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Investigation of a Second Fluorescent Compound in Sycamore Trees

This summer, I worked with professor Muyskens and Janice Wharton to investigate the fluorescence of scopoletin in sycamore trees. In past summers, the Muyskens lab has been interested in the natural fluorescence of some trees; more recently, we have focused on sycamore trees. The Muyskens lab has identified scopoletin, a coumarin, as the primary fluorescent compound in sycamore trees. In the literature, scopoletin is shown to be a molecule that is produced in response to a stress on certain plants. This stress can be fungal or bacterial infection or even wounding the tree.

My research this summer was focused on a mystery fluorescent compound that Professor Muyskens and I noticed in one of our samples. After running an extraction of wood shavings in water and some base, we diluted the sample with some buffer and used a fluorimeter to measure emission and excitation. When taking an emission spectrum, we excite the sample with 350 nm light and scan across a range of wavelengths for emitted light. If scopoletin was the only thing fluorescing, we would expect a single peak in the spectrum. However, for our sample, we found that there was a hump to the left of the peak which suggested the presence of a second fluorescent molecule that absorbed a smaller wavelength of light than scopoletin. We took an excitation spectra by exciting the sample with a range of wavelengths and scanning for the wavelength of the peak of the emission. With an excitation spectra taken at the hump and the peak, we were able to get an emission spectrum for each of the fluorescent molecules. We did extractions of other sycamore samples and found that they only had scopoletin, which makes the second fluorophore unique.

We were interested in trying to model the separation of these emission peaks by using pure solutions of different coumarins. We tried different concentrations of mixes and found that only scopoletin and the coumarin, herniarin, had the peak and hump that we saw in the sample. However, the excitation spectrum of herniarin does not agree well with the excitation spectrum of our mystery compound. We also measured how emission and excitation spectra change with changes in the concentrations of the coumarins. I found that all of our coumarins have a peak emission at some concentration in the range of 100-200 μM; at this range, the highest fraction of molecules in the solution are excited by the beam in the fluorimeter. At lower concentrations, there are fewer molecules that can be excited and at higher concentrations, the beam hits too many molecules at the face of the cell that our solution is in.

We also did some work with high-performance liquid chromatography to try to separate the two fluorescent compounds. Using concentrated extracts, we are able to see two prominent peaks in the chromatograph of the fluorescence detector. The retention time for one of these peaks agrees well with scopoletin and is seen when we do a run with some samples that do not have the second fluorescent compound. However, the peak which we attribute to the mystery compound does not have a retention time similar to any coumarin we have in the lab and it does not show up in the chromatographs of other extracts. We are interested in continuing to use HPLC, now with high resolution mass spectrometry, to identify the mass of the mystery compound.