Resveratrol Photoisomerization: An Integrative Guided-Inquiry Experiment

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Our department has recently introduced an integrative guided-inquiry laboratory course directed at our third-year undergraduate honors chemistry students as a way to bridge the gap between introductory laboratory experiences and a cumulative fourth-year independent research project. Although there have been several guided-inquiry based experiments targeting first-year general chemistry *(1),* there are few reports of more advanced experiments that provide students with an integrative approach towards scientific research *(2)* illustrating fundamental chemical concepts related to biologically relevant molecules. Resveratrol (3,5,4′-trihydroxystilbene) and its derivatives are a class of recently discovered phytoalexins derived from grape skins, which were first detected in red wine in 1992 *(3).* In addition to its natural antifungicidal properties, resveratrol is increasingly being studied as a novel antioxidant and anticancer phytochemical beneficial to human health *(4).* Recent evidence *(5)* has also demonstrated resveratrol to be a promising chemopreventative agent to extend lifespan in several organisms.

As an extension to previous reports involving trans–cis photoisomerization of stilbenes *(6)* and cinnamic acid derivatives *(7),* this experiment features a comprehensive qualitative and quantitative study of the kinetics of resveratrol photoisomerization (Figure 1) using three different instrumental techniques routinely used in research laboratories. UV irradiation of *trans*-resveratrol is performed directly in a NMR tube during which aliquots of sample are removed at specific time intervals and diluted for UV absorbance and HPLC measurements. The NMR spectra are performed directly in the original sample. Groups of three students are directly involved in the planning and organization of the experiment along with subsequent data analysis, which includes a statistical comparison of apparent rate constant (k_{cis}) and half-life ($t_{1/2}$). This integrative guided-inquiry laboratory is performed in a multiday format to allow students to repeat experiments to improve their technique, as well as verify the reliability of measurements as performed in a typical research environment. Moreover, this experiment strives to stimulate critical thinking, problem solving, and group collaborative skills while students learn fundamental concepts relevant to chemical biology. Students are assessed individually based on their in-lab performance and a written formal report, whereas an oral presentation by the student group is assessed via student peer review and instructor evaluations. The specific objectives of the experiment, as well as general experimental strategies, are provided in the Supplemental Material.[W]

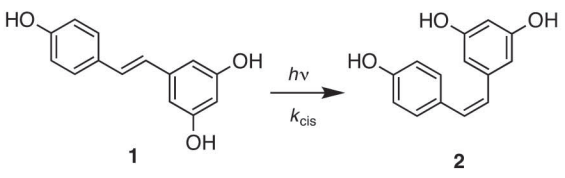

Figure 1. Photoisomerization of *trans*-resveratrol (**1**) into the sterically hindered *cis*-resveratrol (**2**) by UV irradiation in methanol-d_4.

Experimental Procedure

Guided-Inquiry Philosophy of Experiment

As this multiday experiment is designed for third-year honors chemistry students, a detailed "cookbook" description of procedures is omitted. Emphasis is placed on student discovery. A general experimental description, discussion of results, and relevant references are highlighted to provide student groups with sufficient information to organize their own procedure prior to starting the lab. It is suggested that the first lab period is used to perform a trial of the experiment, whereas the three additional lab periods are used to further improve technique with sufficient time for in-lab spectral interpretation and calculations. The major goal of this experiment is to provide students with an integrative approach to research by exploring complementary techniques for studying the photochemical properties of resveratrol. Further details are provided for students and instructor handouts in the Supplemental Material.[W]

Photoisomerization of trans*-Resveratrol*

Since resveratrol is only commercially available as its more stable trans stereoisomer, photoisomerization experiments were performed with 20 mM *trans*-resveratrol prepared in 1.5 mL of degassed methanol-d_4 using a micropipettor. The resveratrol solution was mixed in a 1.5 mL microcentrifuge tube and then transferred into a clean NMR tube using a long-neck Pasteur pipet. The NMR tube was then suspended inside a photochemical reactor chamber (Rayonet Photochemical Reactor, RPR-100, Hamden, CT) exposing the resveratrol solution to 350 nm radiation (RPR-3500 lamp) over a 48 min total time interval. At six carefully timed intervals during the study, the NMR tube was removed from the photochemical reactor and then gently mixed prior to withdrawing about a 30 μL aliquot using a disposable glass long-neck Pasteur pipet into a 0.5 mL microcentrifuge tube. This aliquot was then used to prepare diluted 40 μM and 120 μM resveratrol solutions for UV absorbance (4.0 mL) and reverse-phase HPLC (1.0 mL) experiments, respectively. Students should note the resveratrol concentrations suggested in the experiment reflect the different inherent sensitivity of each technique. It is important to clarify that the 30 μL aliquot was only an approximate volume sufficient for preparing subsequent dilutions. Students should practice using the long-neck Pasteur pipet for sampling directly from the NMR tube prior to photoisomerization. All

sample dilutions were accurately prepared using micropipettors in 0.5 mL, 1.5 mL, or 15 mL centrifuge tubes with sufficient volumes for rinsing cuvettes or injection ports. Once the aliquot was obtained, the resveratrol solution in the NMR tube was scanned directly by ^{1}H NMR. Afterwards, the NMR tube was resuspended into the UV photochemical reactor and this procedure was repeated throughout the experiment. It is important that groups organize the role of each student, as well as perform calculations of the required dilutions prior to starting the experiment. Note that it was necessary that all NMR and centrifuge tubes were covered in aluminum foil to avoid photodegradation in ambient light during sample handling prior to analyses. Also, preliminary measurements of *trans*-resveratrol at 0 min were completed before starting the photoisomerization.

^{1}H NMR Spectrometry

All ^{1}H NMR spectra of resveratrol trans–cis photoisomerization were performed on a Bruker AV 200 NMR spectrometer locked on methanol-d_4 using a standard single pulse acquisition at 30° spin angle with 24 average scans. Upon spectra acquisition of all samples, it is suggested that students carefully assign the resveratrol stereoisomers, **1** and **2**, based on characteristic chemical shifts, coupling constants, and relative peak integrals. For quantitative estimation of the relative percentage of *trans*- and *cis*-resveratrol during photoisomerization based on relative peak integration, students are recommended to select a pair of associated resolved proton resonance peaks for **1** and **2**. There was no evidence of photodegradation during the 48 min interval from NMR spectra, thus the total concentration of resveratrol at any time was 20 mM.

UV Absorbance Spectrometry

All UV absorbance measurements were performed on a dual-beam Varian Cary 100 spectrometer and 40 μM resveratrol solutions in methanol were scanned over a range of 200–400 nm at a resolution of 100 nm/min after background correction with solvent. Since there is no *cis*-resveratrol standard available, estimation of the average molar absorptivity of **2** was determined by using mole percent (%) *cis*-resveratrol derived from ^{1}H NMR experiments. Calculation of the percent of *trans*-resveratrol versus *cis*-resveratrol was then assessed by dual-wavelength UV absorbance measurements based on a binary mixture using the Beer–Lambert law. It is recommended that students carefully examine spectral changes upon resveratrol photoisomerization, as well as identify characteristic absorption bands for **1** and **2**.

Reverse-Phase HPLC

Reverse-phase HPLC experiments were performed using a Varian ProStar 230 system with 325 UV–vis detector at a flow rate of 1.0 mL/min. The stationary phase consisted of a 5 μm Varian Pursuit C18 (25 cm × 0.46 cm) column and the mobile phase was a binary solvent system of deionized water and methanol with single UV wavelength detection at 300 nm. A linear step gradient elution was programmed between 4–6 min using 0–100% methanol to ensure sufficient resolution of *trans*- and *cis*-resveratrol under 8 min. Reequilibration of the column was then performed for 4 min in deionized water prior to subsequent analyses. Owing to differences in resveratrol isomer absorbance responses at 300 nm, a correction factor was calculated based on the trans/cis molar absorptivity ratio from UV absorbance spectrometry measurements as a way to normalize resveratrol peak areas determined by HPLC. In addition, the retention factors, selectivity factor, and resolution of reservatrol stereoisomers were calculated directly from their chromatograms.

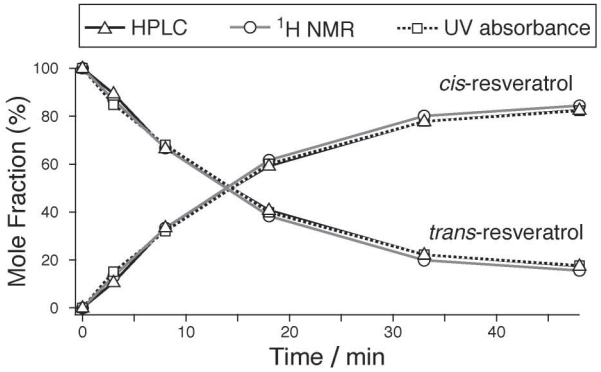

Figure 2. Assessment of the rate of photoisomerization of *trans*-resveratrol into *cis*-resveratrol by ^{1}H NMR, UV absorbance, and HPLC.

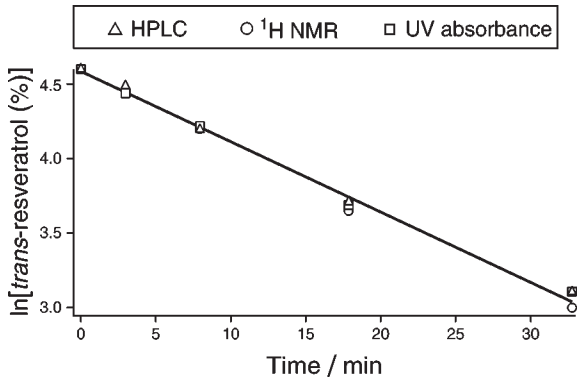

Figure 3. Determination of apparent rate constant (k_{cis}) and half-life ($t_{1/2}$) of *trans*-resveratrol photoisomerization based on pseudo-first-order kinetics by ^{1}H NMR, UV absorbance, and HPLC.

Hazards

Since UV radiation is harmful to the eyes, students who are involved with the photoreactor should use UV safety glasses or goggles. Methanol should be handled with caution since it is toxic, flammable, and a potential fire hazard.

Results and Discussion

The changes in the mole percent (%) of *trans*- versus *cis*-resveratrol upon UV irradiation as a function of time as determined by ^{1}H NMR, UV absorbance, and HPLC are shown in Figure 2. The data are based on triplicate measurements by students over three different days. It is apparent that after 48 min of UV irradiation, greater than 82% of *trans*-resveratrol has been photoconverted to its cis-stereoisomer, as supported by a recent study *(3)*. Moreover, it is evident that all three techniques were able to quantitatively measure resveratrol photoisomerization with good precision (CV < 5%) that yielded similar results. This integrative experiment demonstrates that each technique is complementary since information provided by ^{1}H NMR was used to estimate the molar

absorptivity of *cis*-resveratrol without the need for its purification, whereas UV absorbance data were used to normalize the different resveratrol responses derived from chromatograms using HPLC with single wavelength UV detection. The overall goal of this study was to determine and statistically compare k_{cis} and $t_{1/2}$ of *trans*-resveratrol photoisomerization based on pseudo-first order kinetics. Figure 3 depicts a first-order rate plot of *trans*-resveratrol photoisomerization within a 33 min time interval, using data from Figure 2, that clearly demonstrates good correlation among the three different techniques with excellent linearity as reflected by a R^2 of 0.9972. Comparison of k_{cis} and $t_{1/2}$ values generated by each procedure confirmed that the results were statistically equivalent at 95% confidence level with an overall mean of (0.0472 ± 0.0021) min^{-1} and (14.7 ± 0.7) min, respectively. Note that $t_{1/2}$ corresponds to the intersection point of *trans*- and *cis*-resveratrol photoisomerization curves in Figure 2. Since the stereochemistry of resveratrol can influence its biological activity in vivo, a fundamental understanding of its photostability along with methods to characterize its isomer composition is relevant to its application as a promising therapeutic agent in future clinical studies *(4, 5)*. In fact, HPLC with photodiode array detection *(8)* is often used for analysis of resveratrol metabolites present in human plasma samples owing to its sufficient sensitivity and selectivity.

Supplemental Material

Handout for students and information for instructors in adopting this experiment, as well as resveratrol spectral data and calculations, are available in this issue of *JCE Online*.

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