Research and Creative Experience for Undergraduates Program (RCEU) Summer 2018

Analysis of Interactions Between Caffeine and Berberine Anti-Cancer Drug in Aqueous Solution Using NMR Spectroscopy

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Background

- Cancer is a disease of uncontrolled cell proliferation. DNA replication occurs more frequently due to loss of cell cycle control mechanisms, leading to tumor propagation.
- Berberine is a planar, aromatic compound under investigation as a treatment for certain cancers. Berberine may induce cancer cell apoptosis by intercalating into the bases of the cell's DNA, thus inhibiting replication and suppressing tumor growth.

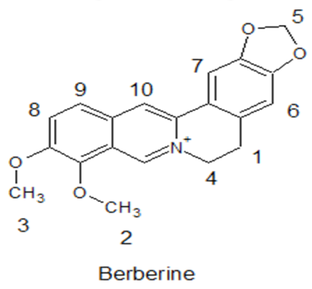

Figure 1: Structure of Berberine. Number labels represent peak positions on berberine's ^{1}HNMR spectrum.

- Caffeine is also a planar molecule which may interfere with the effectiveness of anti-cancer intercalating agents such as berberine by directly binding with the drug and preventing it from targeting the DNA of cancer cells.

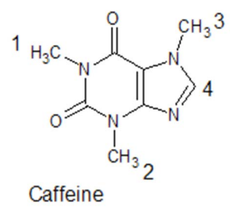

Figure 2: Structure of Caffeine. Number labels represent peak positions on caffeine's ^{1}HNMR spectrum.

- NMR Spectroscopy was used to analyze the chemical shifts of aqueous samples of caffeine and berberine molecules individually and in mixture to determine association constants (K_a) and derive points of interaction between the two molecules. Understanding these interactions will contribute to understanding effectiveness of Berberine as an anticancer drug and caffeine's role in inhibiting the effects of berberine in fighting cancer.

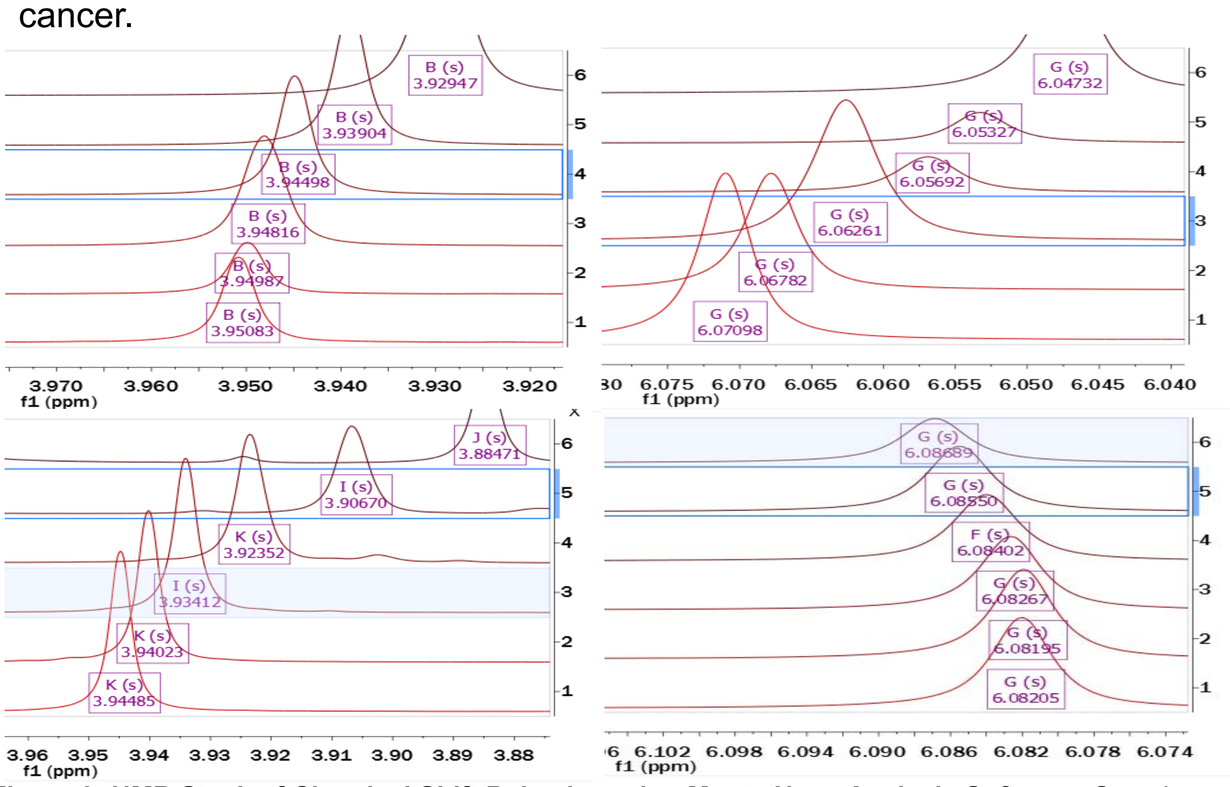

Figure 3: NMR Stack of Chemical Shift Behavior using MestreNova Analysis Software. Samples range from high concentration (top of each stack) to low concentration (bottom of each stack) for each sample set. Peak 3 of caffeine in Caffeine Standard sample set at 25 degrees (top left); Peak 3 of caffeine in Berberine/Caffeine Mixture sample set at 25 degrees (bottom left); Peak 5 of berberine in Berberine 5mM Standard sample set at 25 degrees (top right); Peak 5 of berberine in Caffeine/Berberine Mixture sample set at 25 degrees (bottom right).

Acknowledgements

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References

1. Knott, E. (2014) Caffeine Modulation of Anticancer Drug Activity Against Topoisomerase II Thesis submitted to the Department of Chemistry and the School of Graduate Studies at the University of Alabama in Huntsville.
2. Redmon, W. (2017) Interactions of Caffeine with Aromatic Anticancer Drugs in Aqueous Solutions Studied by Nuclear Magnetic Resonance Spectroscopy Thesis submitted to the Department of Chemistry and the School of Graduate Studies at the University of Alabama in Huntsville.

Methods

- Samples were prepared using serial dilutions of caffeine and berberine in D_2O with 4,4-Dimethyl-4-silapentane-1-sulfonic acid (DSS) reference standard, providing a range of concentrations for each sample set.
- Caffeine samples and Berberine samples were each tested at 25° C and 35 ℃ and compared to mixtures of the two compounds together at the same temperatures.
- ^{1}HNMR experiments were run on a Varian Unity INOVA® 500MHz NMR.
- An NMR stack of chemical shift behavior was prepared for each sample using MestreNova analysis software. Peak chemical shifts were analyzed for potential interaction using K_a calculations on MestreNova and Microsoft Excel.

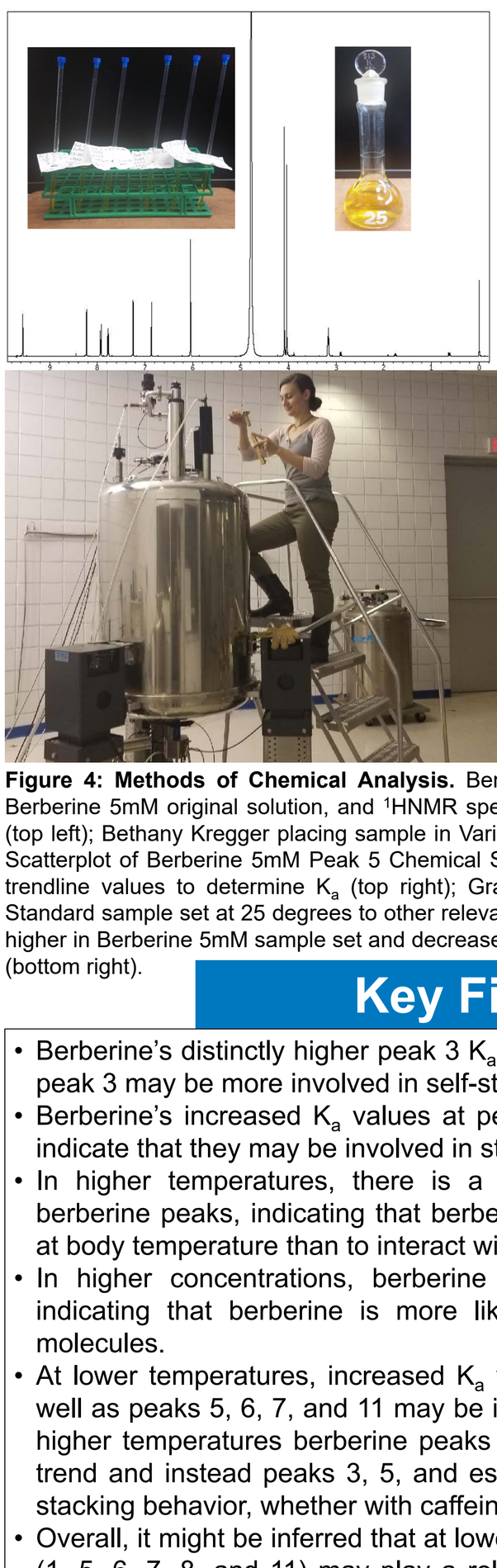

Figure 4: Methods of Chemical Analysis. Berberine 5mM sample set in NMR capillary tubes, Berberine 5mM original solution, and ^{1}HNMR spectrum of Berberine 5mM Sample 1 at 25 degrees (top left); Bethany Kregger placing sample in Varian Unity INOVA® 500MHz NMR (bottom left); Data Scatterplot of Berberine 5mM Peak 5 Chemical Shift using experimental values at 25 degrees and trendline values to determine K_a (top right); Graph of Change in K_a value from Berberine 5mM Standard sample set at 25 degrees to other relevant sample sets, where increase indicates that K_a is higher in Berberine 5mM sample set and decrease indicates that K_a is higher in compared sample set (bottom right).

Key Findings

- Berberine's distinctly higher peak 3 K_a in standard than in mixture indicates that peak 3 may be more involved in self-stacking than in interacting with caffeine.
- Berberine's increased K_a values at peaks 1, 6, and 7 in mixture with caffeine indicate that they may be involved in stacking with caffeine.
- In higher temperatures, there is a trend toward increase in K_a values of berberine peaks, indicating that berberine may be more likely to self-associate at body temperature than to interact with caffeine.
- In higher concentrations, berberine peaks tends to increase in K_a value, indicating that berberine is more likely to self-associate than to repel like molecules.
- At lower temperatures, increased K_a values indicate that berberine peak 1 as well as peaks 5, 6, 7, and 11 may be involved in stacking with caffeine, while at higher temperatures berberine peaks 1, 6, 7, 8, and 10 all show the opposite trend and instead peaks 3, 5, and especially 9 may become more involved in stacking behavior, whether with caffeine or in self-stacking.
- Overall, it might be inferred that at lower temperatures, multiple berberine peaks (1, 5, 6, 7, 8, and 11) may play a role in associating with all caffeine peaks - especially caffeine's peak 4 – and in self-association. However, as temperatures increase to conditions similar to body temperature, berberine may dissociate from caffeine and increase in self-association at peaks 3, 5, and 9 while caffeine may increase in self-association at peak 4.
- Further studies may benefit from measurements using different reference standards for comparison, as there was some indication that the molecules being studied may have had some level of interaction with the DSS reference standard.