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Liquid CO_2 Extraction and NMR Characterization of Anethole from Fennel Seed: A General Chemistry Laboratory

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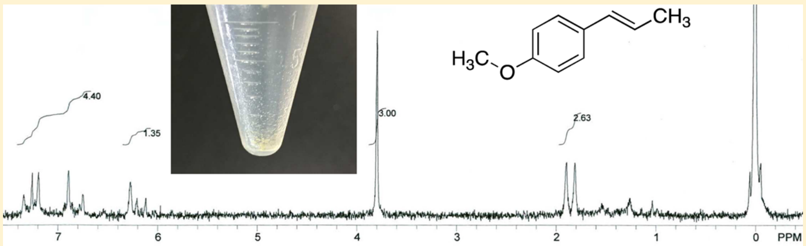
Ⓢ *Supporting Information*



ABSTRACT: An established experiment of liquid CO_2 extraction has been expanded to extraction of natural products from spices and adapted to incorporate NMR spectroscopy into the undergraduate general chemistry laboratory. The first week of this two-week experiment involves the extraction of anethole from fennel seeds using liquid CO_2. The second week includes analysis of the fennel extract by ^{1}H NMR spectroscopy and thin layer chromatography.

KEYWORDS: *First-Year Undergraduate/General, Laboratory Instruction, Hands-On Learning/Manipulatives, Food Science, Natural Products, Green Chemistry, Phases/Phase Transitions/Diagrams, Chromatography, NMR Spectroscopy*

INTRODUCTION

Unique, contemporary, and challenging laboratory experiments, particularly in introductory college chemistry, are necessary to engage students of all calibers. Development of multidisciplinary experiments allows for more in-depth study and opportunities for connections across diverse general chemistry topics. Green chemistry has been increasingly the focus of a wide array of experiments at all levels of chemistry to impress upon students the importance of cleaner and less hazardous techniques. In particular, liquid carbon dioxide extraction of fruit and seed oils was the focus of a seminal work[1] and subsequent extensions[2,3] that have made the procedure accessible for a general chemistry laboratory.

While green chemistry is becoming much more commonplace in introductory chemistry, nuclear magnetic resonance (NMR) spectroscopy still remains a nontraditional topic that is primarily introduced in organic chemistry. However, there are several experiments incorporating NMR spectroscopy in general chemistry that make its presentation accessible and effective.[4−7] The significance of NMR spectroscopy in general chemistry is that it can supplement the basic introduction to organic structures and functional groups often studied in general chemistry and can provide a foundation for more in-depth study for the bulk of students who go on to enroll in organic chemistry.

The goal of this experiment is to combine green chemistry and NMR spectroscopy into one common experience that blends the concepts of organic chemistry, phase changes and phase diagrams, and intermolecular forces. The two-week laboratory is performed while the same topics are simultaneously presented in a classroom curriculum, making for a highly concerted approach to convey and reinforce the material. This experiment has been performed in the spring semesters of 2014 and 2015 for approximately 60 students per year in a second-semester general chemistry laboratory course.

EXPERIMENTAL OVERVIEW

The present experiment is a significant modification of and addition to two published experiments.[1,2] While extraction of limonene from orange peel was very successful, anethole (Figure 1) is the target of extraction from fennel seed (*Foeniculum vulgare*). Liquid CO_2 extraction of anethole from fennel seed is advantageous in the present experiment for many reasons:

1. Fennel seed is cheap and readily available.
2. Anethole is nontoxic and generally regarded as safe (GRAS).[8]

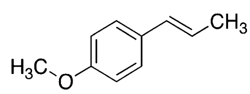


Figure 1. Chemical structure of anethole, the main constituent of fennel seed extract.

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3. Anethole is obtained in good yield and represents the majority of the material obtained from various isolation methods including steam-distillation and supercritical CO_2 extraction.[9−12]
4. The ^{1}H NMR spectrum of the extract exhibits well separated signals, facilitating interpretation for students experiencing NMR spectroscopy for the first time.

In the first week of the experiment, students working in groups of two or three focus on the extraction of anethole from fennel seed via liquid CO_2. In the intervening week, students are provided with a primer on NMR spectroscopy and complete basic NMR exercises. For the second week, student groups are assigned 30 min blocks of time during which they prepare an NMR sample from their extract and acquire and begin analysis of its ^{1}H NMR spectrum. After each group is finished using the NMR spectrometer, they perform TLC analysis on the extract, co-spotting with several other components often found in fennel seed. A brief summary of the experiment follows and complete details can be found in the Supporting Information.

Experiment

Week One: Extraction. Ground fennel seeds (approximately 1.4 g) are sealed in a tea bag pouch. This pouch is placed in a 15 mL polypropylene centrifuge tube, packed with finely crushed dry ice, tightly capped, and placed in a plastic cup filled with hot tap water (approximately 50−60 °C). The subsequent sublimation and condensation of the solid CO_2 caused by the high internal pressure of the tube forms liquid CO_2 that submerges the pouch allowing for extraction of anethole and other components. After the liquid boils off through minute gaps in the thread seal (approximately 4−5 min), and upon removal of the pouch, the extracted anethole is obtained as a coating on the bottom of the tube. The extraction procedure is repeated with the same pouch. The tube is tightly capped, labeled, and stored in a freezer at 0 °C.

Week Two: NMR Spectroscopy and TLC. *NMR Spectroscopy.* For each 30 min block, a student group retrieves their extract from the freezer and prepares an NMR sample by adding 0.50−0.75 mL of deuterated chloroform directly to the centrifuge tube. The dissolved extract is transferred with a pipet into an NMR tube. With guidance from an instructor, students perform all of the operations to acquire an FID and produce a properly processed spectrum.

TLC. The deuterated chloroform solution is spotted onto a TLC plate next to pure samples (neat or as ethanolic solutions) of the reported most high yield components of fennel seed extract for comparison: anethole, fenchone, estragole, and α-pinene.[10] The plates are developed in a *n*-heptane/ethyl acetate (80:20, v/v) mobile phase and visualized with a UV lamp. R_f values are calculated for each spot.

HAZARDS

Safety goggles should be worn throughout the duration of each week of the laboratory. Dry ice is extremely cold and can cause frostbite in a very short period of time touching bare skin or through the plastic of the centrifuge tube. Insulated gloves should be used for any dry ice manipulations. Because the sealed tubes with dry ice represent an explosion hazard, all extractions should be performed in a fume hood with a lowered sash. A clear plastic cup, as opposed to any other glass container, is the best choice for a warm water bath. Rarely will a pressurized tube explode, but it is imperative that tubes not be sealed until they are safely behind the hood sash. Anethole is generally regarded as safe,[8] but it can be a skin and respiratory irritant. Fenchone is flammable and a skin irritant. Estragole is flammable, a skin irritant, and a suspected carcinogen. α-Pinene is a skin and respiratory irritant. Chloroform-*d* is a carcinogen. Ethanol, *n*-heptane, and ethyl acetate are flammable and toxic if swallowed. All liquids should be transferred to a waste container for proper disposal.

RESULTS AND DISCUSSION

Liquid CO_2 extraction of anethole from fennel seed is performed similarly to the extraction procedure developed by McKenzie et al.[1] with several modifications (Figure 2). The extraction yields approximately 15−30 mg of product. ^{1}H NMR and TLC analyses of the product reveal that the major component is anethole.

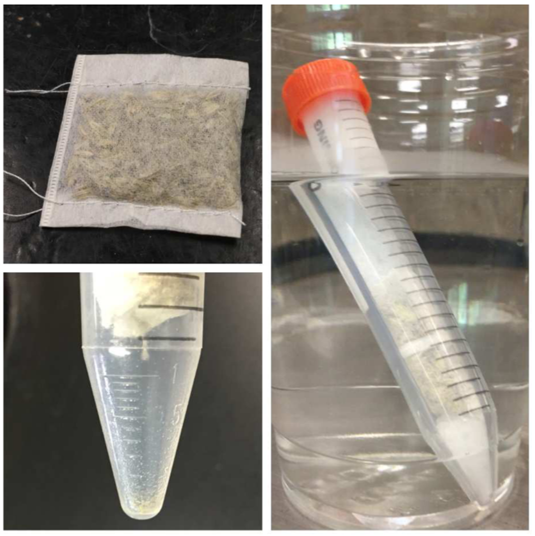

Figure 2. Liquid CO_2 extraction of anethole from fennel seed. Starting in upper left and then clockwise: Sewn tea bag pouch with fennel seed prior to extraction; centrifuge tube packed with dry ice and pouch and placed in warm water bath; bottom of centrifuge tube after extraction showing extracted residue.

This general chemistry experiment was designed to be a new and contemporary way to emphasize multiple concepts in a connected, two-week lab, and also to begin an introduction to build excitement for organic chemistry. The extraction section of the experiment was an opportunity for students to observe unusual phase changes, to make estimations of pressures and temperatures within the tube, and to explore how intermolecular forces influence extractions. Additionally, comparisons are made to other less green methods of extraction (organic solvent extraction, steam-distillation), and their advantages and disadvantages evaluated.

The question after the extraction was how to identify the components of the extract. There are many methods to ascertain the identity of the extract, but this experiment focused on NMR spectroscopy and TLC. Students had previously utilized TLC to separate pigments from plant material,[13] but had not co-spotted with pure material, so this experiment served to reinforce and extend these concepts. Students were introduced to chemical drawing software for the first time and completed structures of all the molecules spotted on their plates. Students calculated R_f values and considered how those

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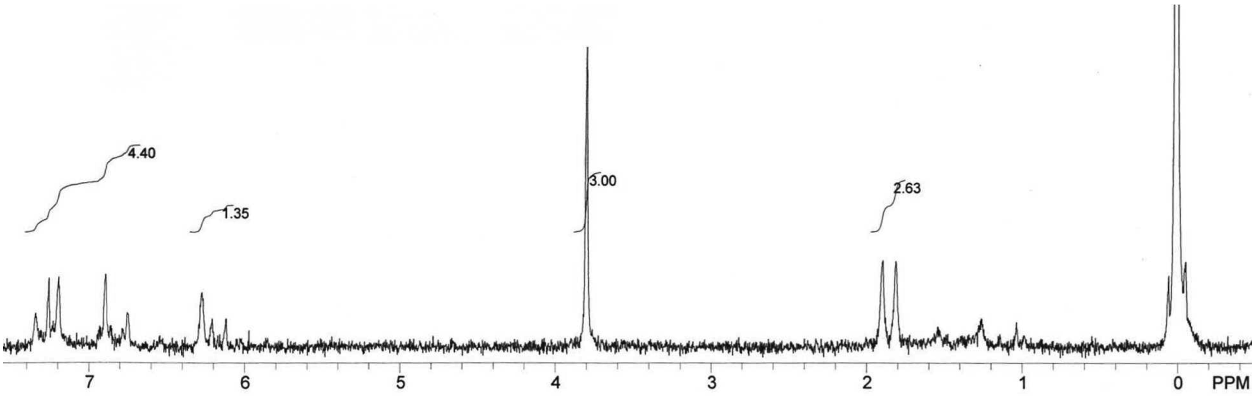

Figure 3. Representative student ^{1}H NMR spectrum of fennel seed extract, showing nearly pure anethole.

values are influenced by molecular structure and TLC mobile phase composition.

NMR spectroscopy represented a fairly sophisticated concept to cover in an introductory chemistry course, but treated simply as another type of spectroscopy (i.e., as a relative of visible spectroscopy with which the students were familiar) and covering only the very basic concepts, students were able to begin to understand this technique.

Fennel seed was chosen very carefully for this experiment because its extract yields almost completely pure anethole, whose ^{1}H NMR spectrum is characterized by well-separated and well-defined signals (Figure 3). In that sense, it was a simple task for students to find a reference spectrum of pure anethole online and to confirm that their spectrum was a match. A more thorough analysis of the spectrum was assigned for students to explain chemical shifts, splitting patterns, and integrations of each signal. In particular, a focus was placed on the signals for the two methyl groups to explain their different splitting patterns and unique chemical shifts. Students are able to identify the peaks at δ = 3.8 and 1.8 ppm as methyl groups based on their integration values. Students then assign the singlet to the methoxy methyl due to the absence of neighboring protons that would split the signal.

Students were asked to consider explanations for signals in their spectrum that did not correlate with anethole. Utilizing reference tables of functional group chemical shifts and common impurities,[14] students are able to identify the residual solvent peak for chloroform (δ = 7.2 ppm), TMS (δ = 0.0 ppm), and water (δ = 1.5 ppm). Students were directed to find reference spectra for the other fennel seed components (such as those used in the TLC portion of the experiment) to attempt to identify signals appearing between δ = 1−2 ppm, however identification was inconclusive.

This introduction to NMR spectroscopy laid a foundation on which students will build a deeper understanding in organic chemistry. The lab worksheet provided assessment of the pedagogic goals of the lab: use of the NMR instrument, including sample preparation; interpretation of NMR spectra, specifically splitting patterns and integrations; and calculation of R_f values for TLC.

■ SUMMARY

The combination of a liquid CO_2 extraction with NMR and TLC analyses of an extract represents an accessible and compelling general chemistry experiment. A green, natural product extraction allowed students to recognize the many advantages of this technique compared to other more traditional methods. TLC and NMR analyses of the extract showed how complementary methods of characterization can assist in confirming identities of unknown compounds. This experiment creates a solid foundation for the many students who move on to organic chemistry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00689.

> Student handouts and pre-lab assignments to prepare for each week of the laboratory; student worksheets for post-lab analysis; instructor notes, including answers to pre-lab and post-lab assignments; representative ^{1}H NMR spectrum of fennel seed extract with peak assignments; representative TLC plate with R_f values (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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