

Behind the Scenes in Pharmacognosy

Clearing the Air on the Smoke Tree

by Amy Keller

In January of this year, the article entitled, "Metabolites of the 'Smoke Tree', *Dalea spinosa*, Potentiate Antibiotic Activity against Multidrug-Resistant *Staphylococcus aureus*" appeared in the *Journal of Natural Products* 69th volume. *The Newsletter* interviewed first author and ASP member Dr. Gil Belofsky, who graciously gives us insight into an interesting study.

How did you become interested in plant metabolites?

My first experience with plant metabolites and natural products chemistry was at Colorado State University in Frank Stermitz's lab. At the time I loved the idea that chemistry could be involved in what is essentially natural history. The "old school" naturalists style of study always appealed to me, but until I started work in this area, I never thought it was something chemists could do for a living. The idea of doing field work was also a big attraction, and still is.

That was when I got my master degree, back in 1987 and, not surprisingly, Frank's influence is clearly seen in the *Dalea* work. He and Kim Lewis pioneered the research that one molecule, acting as a pump inhibitor, could work in concert even with a weak antibiotic to overcome certain resistance mechanisms.



Dr. Gil Belofsky collecting in Tallgrass Prairie Preserve near Pawhuska, OK. He says, "I'm not smiling because I know chiggers, ticks, and who knows what else are crawling on me."

Who in your laboratory carried out the research?

The chemistry work was done at The University of Tulsa, and the bioassay work at the Northeastern University. The plant material was collected in California's Anza-Borrego Desert by an undergraduate, Mr. Kavon Azadi, who is now in medical school at The University of Oklahoma, and me. During the trip the SUV got stuck on a boulder and was only extricated after extensive digging in the sand. The next day, still congratulating myself on the engineering of the rescue, I backed into a telephone pole and crushed the back end of the vehicle. D'oh! The chemistry side of the lab work was done by Roberto Carreno, now in graduate school in Biochemistry at the University of Houston, and me. Roberto did large portions of the extraction, isolation, and initial 1D NMR work and I did some of the final isolation and all the 2D NMR work. As a professor at The University of Tulsa, I still very much enjoyed working in the lab myself. It kept me closely involved, and in many ways was essential given the time and experience limitations of the undergraduate researchers. Despite this, I was always pleasantly surprised at how much some of the undergraduates were able to accomplish.

The bioassay work was done by Anthony Ball as part of his Ph.D. thesis work, and Dr. Gabriele Casadei who was working as a postdoctoral fellow in the laboratory of Professor Kim Lewis at Northeastern. Dr. George Tegos, formerly of the same laboratory, consulted on the project from his current position at Harvard University Medical School's Wellman Laboratories at Massachusetts General Hospital.

Could you provide a brief explanation of the work and results in your own words? In what way are the data in your paper new?

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(from left to right) Anthony Ball, Kim Lewis, Gil Belofsky, George Tegos, Roberto Carreno, and Gabriele Casadei.



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A growing body of evidence suggests that many plant phenolic compounds such as flavonoids, benzofurans, chalcones, etc. have the ability to interact with so-called "efflux pumps", such as Nor A, that play a key role in expelling antibiotics as a mechanism of resistance in bacteria such as *Staphylococcus aureus*. The concept is that if the pump is inhibited, even a weak antibiotic can penetrate and stay inside the cell, leading to cell death. This was elegantly demonstrated in Stermitz and Lewis' earlier work (*Proc. Natl. Acad. Sci. U.S.A.* 2000, 97, 1433-1437). This paper is really a follow-up on our laboratory's earlier paper on pump inhibitors from *Dalea versicolor* (*J. Nat. Prod.* 2004, 67, 481-484). With the *D. spinosa* work (*J. Nat. Prod.* 2006, 69, 261-264), we were able to further investigate the chemistry of the genus *Dalea*, in addition to finding new compounds with this interesting activity. My colleagues also expanded their biological testing to further determine the extent that the compound(s) activity may be attributed specifically to NorA-associated effects, or to inhibition of other efflux pumps known to be present, but historically less studied. We achieved somewhat limited success in that effort, but it was a start in that direction.

I thought it would be interesting to look at *D. spinosa*, the largest member of the genus and the only real tree, and also had the idea that compounds residing in bark over long periods of time may be metabolized (or oxidized) to some interesting variations of the phenolic compounds present in other *Dalea spp.* The results suggested that this may be true with the discovery of the aldehyde compounds described in the paper. Structurally, one might speculate that they result from the oxidative cleavage of a coumestan or pterocarpan.

What impact does this research have?

Plant phenolics aren't going to blow anyone away with their structural complexity, but they are diverse, bioactive, and perhaps underutilized in standard medical practice. They are ubiquitous in herbal therapies of course. One interesting aspect of the work to me is that, not having the test capacity to do "full-on" bioassay guided fractionation, we just went after interesting chemical signatures, in this case the aldehyde signals in the NMR.

Determination of synergistic effects is one of the major challenges in our field. This kind of research hopefully adds to the knowledge of how to elucidate such effects in mixtures. Stermitz, for example, used a method of screening for antimicrobials in which each fraction being tested was "spiked" with a weak antimicrobial, and increased activity relative to "unspiked" fractions was observed. This resulted in the isolation of a pair of compounds, the original antimicrobial and the pump inhibitor, working in synergy. With the increased prevalence of resistance in *S. aureus* even to "last resort" drugs like vancomycin, we may not have to discover a whole new arsenal of drug. We may just need to pair them up with pump inhibitors or other substances that allow us to overcome the resistance mechanisms.