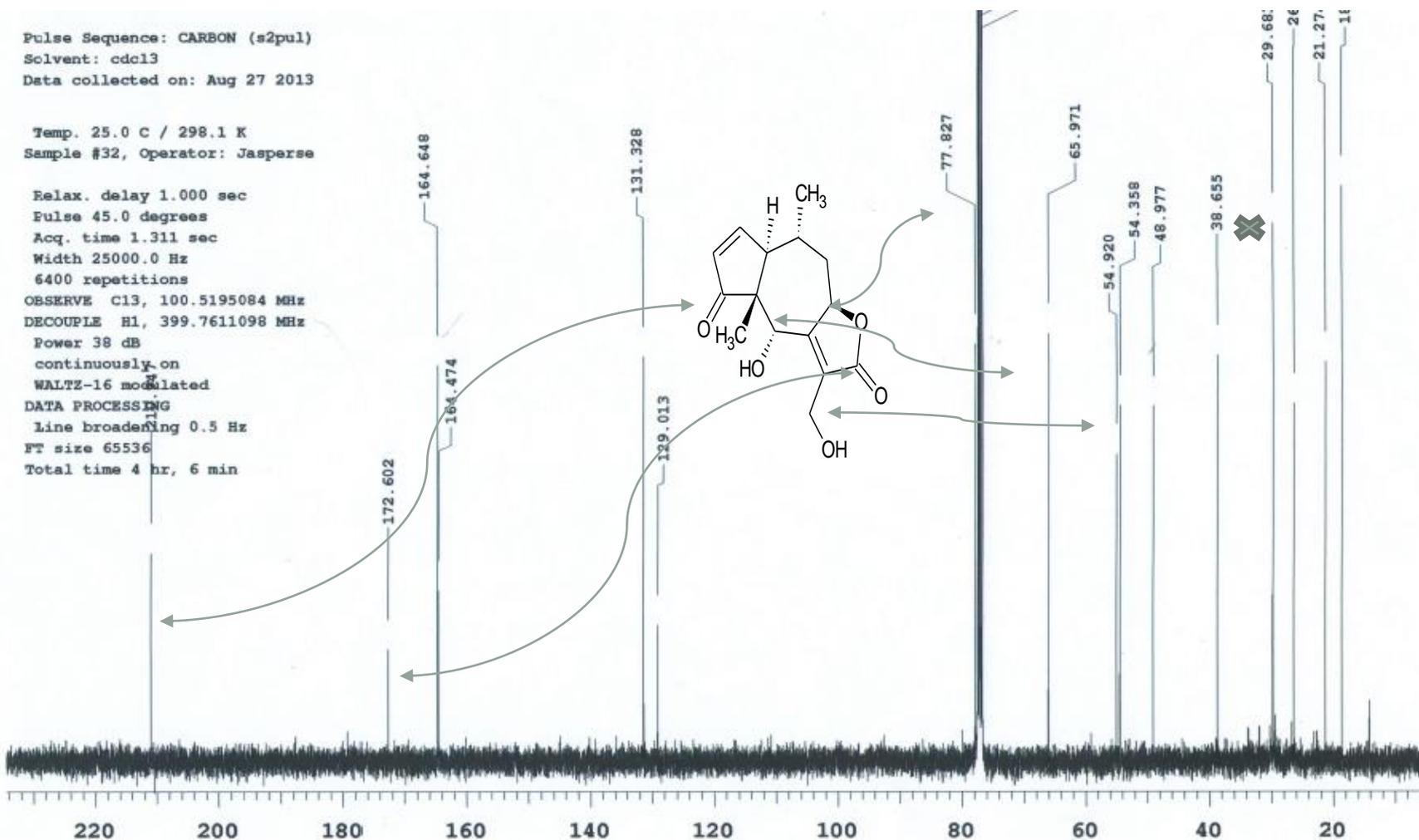
# TLC of isohelenol and related fractions



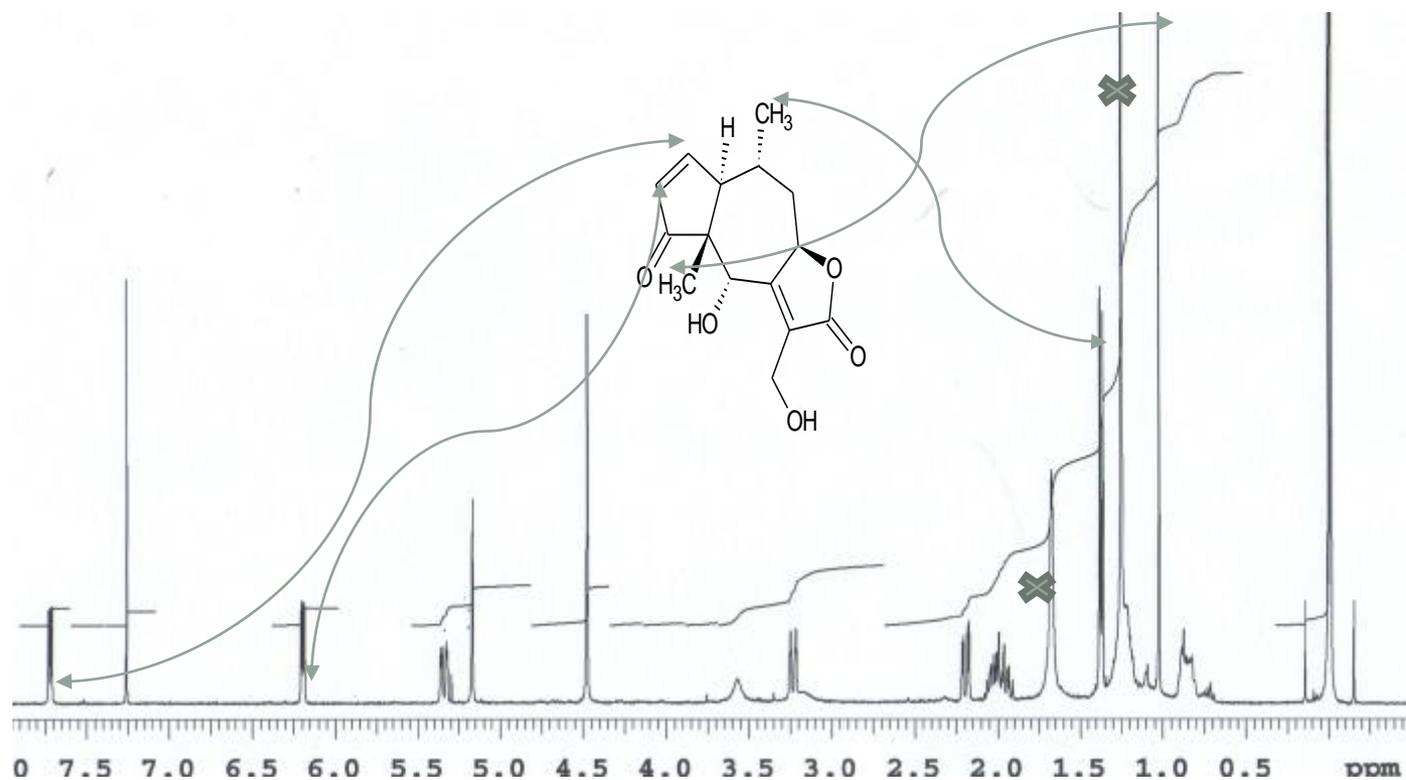
# Results

- Thin Layer Chromatography and  $^1\text{H}$  NMR analysis of these fractions suggested the presence of closely related compounds.
- Isohelenol, a sesquiterpene lactone, was characterized using 1D and 2D NMR.
- It was previously reported from *Helenium microcephalum* and was reported to have *in vivo* activity against mouse P388 lymphocytic leukemia

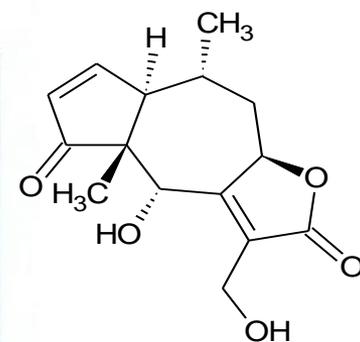
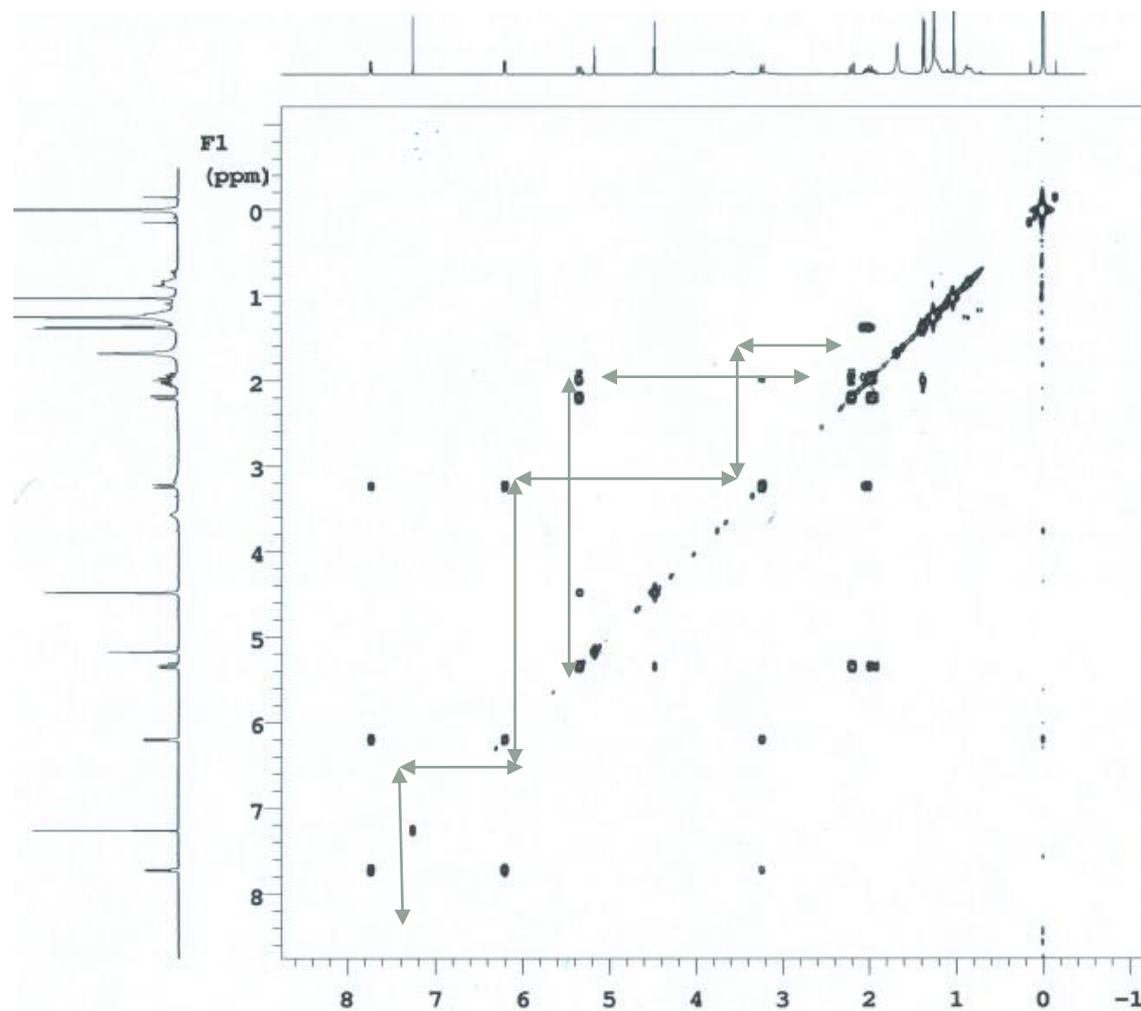
# $^{13}\text{C}$ NMR spectrum of Isohelenol



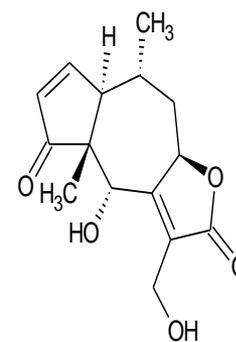
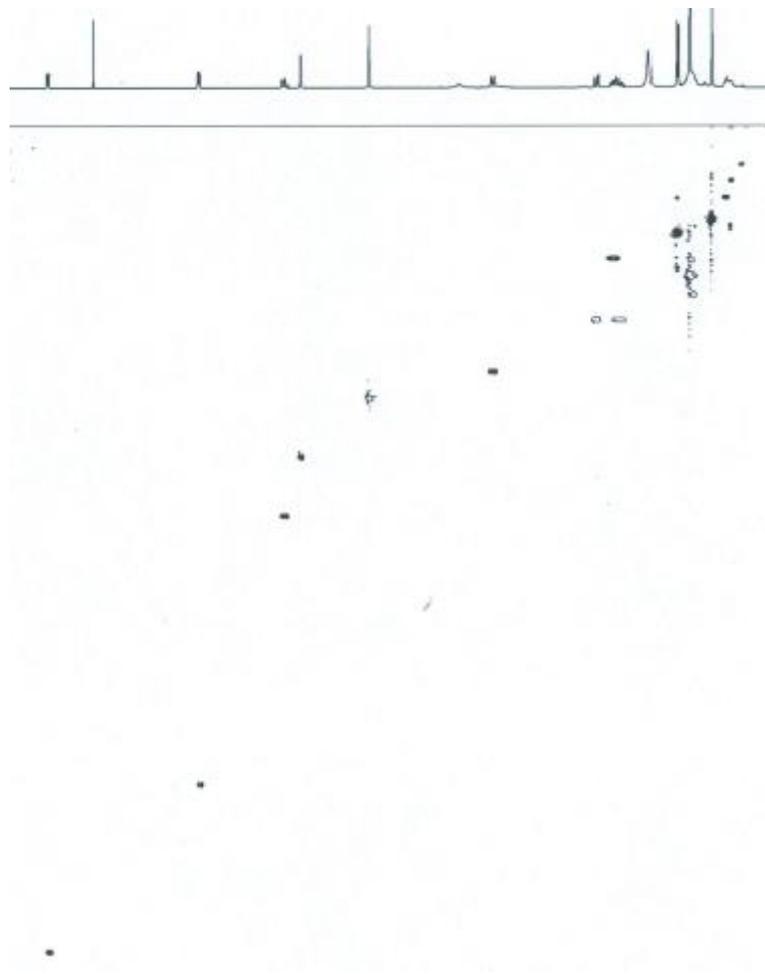
# $^1\text{H}$ NMR spectrum of Isohelenol ( $\text{C}_{15}\text{H}_{18}\text{O}_5$ )



# $^1\text{H}$ - $^1\text{H}$ COSY spectrum of Isohelenol

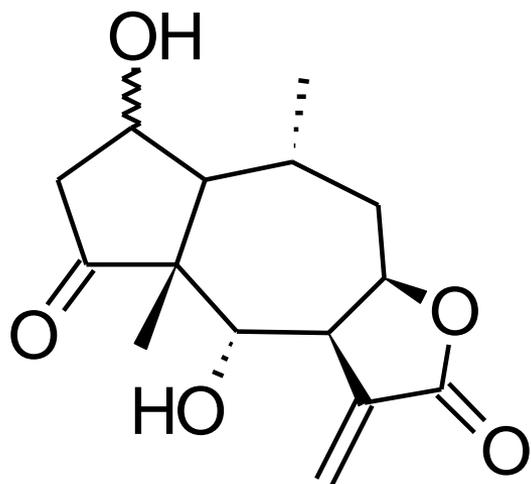


# HMQC spectrum of Isohelenol

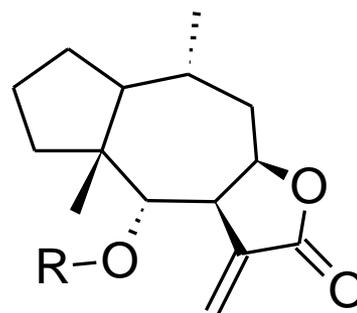


# Other compounds in the process of characterization

F051

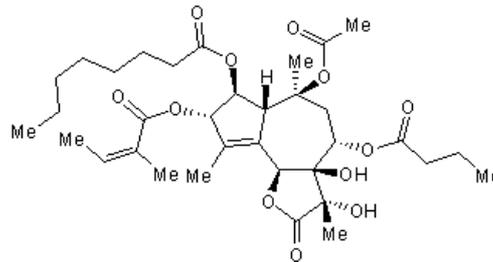
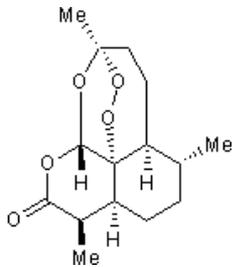


F037



# Sesquiterpene lactones

- Sesquiterpene lactones (SLs) are plant-derived compounds often used in traditional medicine against inflammation and cancer.
- A few sesquiterpene lactone derived drugs from artemisinin, thapsigargin and parthenolide are currently in clinical trials.



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# Importance of SLs

- The chemical properties of SLs comprise alkylating center reactivity, lipophilicity, and molecular geometry and electronic features<sup>4</sup>.
- These drugs are selective toward tumor and cancer stem cells by targeting specific signaling pathways, which make them lead compounds in cancer clinical trials.

• 4. Akram Ghantous *et al.*, *Drug Discov. Today*, **2010**, 15-16, 668-678

# Future work

- Complete the characterization of SLs from *H. flexuosum*
- Send them for testing to NCI.

## Acknowledgments

- I am grateful to the Grant-in-Aid, University of Minnesota for summer salary, the UROP program, for stipend and travel support.
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