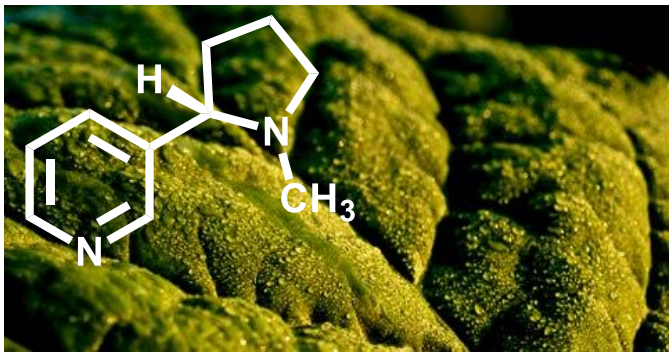
Belofsky Lab - Plant Natural Products Research

Natural products chemistry involves the isolation, characterization, and biological testing of organic compounds from natural sources such as plants, fungi, bacteria, insects, and other organisms.

Philosophy

There are unifying principles of natural products research:

- Bioactive natural products bind to specific biochemical receptors as a result of natural selection.
- Having evolved in close association with nature, human biosynthetic pathways have likely conserved large structural portions of the same or similar receptors. These may therefore be subject to pharmacological effects upon exposure to bioactive natural products (e.g. nicotine below).
- Biological and chemical screening of natural product extracts, using widely available methods of isolation and characterization, will result in the discovery of compounds that exhibit unique properties.
- Natural product-based pharmaceuticals are complementary to synthetic drugs. Whether simple or complex in structure, they typically do not contain problematic chemical functionalities that can lead to short- or long-term adverse effects.



The naturally-selected role of the toxin nicotine, from *Nicotiana tabacum* (tobacco) is to deter insect herbivores from feeding on the plant. The compound exerts different pharmacological effects on the human brain and nervous system.

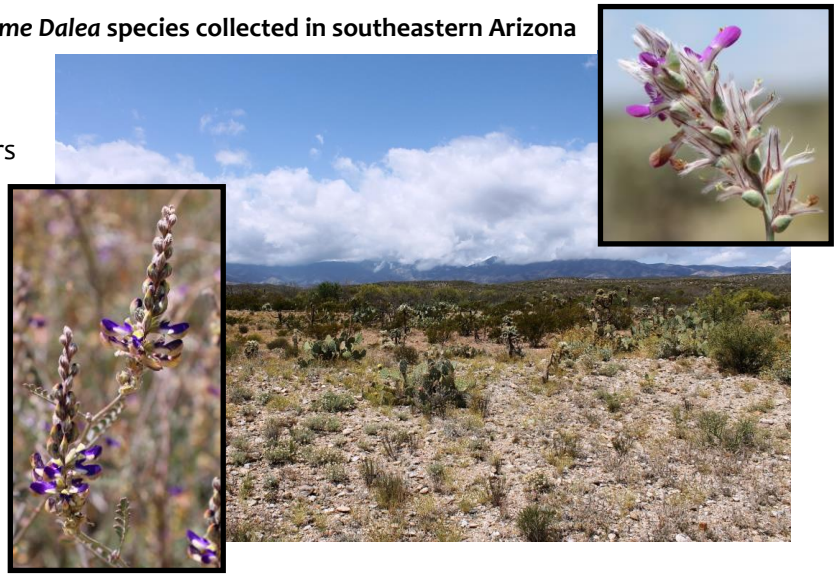
General Research Interests

We are currently interested in studying the activity of plant compounds for antiparasitic activity. We have also published work on compounds having antibacterial, antifungal, and antiinsectan activity.

This work entails the…

- Field collection of wild plants for study.
- Interdisciplinary collaborations with researchers in biology and pharmacology for testing of natural product isolates.
- Use of classical chromatographic and state-of-the-art spectroscopic techniques to isolate and characterize compounds of interest.
- Use of biological assays to evaluate interactions of compounds with macromolecular biological targets.

***Some Dalea* species collected in southeastern Arizona**



Natural products chemistry educates students in transferable, high-demand skills of chromatography and spectroscopy.

Biological Testing

Biological testing of crude extracts, chromatographic fractions, and isolated compounds is a critical component of natural products research that requires extensive knowledge in complementary fields such as biology and pharmacology. Testing may be accomplished through collaborations with researchers in academic, government, and private facilities.

Selection of Organisms for Study

Plant sources offer the advantages of relative abundance of material, ease of collection and subsequent extraction, and a track record of success for medicinal applications. Plant natural products research is well suited to the timeframe of student projects at all levels. Collection of plants in the US offers the additional advantage of lower travel costs, avoids the difficulties associated with international collecting, and to some extent, may serve conservation efforts in the region.

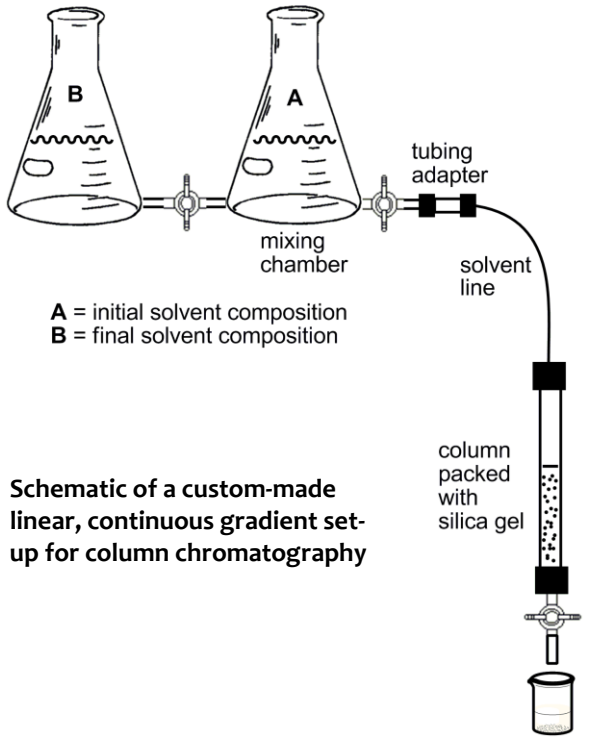
Of consideration in choosing plant sources:

- Ecological – Species exhibiting chemical defenses or communication.
- Geographic – Plants from biologically diverse regions.
- Taxonomic - Infrequently or never before studied species.
- Ethnographic – Species with documented traditional uses.
- Informatic – Based on prior knowledge, and by analogy to known natural or synthetic substances.

There are numerous plants of potential interest that meet at least two or more of the above criteria. Many were used in Native American traditional medicine or in folk medicine of various regions, and have never been chemically-investigated in detail. An example of a valid taxonomic/informatics approach would be when a plant extract of a particular species is found to be active in biological testing. In this case, it is reasonable to seek other species of the same genus that likely contain natural analogs of those found in the first species.

Methods of Isolation and Characterization

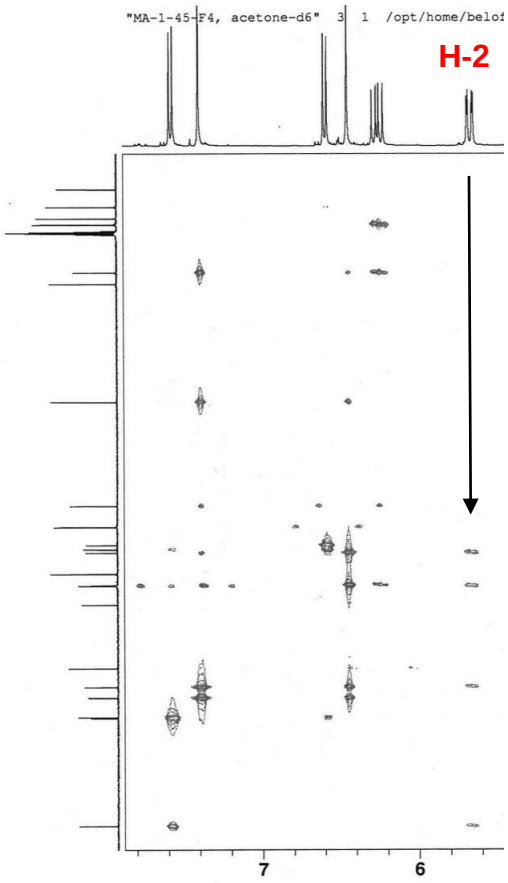
There are many options for extraction and chromatographic purification of natural products on a preparative scale. Traditional methods, like solvent partition and open-column silica gel chromatography are still extremely effective. Methods that utilize polymer gel stationary phases like lipophilic Sephadex have proven invaluable in modern natural products. Preparative high performance liquid chromatography (HPLC), although typically more labor intensive, is useful in cases where higher resolution is sought. Gas chromatography (GC) and analytical scale HPLC, in tandem use with ultraviolet (UV) spectroscopy and/or mass spectrometry (MS), are powerful tools for the identification of known compounds.

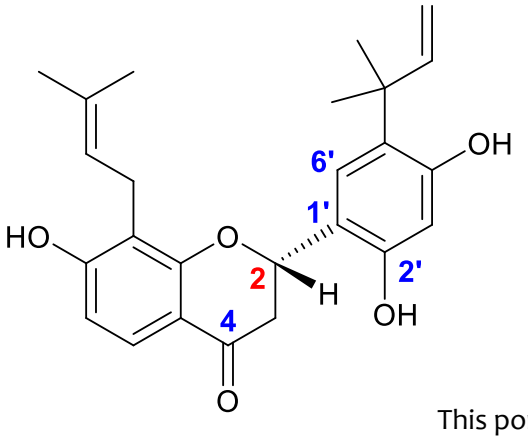


Schematic of a custom-made linear, continuous gradient set-up for column chromatography

Characterization of Natural Products

Techniques of high resolution nuclear magnetic resonance (NMR) spectroscopy and MS have advanced rapidly in recent years and can be applied to previously unsolvable problems in natural product structure elucidation. A two-dimensional heteronuclear multiple bond correlation (HMBC) NMR spectrum is a standard tool for the structure determination of natural products. The example below shows how HMBC was used to establish part of the structure of a new compound, malheuran B.





malheuran B

This portion of an HMBC NMR spectrum shows four clear correlations from hydrogen **2** to carbons **4**, **1'**, **2'**, and **6'**, thereby establishing the connectivity between the two rings involved.

Current Area of Focus

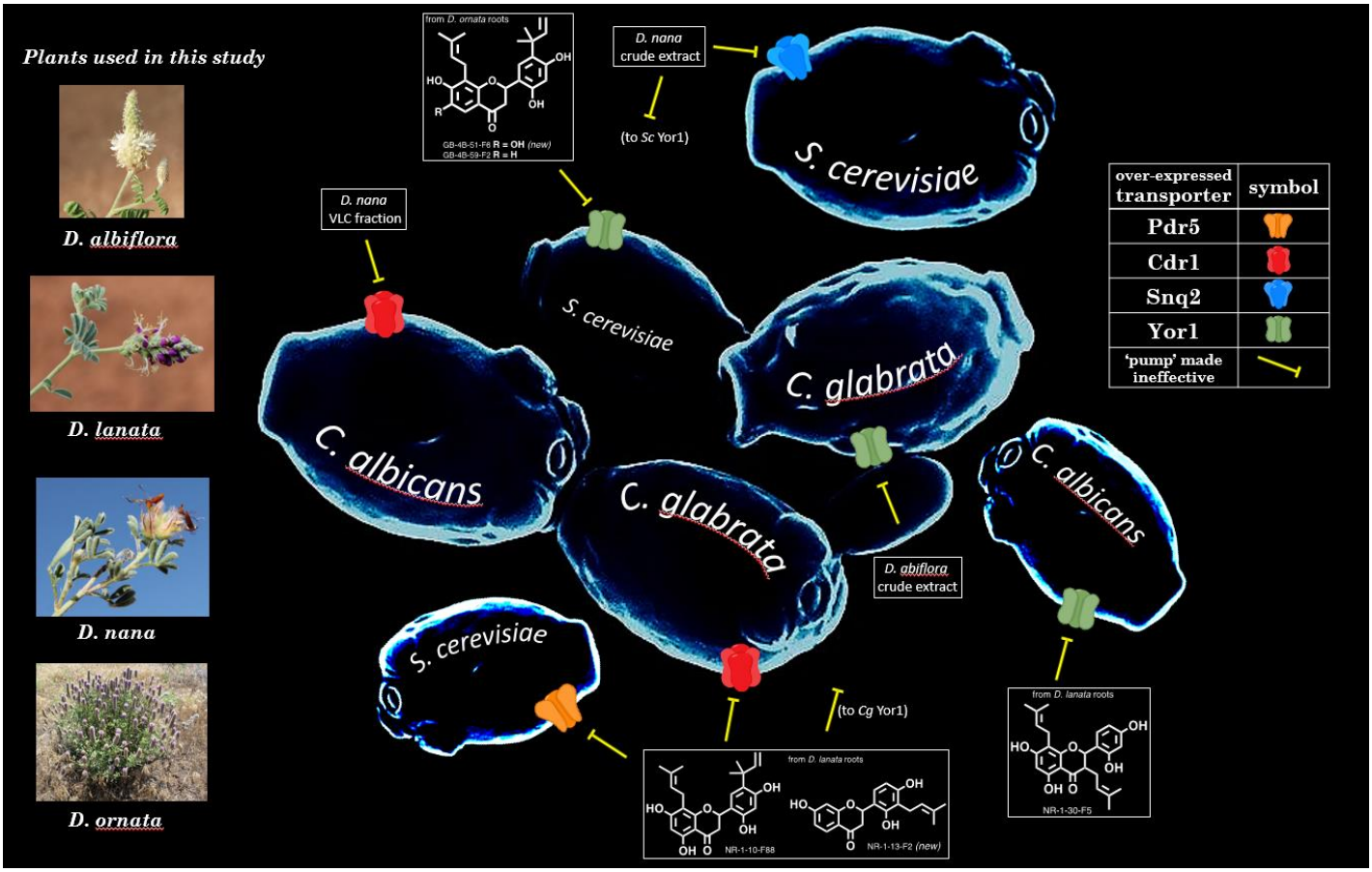
The increased prevalence of fungal multidrug resistance (MDR) is alarming. There are few highly effective antifungal antibiotics, and most are of the same chemical class of azoles. Recent headlines reported on *Candida auris*, a new pathogenic, infectious MDR yeast with very high (>50%) patient mortality.

Our current work involves the isolation, characterization, and activities of phenolic metabolites of *Dalea* spp. toward engineered strains of *Candida* and *Saccharomyces* that under- and overexpress MDR transport proteins. In this work, we collaborate with the research group of Dr. Marcin Kolaczkowski, of the Wroclaw Medical University, Poland.

Dr. Kolaczkowski's assays reveal growth inhibition (direct cell killing), inactivation of specific transporters (e.g., *Candida* proteins Cdr1, Snq2, Yor1, and *Saccharomyces* Pdr5, Snq2, Yor1), synergistic, and antagonistic activities. Examination of the differential activities of flavonoid and other molecular probes toward these specialized strains of *Candida* and *Saccharomyces* may reveal weak points in the mechanisms of transport-based fungal MDR that can be exploited with combination drug therapy.

More recently, we have begun to explore activity for extracts of other plant genera that do not contain flavonoids. These other plants likely contain sesquiterpenes and related compounds.

In the figure below, we see differential activity of various plant materials, some pure compounds, some not yet purified, toward the various engineered strains of Candida and Saccharomyces. The yellow bars indicate inactivation of fungal cell membrane transporters that are typically over-expressed in multidrug resistant strains. Shutting down these transporters would allow an antifungal drug to penetrate the cell wall and not get pumped out before completing its job of killing the cells.



Some preliminary observations and conclusions

- An increase in potency with higher purity is minimal for phenolic compounds of *Dalea* spp.
- Minor structural variations in compounds of *Dalea* spp. appear to lead to distinct differences toward the inhibition of the cell membrane transporters tested.
- Root metabolites appear much more effective at both direct inhibition and transport inhibition, than metabolites of aerial parts (data not shown), likely due to the greater exposure of roots to fungi and other microorganisms in the soil environment.
- The production of multiple congeners of flavonoid metabolites in each *Dalea* spp. may typify a `shotgun´ approach to antimicrobial activity in the wild. Plant survival should be enhanced, and the development of resistance slowed, through a combination of direct inhibition and transport inhibition.
- *Dalea* spp. continue to yield a prolific variety of new compounds with each successive species studied.

Scientific Society

"Pharmacognosy" derives from two Greek words, "pharmakon" or drug, and "gnosis" or knowledge. Like many contemporary fields of science, Pharmacognosy has undergone significant change in recent years and today represents a highly interdisciplinary science that is one of five major areas of pharmaceutical education.

The American Society of Pharmacognosy (ASP) is international in scope and brings together those dedicated to the promotion, growth, and development not only of pharmacognosy but all aspects of those sciences related to and dealing in natural products. The Society currently has over 1,100 active and associate members. Approximately 40 percent of the active members of the Society reside outside of the U.S. and Canada, and represent more than 60 countries throughout the world. The ASP co-publishes its flagship journal, the *Journal of Natural Products*, with the American Chemical Society.

Pharmacognosy is the study of natural product molecules (typically secondary metabolites) that are useful for their medicinal and other functional properties. The sources of compounds of interest span all biological kingdoms, most notably marine invertebrates, plants, fungi, and bacteria. Natural products chemistry is steadily refreshed with techniques and new findings from associated scientific fields. This is one reason why studying pharmacognosy is a good choice for those who like to work at the interface of many different, but complementary, areas of science that relate to the natural world.

Selected Publications

Belofsky, G.; Aronica, M.; Foss, E.; Diamond, J.; Santana, F.; Darley, J.; Dowd, P.F.; Coleman, C.M.; Ferreira, D. "Antimicrobial and Antiinsectan Phenolic Metabolites of *Dalea searlsiae*" *J. Nat. Prod.* **2014**, *77*(5); 1140-1149.

Belofsky, G.; Kolaczkowski, M.; Adams, E.; Schreiber, J.; Eisenberg, V.; Coleman, C.M.; Zou, Y.; Ferreira, D. "Fungal ABC Transporter-Associated Activity of Isoflavanoids from the Root Extract of *Dalea formosa*" *J. Nat. Prod*. **2013**, *76*(5); 915-925.

Belofsky, G.; Carreno, R.; Goswick, S.M.; John, D.T. **"**Activity of Isoflavans of *Dalea aurea* (Fabaceae) against the Opportunistic Ameba *Naegleria fowleri*" *J. Nat. Prod*. **2006**, *72*(4); 383-386.

Belofsky, G.; Carreno, R.; Lewis, K.; Ball, A.R.; Casadei, G.; Tegos, G.P. "Metabolites of the "Smoke Tree", *Dalea spinosa*, Potentiate Antibiotic Activity against Multidrug-Resistant *Staphylococcus aureus*" *J. Nat. Prod.* **2006**, 69, 261-264.

Belofsky, G.; Percivill, D.; Lewis, K.; Tegos, G.P.; Ekart, J. "Phenolic Metabolites of *Dalea versicolor* that Enhance Antibiotic Activity against Model Pathogenic Bacteria" *J. Nat. Prod.* **2004**, 67, 481-484.

Belofsky, G.; French, A.F.; Wallace, D.R.; Dodson, S.L. "New Geranyl Stilbenes from *Dalea purpurea* with in Vitro Opioid Receptor Affinity" *J. Nat. Prod.* **2004**, 67, 26-30.