Investigation of a Second Fluorophore in Sycamore and Other Naturally Occurring Coumarins

Isaac Izaguirre and Professor Mark Muyskens
Calvin College, Grand Rapids, Michigan

Introduction

Professor Muyskens lab has been interested in the fluorescence of trees for a few years. More recently the focus has been on local trees, more specifically, Sycamore trees.

The main fluorophore in Sycamore trees is scopoletin, a coumarin which emits blue light when excited with ultraviolet light. However, in one sample from 2016 we found evidence of a second fluorescent compound, other than scopoletin, in the emission spectra of the sample.

My research this summer was focused on trying to identify this mystery fluorophore that we found.

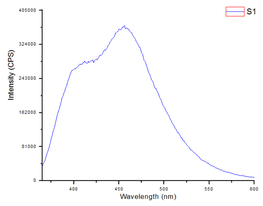


Figure 1

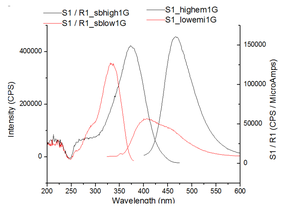


Figure 2

Figure 1 is the emission spectra from a sample from Griggs Street. The hump to the left of the peak is not usually seen in our other samples and is evidence of some other fluorescent compound.

Figure 2 is of four spectra. The two excitation spectra are taken at different points in the emission spectra. The peaks of those excitation spectra give us wavelengths to excite at to generate different emission peaks that further indicates a new fluorophore.

Objectives

Primary Objective

To identify a second fluorescent molecule that was evident in the emission spectra of some Sycamore samples.

Secondary Objectives

- To investigate the properties of different naturally occurring coumarins
- Model the appearance of a hump using different mixtures of coumarins
- Use HPLC to try to identify coumarins and their concentrations in concentrated extracts

Methods

Fluorescence Measurements

We used a FluoroMax-4 spectrofluorometer to measure emission and excitation. For the emission spectra of our samples we excited them at 350 nm and scanned for an emission range from 365 nm to 600 nm. For excitation spectra, we scanned a range of excitation wavelengths for the peak emission wavelengths

Peak Separation

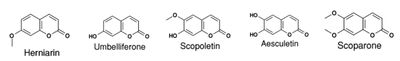
To separate peaks in the emission, an excitation spectrum was taken at either side of the peaks, that is, to the right of the peak and the left of the hump in Figure 1. Then, an emission spectrum was taken at points near the two excitation peaks which had a small degree of overlap. The two different excitation and emission spectra are shown in Figure 2.

HPLC Separations

HPLC separations were done on an Agilent 1100 Series HPLC. We used a C-18 column, our solvent was a mix of 93% Type-1 H_2O and 7% ACN with a gradient of increasing ACN to 37% in 47 minutes. We used a diode array detector and a variable wavelength detector to scan for absorbances, and a fluorescence detector to scan for fluorescence.

Results

Comparison of Mystery to Known Coumarins



Peak Emission Wavelength (nm)	410 nm	393 nm	453 nm	462 nm	467 nm	433 nm
Peak Excitation Wavelength (nm)	336 nm	325 nm	366 nm	380 nm	362 nm	343 nm

Our mystery fluorophore has a peak emission at around 410 nm and a peak excitation around 336 nm. None of the known coumarins that we investigated had emissions and excitations that were comparable to the mystery compound.

Creating a Model for the Hump

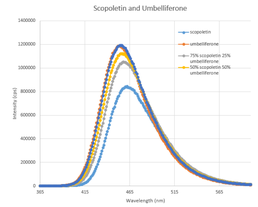


Figure 3

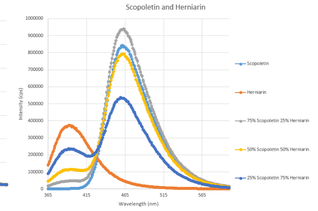


Figure 4

The hump in the emission spectrum was difficult to model, especially when the two fluorophores have similar peak absorbances as shown in Figure 3. Scopoletin and herniarin (Figure 4) provided a decent model since their peak absorbance and emission wavelengths are different enough to produce a separate peak in the emission.

Characterizing Fluorescent Compounds in Extracts

Concentration Tests:

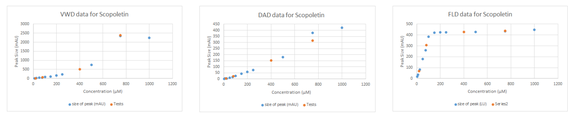


Figure 5

Figure 5 shows the concentration dependent peak sizes for each detector. At low concentrations there is good linearity in the data, especially from 5 µM to 50 µM. At concentrations that are too high, the fluorescence detector becomes saturated and the readings are unreliable. The linearity in the data can be used to estimate a concentration of scopoletin in our samples.

Separating the Fluorescent Compounds:

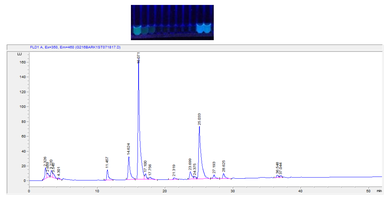


Figure 6

Using the fluorescence detector, we have more evidence in a second fluorescent compound in the extract of our sample. We separated the effluent into a different test tube every minute. The 25th and 26th minutes were fluorescent and corresponded well with the known retention time for scopoletin. The 16th minute was also fluorescent but did not correspond well with the retention times for any of our known coumarins, suggesting that this is our mystery compound.

Conclusions

- Our mystery fluorophore is not one of the coumarins we have tested.
- We can use excitation spectra to separate two peaks from an emission spectrum.
- We can use HPLC to identify the concentration of scopoletin in a sample, given that the pH of our standards are consistent with the samples.
- We can use the HPLC to separate and collect the mystery compound for further testing.

Future Work

- Continue to try to identify the mystery fluorescent compound using different method like LC-MS
- Obtain more fresh samples from the same tree and track the appearance of the mystery compound in the spectra.
- Try to find more samples with the mystery compound and identify what makes them unique

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