ORGANIC

UNDERGRADUATE EXPERIMENT

Food Additives

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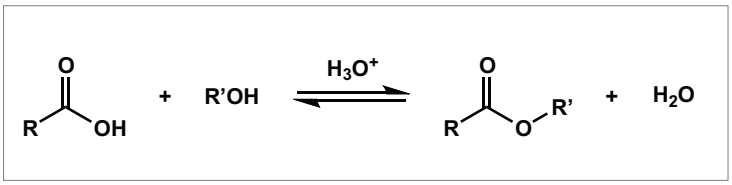
INTRODUCTION

The laboratory component of an undergraduate chemistry course can often be the largest contributor to productive learning, as students are able to relate challenging in-class material to real-life scenarios via hands-on experiments and visual aids. During the laboratory component, students gain experience with fundamental concepts including polarity, solubility, extraction/isolation, and characterization techniques (*e.g.*, ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy, ultraviolet-visible absorption spectroscopy, etc). Nuclear magnetic resonance (NMR) spectroscopy is one of the most valuable tools available to organic chemists in terms of molecular characterization, as it provides critical information for structural elucidation, which includes atom connectivity, chemical environment, and functional group identification. Although NMR can provide such a plethora of information, a large factor contributing to it sometimes being overlooked as a technique relates to the costs required to operate and maintain traditional high-field spectrometers. However, with the increasing popularity of benchtop NMR instruments due to their affordability, practicality, and small footprint, students are effectively able to collect NMR spectra during their laboratory blocks with little to no unnecessary time expenditures.

An important component that plays a key role in keeping food safe for consumption are food additives.[1] Food additives are widely used throughout the food industry and are generally recognized as safe, meaning they do not pose a health risk upon human consumption, provided that they are not used over the maximum amount proposed by the governing safety standards.[1-3] Although there are many different groups and subgroups of food additives, as well as many molecules within these groups, this sample experiment will focus on propyl gallate. Propyl gallate is used as an antioxidant that prevents peroxides from forming in fat/oil-containing food to allow the lifetime and therefore shelf-life of said food to be prolonged.[2] Generally, oxidation is a naturally occurring reaction where chemicals will react with the oxygen in the air and begin to deteriorate via free radical formation, essentially creating a chain reaction upon propagation of this radical.[1] Propyl gallate, known for its radical scavenging

properties, inhibits/delays this by interrupting the self-propagating chain reaction.[1,4]

The work performed in this sample experiment is based on a recent publication by Borges *et al.* in *The Journal of Chemical Education*.[1] Herein, propyl gallate is synthesized via a commonly studied first-year organic chemistry reaction, the Fischer esterification, a general reaction scheme for which is shown in **Scheme 1**.



Scheme 1. *General reaction scheme for the Fischer esterification reaction.*

In their paper, ^{1}H and ^{13}C{^{1}H} NMR spectroscopy was used to confirm formation of the product and the antioxidative properties of propyl gallate were studied using an ultraviolet-visible (UV-Vis) spectrophotometer. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) the antioxidative ability of propyl gallate and gallic acid were measured against the standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), which is used as the baseline for antioxidative strength.

In this work, the ^{1}H and ^{13}C{^{1}H} NMR spectra were acquired using a 60 MHz benchtop spectrometer, showcasing the benefits of benchtop NMR incorporation into undergraduate teaching labs and allowing students access to multiple characterization techniques.

PROCEDURE

Materials

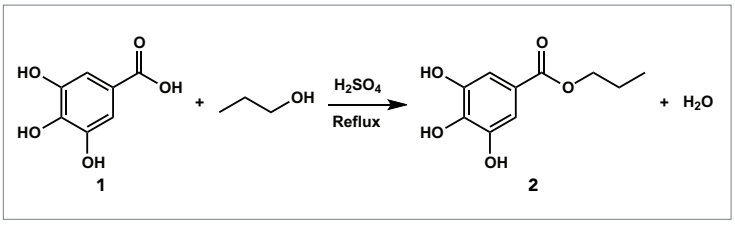
Gallic acid monohydrate (≥98.0%), sulfuric acid (95-98%), 1-propanol (≥99.9%), anhydrous diethyl ether (≥99.7%), sodium bicarbonate (≥99.5%), magnesium sulfate (≥99.5%), hexanes (≥97.0%), and DMSO-d_6 (99.9%) were purchased from MilliporeSigma and used without further purification.

Instrumentation

All NMR data was obtained using a Nanalysis 60PRO instrument. The ^{1}H experiments were performed using the following acquisition parameters: spectral width, 40 ppm; spectral center, 10 ppm; number of points, 8192; number of scans, 16; dummy scans, 0; interscan delay, 1 second for gallic acid (**1**), 6 seconds for propyl gallate (**2**); pulse angle, 90°; receiver gain, auto. The ^{13}C{^{1}H} experiments were performed using the following acquisition parameters: spectral width, 220 ppm; spectral center, 100 ppm; number of points, 4096; number of scans, 256 for gallic acid (**1**), 4096 for propyl gallate (**2**); dummy scans, 0; interscan delay, 0 seconds; pulse angle, 61.82°; receiver gain, auto. All spectra were manually corrected for phase and baseline distortions using the MestReNova software (v14.1.1).

Synthesis

The synthesis of propyl gallate (**2**) was adapted from a literature procedure outlined by Borges *et al.* and is outlined in **Scheme 2.**[1]



Scheme 2. *Preparation of propyl gallate (**2**) from the reaction of gallic acid (**1**) with 1-propanol under reflux conditions using concentrated sulfuric acid as a catalyst.*

Gallic acid (1.50 g, 8.83 mmol) was dissolved in 1-propanol (15 mL) in a 50 mL round-bottom flask. Concentrated sulfuric acid (1 mL) was added dropwise to the reaction mixture and was heated to reflux for 1.5 hours. Note: Borges *et al.* monitor this reaction by thin layer chromatography,[1] but this step was omitted in our experiments. The reaction was removed from the heat and allowed to cool to room temperature. Water (15 mL) was added and the mixture was extracted using diethyl ether (3 x 15 mL). The organic phases were combined and washed with 10% sodium bicarbonate (3 x 15 mL), followed by water (15 mL). The solution was dried with magnesium sulfate, filtered and concentrated *in vacuo*. The solid was collected and recrystallized using diethyl ether and cold hexanes. The final product was filtered, thoroughly dried *in vacuo* and collected as a white solid (**Figure 1**).

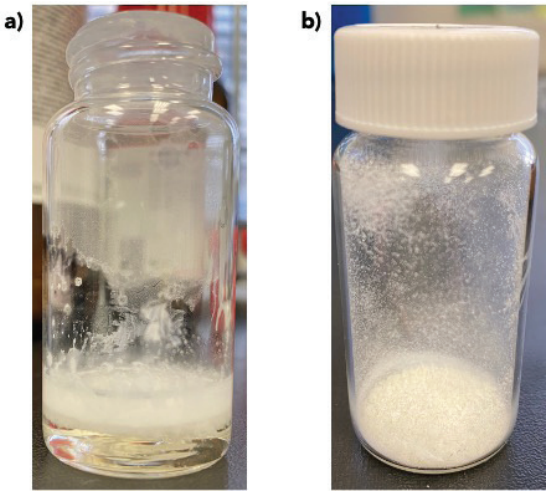


Figure 1. a) *Recrystallization of propyl gallate (**2**) in diethyl ether and hexanes.* ***b)*** *Final purified product.*

RESULTS AND DISCUSSION

The ^{1}H and ^{13}C{^{1}H} NMR spectra of both the starting material (**1**) and final product (**2**) were collected and their spectra were compared. The ^{1}H (top) and ^{13}C{^{1}H} (bottom) NMR spectra for **1** and **2** are shown in **Figure 2** and **Figure 3**, respectively.

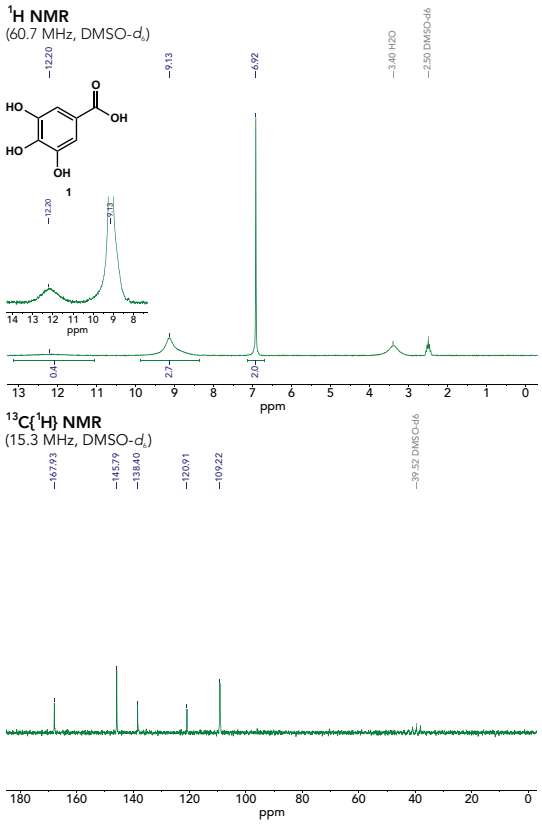


Figure 2. *^{1}H (60.7 MHz, top)) and ^{13}C{^{1}H}) (15.3 MHz, bottom) NMR spectra of gallic acid (**1**) in DMSO-d_6.*

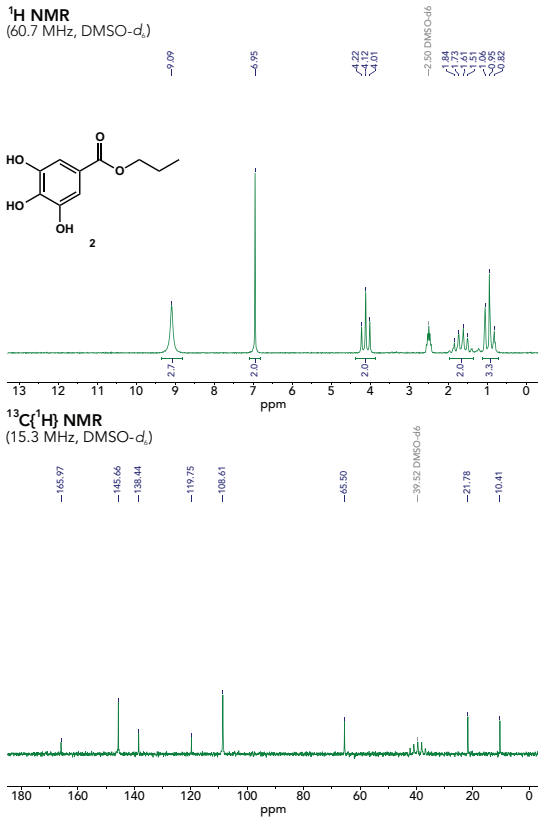


Figure 3. *^{1}H (60.7 MHz, top) and ^{13}C{^{1}H}) (15.3 MHz, bottom) NMR spectra of propyl gallate (**2**) in DMSO-d_6.*

As the starting material and final product are nearly identical, the main difference between the ^{1}H spectra of **1** and **2** is the incorporation of a propyl group, wherein the carboxylic acid moiety is transformed into an ester group. Upon comparison of the ^{1}H spectra of the starting material and product, the conversion of the carboxylic acid group is confirmed by the loss of a broad peak at 12.20 ppm in **Figure 3** (top) and the appearance of the 3 resonances associated with the incorporation of the propyl group. These new peaks are observed as a triplet, multiplet, and triplet centered at 4.12, 1.61, and 0.95 ppm and integrate to 2, 2, and 3.3 protons, respectively, which is expected for a propyl functionality. The remaining signals in the ^{1}H spectra for these two molecules are nearly identical, with two broad peaks at 9.13 ppm and 12.20 ppm in **Figure 2** and one in **Figure 3** at 9.08 ppm that integrate to approximately 3, which relates to the 3 –OH groups on the phenyl ring. Additionally, the singlet at 6.92 ppm in **Figure 2** (6.95 ppm in **Figure 3**) corresponds to the 2 protons in the *meta* position of the phenyl ring.

Similarly, in the ^{13}C{^{1}H} spectra, the characteristic propyl peaks absent in the starting material are observed in the final product. As evident in **Figure 3** (bottom), these characteristic signals at 10.41 ppm, 21.78 ppm, and 65.50 ppm allow for simple elucidation of the propyl fragment of structure **2**. The most shifted peak at 167.93 ppm in **Figure 2** (165.97 ppm in **Figure 3**) is related to the carbonyl carbon, which is where we would expect this peak to be. In total, the phenolic ring gives rise to 4 distinct peaks as the 2 *meta* and 2 *ortho* carbons are in 2 distinct chemical environments. In **Figure 2**, the *meta*, *para* and *ortho* carbon peaks appear at 145.79 ppm, 138.40 ppm, and 120.91 ppm, respectively (145.66 ppm, 138.44 ppm, and 119.75 ppm in **Figure 3**). These are defined in relation to the aromatic carbon adjacent to the carbonyl group, which resonates at 109.22 ppm in **Figure 2** (108.51 ppm in **Figure 3**).

For a thorough discussion on the use of the diphenyl-2-picrylhydrazyl (DPPH) method in determining the antiradical reactivity of gallic acid and propyl gallate using UV-Vis spectrophotometry, the reader is encouraged to read the original publication by Borges *et al.*[1] Therein, it was concluded that the functional group conversion from the carboxylic acid (gallic acid) to the ester (propyl gallate) showed an increased radical-scavenging activity, which was much higher than that of the Trolox standard.[1]

Conclusion

With benchtop NMR spectroscopy, students can more easily add NMR experience to their repertoire of instrumentation knowledge. Specifically, benchtop NMR allows students to work directly with an NMR spectrometer and obtain multinuclear spectroscopic data (*i.e.*, ^{1}H and ^{13}C spectra) during their laboratory period and can therefore elucidate their products in real time. In this sample experiment, a common food additive, propyl gallate (**2**) was synthesized via a simple Fischer esterification from gallic acid (**1**) and 1-propanol using an acid catalyst. The ^{1}H and ^{13}C spectra of **1** and **2** were obtained and compared to validate successful formation of the final product. With the ease of use of the benchtop NMR spectrometer, it can be easily incorporated and combined with other techniques to give students much needed characterization skills in undergraduate laboratories.

References

[1] Garrido, J.; Garrido, E.M.; Borges, F. *J. Chem. Educ.* **2012**, *89*, 130-133.
[2] Hooker, E.; Becker, L. Final Report on the Amended Safety Assessment of Propyl Gallate. *Int. J. Toxicol.* **2007**, *26* (Suppl. 3), 89-118.
[3] European Parliament and Council Directive 94/34/EC. https://www.fsai.ie/uploadedFiles/Dir89.107.pdf (accessed February 2022).
[4] Medina, M.E.; Luga, C.; Alvarez-Idaboy, J.R. *Phys. Chem. Chem. Phys.* **2013**, *5*, 13137-13146.
[5] Garcia, E.J.; Oldoni, T.L.C.; Alencar, S.M.; Reis, A.; Loguercio, A.D.; Grande, R.H.M.; *Braz. Dent. J.* **2012**, *23*, 22-27.