Sesquiterpene Lactones from *Helenium flexuosum*

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March 31, 2015

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Introduction

• Biodiversity is the diversity of life including plants, animals, and microorganisms.

• Chemotaxonomy is the biological classification of organisms based on their similarities in chemical composition.

• Primary metabolites are chemical compounds in living organisms include DNA, RNA, proteins, lipids, and carbohydrates.

• Secondary metabolites are smaller chemical compounds produced by all living things often for purposes of survival, defense, and communication.¹

Natural Products

• Natural Products are secondary metabolites - chemical compounds discovered from living organisms.
• Approximately one-half of the U.S. Food and Drug Administration approved drugs are derived from natural products.
• Bio-prospecting is the search for novel natural products found in nature by researchers around the world to analyze and evaluate for potential medicinal drug discoveries.¹

Synthesis of Natural Products

• Once a natural product has been discovered and passed multiple clinical trials it is synthesized in a laboratory.
• Many polymers (plastics) and pharmaceuticals are synthesized.
• Organic synthesis involves the use of common reactants under controlled pH and temperature, using organic solvents, to synthesize the desired product.
• Bioactivity of the product is reliant on chirality. Racemic mixtures are separated using chiral resolution.²

Natural Products

- Penicillin was the first antibiotic discovered in 1928, a natural product produced from the fungus Penicillium chrysogenum.³

- Vancomycin is the first line treatment for methicillin resistant Staphlococcus aureus (MRSA) infections. It was discovered in 1953, from soil samples in the country of Borneo, from the bacterium Amycolatopsis orientalis.⁴


Natural Products

• Epinephrine (adrenaline) is a compound produced by the adrenal glands of mammals and a hormone that works as a vasopressor in the sympathetic nervous system. It was discovered in 1895. It can be extracted from animal serum or synthesized.\(^5\)

• Acetylsalicylic acid ASA (aspirin) is a natural product produced by willow tree bark sap and used since antiquity as an analgesic (pain reliever).\(^6\)

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Natural Products

- Artemisia annua contains a large concentration of artemisinin. Artemisinin is a chemical compound in the sesquiterpene lactone class. Youyou Tu shared the 2015 Nobel Prize in Physiology or Medicine for the discovery of artemisinin. Artemisinin-based Combination Therapies (ACT) are being used to treat malaria caused by several species of the parasitic Plasmodium protozoan.⁷

Natural Product Research on Helenium Flexuosum

- Helenium Flexuosum is a Native American daisy species, from the Asteraceae family. The Asteraceae family is known for the production of sesquiterpene lactones, a class of compounds with anti-cancer and anti-inflammatory properties.

- A sesquiterpene lactone compound is a 15-carbon compound, formed from three isoprene units, with a lactone ring. Their biological effects such as tumor treatment is due to the $\alpha$-methylene-$\gamma$-lactone ($\alpha$MyL) group.\(^8\)

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Sesquiterpene Lactones

- 3 Isoprene Units (2-methyl-1,3-butadiene)
- Lactone – 5, 6, or 7 member ring ester
- αMγL group is reactive by the process of the Michael Reaction – the addition of a nucleophile to an α-β unsaturated carbonyl group.
- Sesquiterpene lactones are lipophilic, enabling movement across cell membranes, altering transcription of genes in the nucleus and signal transduction pathways.⁸

Gathering Daisies

- *Helenium flexuosum* (Purple Sneezeweed) is a species in the daisy family; an herbaceous perennial wildflower, native to North America. It grows up to 3 feet in height.

- Each globoid flowerhead is 1 inch across and has 8-14 florets. At the apex of the flowerhead there are numerous achenes (small, dry, one-seeded fruits). The achenes are the source of the medicinal sesquiterpene lactones.  

Crude Extract from Seeds
Fractionation

• The seeds were ground using a seed grinder.
• Next the powder was extracted with methanol. The methanolic extract was Kupchan partitioned into hexane, dichloromethane, and aqueous fractions.
• Categorization of fractions were named as such:
  – Helenium flexuosum – HF
  – Fraction F003 is hexane
  – Fraction F004 is dichloromethane
  – Fraction F005 is aqueous
• The fractions under research are HFF004.
• The fractions that showed potential from Thin Layer Chromatography (TLC) included: HFF051, HFF036, HFF037, HFF041, and HFF027.
What is HPLC?

- High Pressure Liquid Chromatography is a type of Chromatography technique which separates compounds based on adsorption.
- HPLC involves two phases – a stationary phase and a mobile phase. The stationary phase is a nonpolar silica-based column. The mobile phase is a mixture of water and methanol or acetonitrile.
- With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily.\(^{11}\)

HPLC Equipment

- Mobile phase
- Degasser
- Pump
- Autosampler
- Column thermostat
- Detector
Separation Method by HPLC

- HFF051, HFF036, HFF037, HFF041, and HFF027 were injected into the analytical column (C18).
- Next the chromatograms were analyzed for potential compounds for isolation.
- Many compounds were isolated using the preparatory column (C18).
- Isolates were collected into sample vials. Of the many compounds, two known compounds were found using 1H NMR spectral analysis.
The compound discovered from Helenium flexuosum is an un-named sesquiterpene lactone; a helenanolide, previously reported from the aerial parts of the plant Gaillardia powellii.  

- 1H NMR  
- 13C NMR  
- ESI MS  
- HMBC  
- HSQC  

HPLC purified
HFF28B-C18 (0.054) Is (1.00,1.00) C20H30O6

HFF28B-C18 98 (3.710) Cm (78:100)

1: TOF MS ES+ 7.92e12

1: TOF MS ES+ 2.90e8

m/z

%
Conclusion

• Sesquiterpene lactones are ubiquitous, yet there is little research done on this class of compounds. Sesquiterpene lactones are most prevalent in the Asteraceae family; Asteraceae plants are in turn the most diverse and plant family in the world.

• Sesquiterpene lactones from Asteraceae play a highly significant role in human health. As pharmaceutical agents, sesquiterpene lactones have been found to sensitize tumor cells to conventional drug treatments.

• Clinical trials on sesquiterpene lactones have found these compounds to have high anti-carcinogenic potential, and high anti-inflammatory potential.\(^8\)

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Acknowledgements

• I am grateful to Dr. Venu Mukku, PhD; Organic Chemistry for our collaboration, as well for inspiration and guidance.
• I am grateful to Gyungyoun Baek, whose previous fractionation work led to our continuing natural product research.
• I am grateful to the University of Minnesota, Crookston; Math, Science, and Technology Department, for the use of University facilities and equipment for our natural product research.