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Laboratory Experiment

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Dual Studies on a Hydrogen−Deuterium Exchange of Resorcinol and the Subsequent Kinetic Isotope Effect

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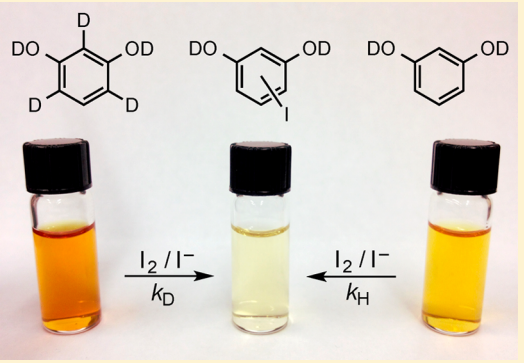
Supporting Information

ABSTRACT: An efficient laboratory experiment has been developed for undergraduate students to conduct hydrogen−deuterium (H−D) exchange of resorcinol by electrophilic aromatic substitution using D_2O and a catalytic amount of H_2SO_4. The resulting labeled product is characterized by ^1H NMR. Students also visualize a significant kinetic isotope effect ($k_H/k_D \approx 3$ to 4) by adding iodine tincture to solutions of unlabeled resorcinol and the H−D exchange product. This method is highly adaptable to fit a target audience and has been successfully implemented in a pedagogical capacity with second-year introductory organic chemistry students as part of their laboratory curriculum. It was also adapted for students at the advanced high school level.



KEYWORDS: *First-Year Undergraduate/General, Second-Year Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Brønsted-Lowry Acids/Bases, Isotopes, Kinetics, NMR Spectroscopy, Electrophilic Substitution*

It is well-understood that hydrogen−deuterium (H−D) exchange reactions are efficient and selective on numerous activated aromatic compounds in D_2O with a catalytic amount of acid. These reactions typically follow general trends for electrophilic aromatic substitution. H−D exchange of aromatic systems has been extensively reviewed in the chemical literature[1,2] but implemented sparingly in experiments within this particular *Journal*.[3−5] Moreover, simple isotopic labeling laboratory experiments with clear results are underrepresented in a teaching context outside of upper-level physical, physical organic, and inorganic chemistry laboratories. Growing interest in deuterated pharmaceuticals[6,7] presents a fascinating application for this avenue of chemistry.

While a handful of kinetic isotope effect (KIE) laboratory experiments and lecture demonstrations with organic compounds suitable for lower-level undergraduate students have been reported in this *Journal*,[8−11] they employ deuterated compounds that have been previously synthesized and/or highly toxic metal catalysts or reagents that are undesirable for use in a teaching laboratory. In contrast, the methodology described herein utilizes simple reagents under metal-free conditions and allows students to both conduct an H−D exchange experiment and subsequently examine the kinetic isotope effect within a single laboratory session. It is a versatile methodology suitable for students at the secondary and undergraduate levels to explore the process of isotopic substitution and its effect on further reactivity.

Aromatic hydrogen−deuterium exchange seems to be underutilized in teaching laboratories because such reactions often take an impractical amount of time for sufficient deuterium incorporation or require undesirable metal catalysts. There is generally no visual confirmation that exchange has taken place, as the product and starting material possess identical appearances and may be difficult to resolve apart from analysis from advanced instrumentation, such as mass spectrometry and NMR.

Additionally, most common electrophilic or nucleophilic aromatic substitution reactions do not exhibit meaningful KIE values, as elimination of hydrogen or deuterium from the intermediate species is rapid compared to the slow addition of electrophiles or nucleophiles to the aromatic ring. Under normal circumstances, for example, there are few, if any, easily observable kinetic isotope effects for nitration, chlorination, or bromination of aromatic systems.[12] However, there are notable exceptions to this trend, including azo-coupling[8] and iodination. Specifically, a substantial KIE for the iodination of phenol with molecular iodine in buffered solution was reported with an observed k_H/k_D up to 6.3 depending on the concentration of iodide.[13,14] Under these conditions, the initial addition of iodide to the aromatic ring was rapid and highly reversible, making elimination of hydrogen or deuterium the slow step in comparison.

Resorcinol is deuterated much more rapidly under acidic conditions than phenol while retaining a comparable isotope effect. Much like phenol, resorcinol is inexpensive and highly soluble in acidic D_2O solution. These qualities make it an optimal substrate for a laboratory experiment that combines an

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H−D exchange reaction with a subsequent visualization of the kinetic isotope effect. Herein, a convenient way to label a simple aromatic molecule with deuterium is described, and its reactivity compared to the unmodified starting material is demonstrated. The pedagogic goal of the experiment is to improve student understanding of several important concepts, including electrophilic aromatic substitution (EAS), the kinetic isotope effect, and ^{1}H NMR spectroscopy. The described reactions can be examined with or without advanced instrumentation. While employed primarily as part of the laboratory curriculum for undergraduate students in introductory organic chemistry, the chemistry involved is accessible by students at multiple levels of instruction and can also be discussed from several perspectives depending on the intended audience.

EXPERIMENTAL OVERVIEW

Students work in pairs. The reactant is resorcinol, a highly activated aromatic molecule that reacts quickly and efficiently by electrophilic aromatic substitution under acidic conditions. Each student prepares a solution of resorcinol (0.60 mmol) in D_2O (1 mL) containing H_2SO_4 (20 μL). Both solutions are refluxed for 30 min and are cooled to room temperature. One reaction solution is analyzed by ^{1}H NMR spectroscopy, and the other solution is used in the iodination reaction (as the reaction mixture).

Students prepare another solution of resorcinol in acidic D_2O that is not refluxed as a (nondeuterated) control. Students conduct iodination of resorcinol at room temperature by adding a dilute solution of molecular iodine and potassium iodide in ethanol simultaneously to two vials, one containing the reaction mixture and the other containing the (nondeuterated) control.[14,15] The reaction progress is monitored visually via the disappearance of iodine color in the reaction solutions due to the formation of 2-iodoresorcinol and 4-iodoresorcinol. The kinetic isotope effect of the iodination reactions is quantified by analysis of UV−vis spectroscopic data[14,15] provided to students.

Detailed procedures for the reactions are in the Supporting Information.

HAZARDS

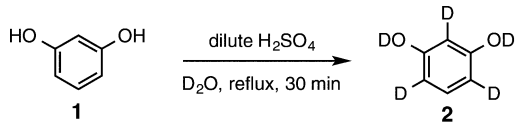
Concentrated sulfuric acid is highly corrosive and should be handled cautiously. A solution of sulfuric acid in D_2O can be prepared in advance for students to use. Resorcinol is harmful if swallowed, inhaled, or absorbed through the skin. No safety information is available for resorcinol-d_5, but similar safety concerns are reasonably expected. D_2O is mild in toxicity. Molecular iodine is corrosive to skin and eyes and harmful if inhaled. The solution of iodine/iodide in ethanol may be corrosive and should be handled with care. The iodination products of resorcinol, including 2-iodoresorcinol, are harmful if swallowed, inhaled, or absorbed through the skin. Students should wear proper personal protective equipment at all times when performing this experiment, including gloves, a lab coat, and safety goggles. Chemical manipulations are recommended to be done in an appropriate fume hood if possible.

RESULTS AND DISCUSSION

This experiment has been completed once in a laboratory session of 3 h by a total of 15 first-semester undergraduate organic chemistry students. The C−H bonds in resorcinol did not undergo significant H−D exchange in refluxing D_2O in a time frame reasonable for use in a classroom setting. However, when D_2O and sulfuric acid were mixed together, the concentration of D_3O^+ in solution and subsequent reactivity were greatly increased. To conduct the reaction, a microscale reflux apparatus, consisting of a small vial containing a spin vane (a boiling chip would be sufficient) with a reflux condenser, was heated on a hot plate. The reaction typically reached equilibrium within 30 min of heating, after which no further net deuteration would occur. The appearance of the reaction mixture did not change over the course of the experiment.

Resorcinol underwent >95% H−D exchange at the 2-, 4-, and 6-positions on the ring (Scheme 1). This result was in accord

Scheme 1. Acid-Catalyzed H−D Exchange of Resorcinol in D_2O



1 2

>95% exchange at 2-, 4-, 6- positions

with a typical EAS mechanism involving slow addition of D+ to the aromatic ring to form an intermediate arenium ion followed by rapid elimination of the proton. Though there was a negligible kinetic preference for elimination of the proton over the deuteron, the high concentration of deuterium in solution resulted in preferential formation of the trideuterated species. The alcohol protons were also exchanged, but this had no effect on the outcome of the experiment.

Students transferred the reaction mixture to an NMR tube for ^{1}H NMR analysis. The extent of deuteration was determined by integrating the remaining proton at the 5-position and comparing it to any remaining proton signals (Figure 1). This proton was *meta* to the activating hydroxyl groups on the ring and thus not activated toward electrophilic exchange. As a result, deuterium incorporation at this position was negligible during the course of the experiment. The NMR signal associated with this proton collapsed from a triplet into a broad singlet, providing strong evidence that neighboring protons were exchanged with deuterium. The inability to observe any H−D coupling in the spectrum, despite deuterium being NMR active (I = 1), typified important differences between H−H and H−D spin−spin coupling and can provide an introduction to more advanced NMR concepts such as the magnetogyric ratio. The spectrum was used as an effective explanation of electrophilic aromatic substitution trends due to the clear distinction between near complete exchange at the positions *ortho* and *para* to the activating hydroxyl groups and very minor exchange at the *meta* position. Representative spectra are in the Supporting Information.

By adapting the procedure from a previous work,[15] students visualized the kinetic isotope effect by adding a dilute ethanolic solution of iodine and sodium iodide to the reaction mixture containing deuterated resorcinol and a control mixture of resorcinol and sulfuric acid in D_2O at ambient temperature (Scheme 2). Before addition of the iodine solution, the deuterated mixture and control (nondeuterated) mixture were clear, nearly colorless, and visually indistinguishable. After initial addition of the iodine solution, both the deuterated mixture and

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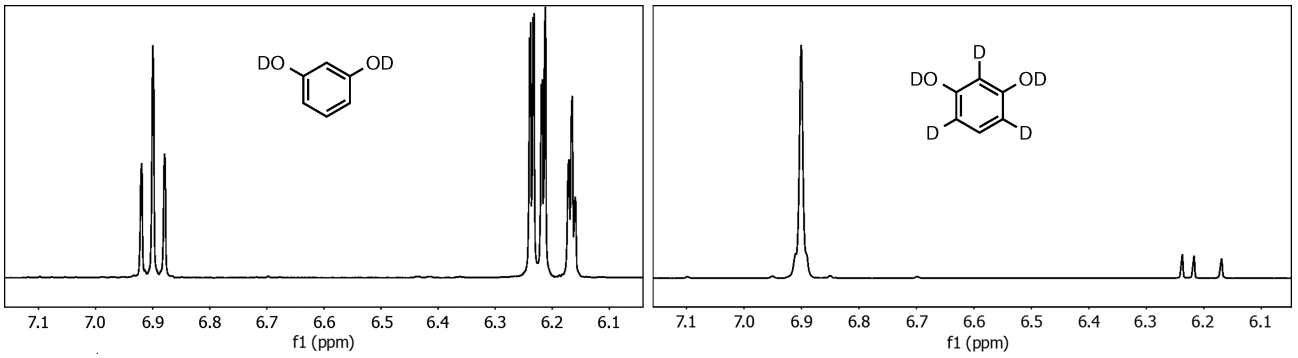
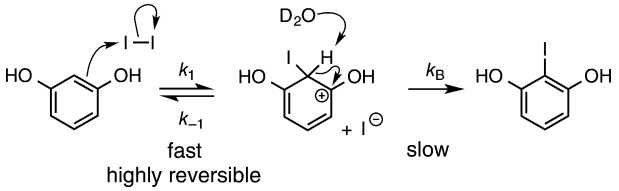


Figure 1. ^{1}H NMR spectra of resorcinol prior to (left) and after (right) H−D exchange.

Scheme 2. Simplified Mechanism for Electrophilic Iodination of Resorcinol



control mixture were translucent and dark orange-red in color. The color faded, and the solutions clarified as the iodine reacted with the resorcinol. In each case, after completion of the reaction, the solution was clear and very light yellow in color, indicating that a trace amount of unreacted iodine may remain.

In the control experiment, the iodine color of the solution faded much more rapidly (i.e., a change was apparent within 5 min) compared to the deuterated example, and the reaction was nearly complete after approximately half an hour. Comparatively, the isotopically labeled resorcinol-d_5 reacted much more slowly, and the reaction remained incomplete after well over an hour. The concentration of iodine remained consistently higher in the vial containing deuterated resorcinol throughout the duration of the experiment, providing a qualitative visual representation of the comparative reactivities of each substrate. The effect was best observed when the experimental and control reactions were conducted side-by-side and a student could observe both results simultaneously. While reaction time and the concentration of iodine varied somewhat from student to student, the trend was very clear in each case and the effect was easily visualized.

The kinetic isotope effect k_H/k_D of the iodination reaction was also determined quantitatively. The reaction of iodine with resorcinol under the aforementioned conditions was found to be second-order overall, providing an excellent opportunity to quantify the KIE by monitoring the UV−vis absorption spectrum of iodine as it reacted. However, because a significant amount of time was required to collect sufficient UV−vis data and the outcome appeared to be somewhat sensitive to minor variances in preparation, previously obtained data were given to students for interpretation. When $1/[I_2]$ versus time was plotted for the control and experimental conditions and the slopes of these plots were compared, a k_H/k_D of approximately 3.4 was calculated. Representative UV−vis absorbance data for this experiment are in the Supporting Information.

STUDENT EXPERIENCES

A key aspect of this experimental methodology is its adaptability to fit the target audience. This experiment was primarily utilized as part of the lab curriculum for first-semester undergraduate organic chemistry students. Prior to conducting the experiment, most students only had a cursory understanding of isotopes and their effect on chemical reactivity. Students were introduced to the concepts of electrophilic aromatic substitution, isotopic exchange, and the kinetic isotope effect as part of the required reading before the experiment. By performing the experiment, students were able to comprehend better EAS and isotopic exchange. Using ^{1}H NMR spectroscopy, they were able to familiarize themselves with the instrumentation and its utility in characterizing such isotopic exchange reactions. Additionally, they interpreted UV−vis absorbance data to quantify the kinetic isotope effect of the iodination reaction and rationalize its rate and mechanism. After the experiment, post-lab write-ups indicated that all students had a significantly greater understanding of EAS and the kinetic isotope effect, and many were interested in the potential applications of the chemistry, including the production of deuterated drugs. The materials given to students, including introductory material as well as pre-lab and post-lab questions, are provided in the Supporting Information.

As a secondary project, the deuteration and iodination reactions were successfully performed and the results interpreted by ambitious high school students looking to gain experience with undergraduate-level chemistry. These students had previous experience with chemistry courses but were not familiar with isotopic substitution reactions or their effects. While some of the more complicated aspects of the transformation were omitted, the fundamental aspects of isotopic exchange could be understood and demonstrated experimentally. Isotope effects were explained by comparing the physical properties of D_2O and H_2O (slightly different molar masses and densities), as well as by monitoring the iodination reaction visually. In each case, students saw firsthand that isotopically substituted compounds often have very similar physical properties but can be differentiated through experimentation. This methodology is amenable to a diverse audience of chemistry students and educators, with the complexity of the material highly adaptable to the desired outcome.

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■ ASSOCIATED CONTENT

Supporting Information

^{1}H NMR spectra from a sample experiment, IR spectra from a sample experiment, UV−vis data from a sample experiment, student laboratory supplement, instructor notes with extensions of the experiment, photo of apparatus. This material is available via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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