Scopoletin is suspected to undergo excited-state proton transfer (ESPT) resulting in fluorescent emission from the anionic structure except in very low pH. Excitation and emission spectra were collected in water of varying pH and were used to determine the pKₐ of ground-state and excited-state scopoletin to be 7.6 ± 0.2 and 1.7 ± 0.2 respectively. This agrees with literature’s calculated value for the ground state and the literature excited-state value for a molecule of similar structure (6). Scopoletin emission spectra were collected using tetrahydrofuran as a solvent to force neutral conditions and added sodium metal to force anionic conditions. These spectra provide further evidence for ESPT occurring in protic solvents as both neutral and anionic fluorescent spectra were found to be distinct in aprotic tetrahydrofuran.

Scopoletin is a coumarin that is naturally found in — and can be isolated from — varieties of chlorella, strutting nettle, and sycamore (2, 7, 13) and commonly found in some whiskies, vinegars, and teas (4). Medicinally, scopoletin has been tested as an antidepressant (1) and antipsychotic (8), and it has been noted as an antifungal and antibacterial agent (5). It is also suspected to undergo excited-state proton transfer (ESPT) and is commonly found in water (2, 14). However, the shift in excitation spectra occurs in the range of pH 6 to pH 9 (with the peak emission wavelength shifting from 340 nm to 380 nm) while the shift in emission spectra occurs in the range of pH 1 to pH 4 (with the peak emission wavelength shifting from 430 nm to 480 nm). This study observes the excitation and emission shifts in an effort to predict pKₐ values for ground-state and excited-state scopoletin.

Methods

Aqueous scopoletin (99% from Indofine Chemical Company, Inc.) solutions with concentrations of 17.3 μM were prepared using water as the solvent. Tetrahydrofuran (99+% HPLC grade) was used as the solvent. Fluorescence spectra were collected using quartz cells in a Cary 100 UV-Vis spectrophotometer. Excitation and emission spectra were collected using a Fluoromax-4 spectrophotometer. Computer modeling of scopoletin was performed using Gaussian 09 software with B3LYP and 6-31+G* and 6-31G* basis sets with PM3 via WebMO software with WebMO, Inc. 3. Ferrari AM, Sgobba M, Gamberini MC, Rastelli G (2007) Relationship between quantum-chemical descriptors of proton affinities and fluorescence characteristics for the normal and anionic forms of isoscopoletin. J Phys Chem B 111:11917–11922. 4. Gálvez MC, Barroso CG, Pérez-Bustamante JA (1994) Analysis of polyphenolic compounds of different vinegar samples. J Agric Food Chem 42:232–238. 5. Hunter T. Pham and Dr. Mark A. Muyskens

Abstract

Scopoletin is suspected to undergo excited-state proton transfer (ESPT) resulting in fluorescent emission from the anionic structure except in very low pH. Excitation and emission spectra were collected in water of varying pH and were used to determine the pKₐ of ground-state and excited-state scopoletin to be 7.6 ± 0.2 and 1.7 ± 0.2 respectively. This agrees with literature’s calculated value for the ground state and the literature excited-state value for a molecule of similar structure (6). Scopoletin emission spectra were collected using tetrahydrofuran as a solvent to force neutral conditions and added sodium metal to force anionic conditions. These spectra provide further evidence for ESPT occurring in protic solvents as both neutral and anionic fluorescent spectra were found to be distinct in aprotic tetrahydrofuran.

Results

Demonstrates Differing Absorption but Similar Emission Between pH 4.96 and pH 9.02

Demonstrates Ground-State pKₐ

Demonstrates Excited-State pKₐ

Demonstrates Distinct Neutral and Anionic Fluorescence in Tetrahydrofuran

Spectra in tetrahydrofuran provided evidence that the observed shift was a result of deprotonation, as has been suggested in literature (9, 10, 12). With this evidence, pKₐ was able to be determined as the midpoint of linearized peak emission wavelength for the ground state and peak emission wavelength for the excited state. This was recorded as 7.6 ± 0.2 for the neutral form and 1.7 ± 0.2 for the anionic form. These numbers agree with literature values for ground-state pKₐ of scopoletin (3) and excited-state pKₐ of a structurally similar molecule, asculetin (8).

Discussion

Further research will include evaluating isoscopoletin under similar conditions. When compared in THF, neutral isoscopoletin had near identical excitation and emission spectra; however, anionic isoscopoletin exhibited a drastically blue-shifted excitation spectra (with a peak around 270 nm) and a red-shifted emission spectra (with a peak around 570 nm). This is likely due to the charge delocalization possible in isoscopoletin but not found in scopoletin. The inability to delocalize the charge lead to a higher energy HOMO in anionic isoscopoletin than in scopoletin, subsequently resulting in a lower energy gap between the HOMO and LUMO of isoscopoletin and a longer emission wavelength.

Conclusions

Further research will include evaluating isoscopoletin under similar conditions. When compared in THF, neutral isoscopoletin had near identical excitation and emission spectra; however, anionic isoscopoletin exhibited a drastically blue-shifted excitation spectra (with a peak around 270 nm) and a red-shifted emission spectra (with a peak around 570 nm). This is likely due to the charge delocalization possible in isoscopoletin but not found in scopoletin. The inability to delocalize the charge lead to a higher energy HOMO in anionic isoscopoletin than in scopoletin, subsequently resulting in a lower energy gap between the HOMO and LUMO of isoscopoletin and a longer emission wavelength.

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Figure 1: Scopoletin Structure

Figure 2: Scopoletin Emission shift with varying pH

Figure 3: Simple differing excitation peak wavelengths, scopoletin emits at 460nm above pH 2.

Figure 4: Scopoletin Excited-State Proton Transfer and Excited-State pKₐ

Figure 5: Calculated spectra alongside experimental spectra for neutral scopoletin in water (top left), anionic scopoletin in water (bottom left), neutral scopoletin in THF (top right), and anionic scopoletin in THF (bottom right).

Figure 6: Related emission spectrum of pH 4.6 subtracted from emission spectrum of pH 4. Assuming all emission at pH 4 to be from the anionic form and some of the emission at pH 4 to be from the neutral form, this would give the predicted excited-state spectrum of neutral scopoletin with a peak wavelength of 460 nm.

Figure 8: Gradual shift of emission spectra with lowered pH. Peak wavelengths were arranged linearly in the midpoint pH was used to determine the pKₐ of excited-state scopoletin to be 1.7 ± 0.2.

Figure 9: Anionic scopoletin on an anionic solvent that will not readily accept a proton, allowing for observation of fluorescent spectra of purely neutral scopoletin. Adding sodium metal irreversibly deprotonates scopoletin, as tetrahydrofuran does not readily donate a proton. This allows for observation of the purely anionic form. Comparison of spectra with varied pH to computational models and spectra in tetrahydrofuran allow for predictions regarding deprotonation in water.

Figure 10: Further research will include evaluating isoscopoletin under similar conditions. When compared in THF, neutral isoscopoletin had near identical excitation and emission spectra; however, anionic isoscopoletin exhibited a drastically blue-shifted excitation spectra (with a peak around 270 nm) and a red-shifted emission spectra (with a peak around 570 nm). This is likely due to the charge delocalization possible in isoscopoletin but not found in scopoletin. The inability to delocalize the charge lead to a higher energy HOMO in anionic isoscopoletin than in scopoletin, subsequently resulting in a lower energy gap between the HOMO and LUMO of isoscopoletin and a longer emission wavelength.

Figure 11: Calculated and experimental peak wavelengths.

Table 1: Calculated and Experimental peak wavelengths.

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