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Laboratory Experiment

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Organic Chemistry and the Native Plants of the Sonoran Desert: Conversion of Jojoba Oil to Biodiesel

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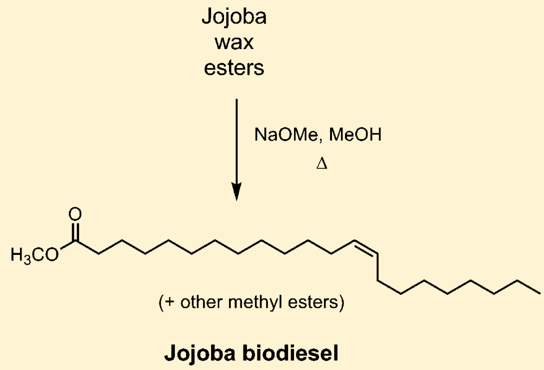
S *Supporting Information*

ABSTRACT: A new, general approach to the organic chemistry laboratory is introduced that is based on learning about organic chemistry techniques and research methods by exploring the natural products found in local native plants. As an example of this approach for the Sonoran desert region, the extraction of jojoba oil and its transesterification to biodiesel are presented. The oil of jojoba seeds contains a large quantity of wax esters that are smoothly converted to biodiesel using various methods. The biodiesel is isolated using preparative thin layer chromatography, and infrared and NMR spectroscopies are used to evaluate the product. Either traditional expository ("cookbook") or guided inquiry approaches may be used.



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■ INTRODUCTION

The Sonoran desert, which encompasses Maricopa County, Arizona, contains hundreds of diverse native plant species. Natural products derived from these plants have found extensive application in traditional medicine[1] and materials,[2] and many of the plants have been used historically as food sources for native peoples.[3] Some applications already are known anecdotally to students in the region, for example, the use of mesquite seedpods to make flour; thus, inclusion of experiments in the organic chemistry laboratory involving these plants carries potential for considerable student interest. Any investigation of the chemistry of Sonoran desert native plants is potentially interdisciplinary—organic chemistry and biochemistry, botany, ecology, ethnobotany, microbiology, and pharmacology all are involved. Further, the chemical profiles of many of these plant species have not been fully characterized, which makes them ideal candidates for further study by students wishing to participate in independent research. Natural product isolation and structure elucidation experiments already are included in many organic chemistry laboratory curricula,[4] but a focus on local species has scant precedent,[5] and existing natural products lab activities generally lack the potential to become more challenging student research experiences. Science education research verifies that heightened student interest and engagement occur when lab activities parallel "real" research methods,[6] and the benefits of an active undergraduate research program are numerous.[7] These findings have encouraged us to revisit the organic chemistry laboratory curriculum to seek ways to provide a more relevant, engaging, and "real world" experience for students that encompasses potential for independent study outside lab courses.

A new pedagogical approach has been initiated in the second-year organic chemistry laboratory courses using the broad utility of Sonoran desert native plants as a focal point for undergraduate education and research. Investigation of plants that are suitable for student experimentation has resulted in the development of several new lab experiments for both semesters of organic chemistry as well as a growing undergraduate research program based on the natural products of local native plants. Project goals are first to introduce students to fundamental organic research methods, instrumentation, and spectroscopic techniques, and second to provide opportunities to explore critical thinking, problem solving, and decision-making in the context of natural product extraction, isolation, characterization, and other activities. Here, one of the experiments is presented.

Jojoba (*Simmondsia chinensis*) is a small waxy-leaved shrub native to the Sonoran and Mojave deserts. Its seeds contain an oil composed of mostly long-chain esters ("wax esters"); 87% of these are derived from the condensation of eicosenoic and docosenoic fatty acids and eicosenol and docosenol[8,9] (Figure 1). This oil, which constitutes up to 55% of the mass of the seeds, is used in numerous cosmetic products and has been explored as a biodiesel fuel.[10−13] Jojoba oil releases considerable energy on combustion and is stable at the high operating temperatures of a diesel engine. The oil itself and the biodiesel produced from it have viscosities slightly outside of

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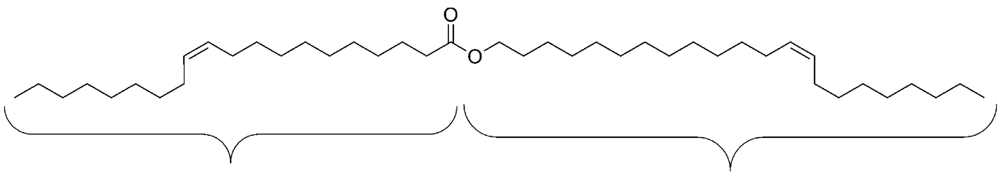

Figure 1. Docosenyl eicosenoate, one of the major wax ester components of jojoba oil.

the normally accepted ranges for diesel fuel, but this issue can be circumvented by using the jojoba-derived biodiesel in a blend with regular diesel.[9−13] Other jojoba biodiesel fuel properties compare favorably to regular diesel. Although jojoba presently is not an economically viable fuel source, it serves as an excellent example of how biodiesel can be synthesized while illustrating the utility of local flora.

Biodiesel, a fuel composed of monoalkyl esters of long chain fatty acids derived from vegetable oils or animal fats has inspired significant research interest.[14] Undergraduate experiments involving production and analysis of biodiesel have been reported extensively in this *Journal* and elsewhere.[15−28] Experiments range from high school[25,26] to undergraduate levels and incorporate organic and analytical[18,22,27] chemistry and guided inquiry approaches. A variety of oil sources have been used (e.g., vegetable,[16,17,20,23] soybean,[24] and seed oils;[19] algae[28]), and all experiments rely on the transesterification of triglycerides, the common component to all the substrates described. However, jojoba is unique in that its oil does not contain significant amounts of triglycerides, the most common biodiesel precursor, but rather wax esters.[10] Its use in the present experiment is also distinctive in that it is a local, readily available plant source; students can actually collect their own seedpods for the experiment. Although jojoba transesterification requires a purification step (either chromatography or crystallization) that is unnecessary for triglyceride transesterification reactions where the water-soluble byproduct is easily removed, students benefit from comparison of the two different starting materials and the methods used to convert each of them to biodiesel.

■ EXPERIMENT

The jojoba transesterification experiment has been used in second-semester organic chemistry laboratory courses every semester since Spring 2011 (30−50 students per semester). The experiment can be conducted comfortably in two or three 3-h lab periods. Because jojoba oil is commercially available, this experiment can be performed in any geographical location, although it was designed using the "local native plant" theme. Detailed information about the experiment is available in the Supporting Information.

The method involves the following steps:

(1) Jojoba oil is extracted from jojoba seeds.
(2) The jojoba oil is transesterified to produce biodiesel.
(3) The components of the reaction mixture are separated chromatographically.
(4) The isolated methyl ester is analyzed spectroscopically.

Students typically work in pairs. Students obtain 12−15 g of jojoba seeds and grind them to meal in a small coffee grinder, reflux the powder in a hydrocarbon solvent, and remove the meal by filtration. The oil recovered from the extraction is transesterified using sodium methoxide in methanol and refluxing for 90 min. Reaction progress is monitored by thin layer chromatography at 30, 60, and 90 min. After neutralization and recovery of the crude biodiesel, the mixture is separated using preparative thin layer chromatography, and the biodiesel fraction is isolated and characterized using infrared (IR), ^{1}H NMR, and ^{13}C NMR spectroscopies.

■ HAZARDS

Gloves, eye protection, and suitable protective clothing should be worn at all times during the experiment, and all portions of the experiment must be conducted in a properly operating fume hood. Sodium hydroxide, potassium hydroxide, and hydrochloric acid are corrosive, harmful if swallowed, and cause severe burns, respiratory, and eye damage. Chloroform-*d* is an irritant on inhalation or ingestion, a suspected carcinogen, and toxic to liver, kidneys, and heart. Diethyl ether, dimethoxyethane, ethyl acetate, heptane, isopropanol, methanol, and petroleum ether are flammable and harmful if ingested or inhaled; sodium methoxide and methanol are toxic. Potassium permanganate is an oxidizer and harmful if swallowed. Sodium sulfate is an irritant on inhalation or ingestion. Silica gel is an irritant if inhaled or ingested. Jojoba oil and the biodiesel product are flammable.

■ RESULTS AND DISCUSSION

Student yields for the isolated methyl ester ranged from 10−50%, depending on the length of time that the reaction was allowed to run and whether students obtained efficient separation in the chromatography step. Most students recovered an amount of biodiesel at the higher end of that range. Spectroscopic data obtained by students showed excellent agreement with literature data for the jojoba methyl esters[10] (examples of student-generated spectra are provided in the Supporting Information). Students successfully located key identifying features in the IR spectrum (e.g., carbonyl absorbance at 1742 cm^{-1}) as well as in the ^{1}H NMR spectrum (e.g., methoxy group characteristic of methyl esters at δ = 3.66 ppm; alkene protons at δ = 5.35 ppm; and α protons at δ = 2.30 ppm) and verified integration values for these and other signals by keeping in mind that the alkenyl chains on the methyl esters will have varying numbers of carbons because of the composition of the jojoba oil that reacted. Students were asked after the experiment to consider methods for converting triglycerides to biodiesel and to compare these methods with the present experiment.

Alternative approaches for the experiment focus on the pedagogical goals of the Sonoran desert project including development of critical thinking skills and experimental design experience. In one alternative method, rather than sequential extraction and transesterification of the jojoba oil, the two processes are combined in one pot[29] by refluxing either commercially available jojoba oil or ground jojoba meal in a

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KOH/methanol/heptane mixture for 30−45 min, thus reducing the class time needed for the experiment. Additionally, an ethereal cosolvent can accelerate the rate of the transesterification reaction.[29] This method minimizes the amount of soapy material formed, which makes the workup more tractable.

Guided-inquiry approaches also may be employed to provide students with an experience that more closely parallels the process in an actual research laboratory. Both guided inquiry approaches that we have used[30] involve experimental design by students. In each method, students are presented with a brief background (plant oils have been investigated in recent decades as renewable energy sources) and a specific research "problem": how can we make biodiesel from jojoba oil? In the first method, students are given different sets of experimental conditions from which to choose including solvent, base, workup conditions, and extraction solvent. For example, extracting solvent choices must be made based on student knowledge of ester solubility and solvent properties such as polarity. Other choices (e.g., drying agent) are made based on previous student lab experience. In the second method, students read papers from the scientific literature and are asked to propose an experiment to extract and transesterify jojoba oil. Papers are provided to the students that describe biodiesel production from oils other than jojoba[31,32] to encourage students to discern differences and similarities between jojoba and other plant oils, and to learn how to apply information from one chemical system to another. Students read the literature, design and run experiments, and verify whether the experiment succeeded by acquiring spectroscopic evidence. Students were able to locate open-source articles on biodiesel production; in an institution with more extensive journal access, independent library research would add a very desirable component to the experience.

With either method, students meet as a group before they actually conduct the experiment. Student proposals are presented, and pros and cons are discussed as a class with instructor guidance. In this manner, undergraduate students with little or no research experience are asked in a no-risk environment to design an experiment that relates to "real world" issues and given immediate feedback from their peers and instructor about their decisions.

Detailed descriptions of the alternative methods are found in the Supporting Information.

Aside from the fieldwork (collection of jojoba seedpods), students gained exposure to a variety of lab processes including solvent extraction of a natural product; running, monitoring, and working up a reaction; preparative thin-layer chromatography; and spectroscopic structure analysis. In the guided inquiry versions of the experiment, students gained experience using the literature, selecting and testing reaction conditions, and analyzing spectroscopic data to assess the outcome of the experiment and to confirm the efficacy of their chosen method. The results of a series of formative assessments conducted by an external evaluator for the National Science Foundation (NSF) project over the past three years consistently reflected a high level of student interest in the native plant theme and in lab experiences that provide a connection between "real world" research issues and finding local solutions, as well as training on instrumentation (NMR) that is routinely used by chemists in actual research settings. Formative assessments included lab observations, student lab notebook reviews, and student interviews and have been performed at least once each semester since Fall 2012.

■ ASSOCIATED CONTENT

Supporting Information

Student instructions, instructor notes, chemical hazards, and sources. The Supporting Information is available on the ACS Publications website at DOI: 10.1021/ed500773k.

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Notes

The authors declare no competing financial interest.

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