

Introducing Organic Chemistry Students to Natural Product Isolation Using Steam Distillation and Liquid Phase Extraction of Thymol, Camphor, and Citral, Monoterpenes Sharing a Unified Biosynthetic Precursor

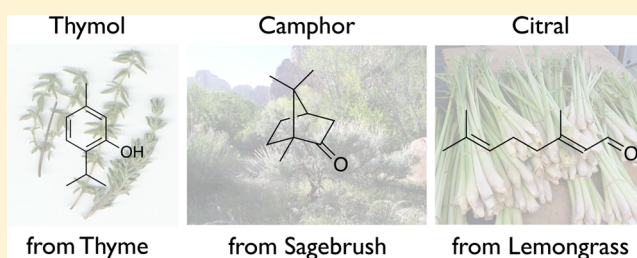
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S Supporting Information

ABSTRACT: Plants have provided and continue to provide the inspiration and foundation for modern medicines. Natural product isolation is a key component of the process of drug discovery from plants. The purpose of this experiment is to introduce first semester undergraduate organic chemistry students, who have relatively few lab techniques at their disposal, to the process of natural product isolation. In one, 3 h lab period, students use steam-distillation and liquid-phase extraction to isolate a single medicinally relevant molecule from one of three plants. The identity of the molecule can be demonstrated using infrared spectroscopy and thin-layer chromatography. Students choose to isolate thymol from thyme (*Thymus vulgaris*), citral from lemongrass (*Cymbopogon*), or camphor from sagebrush (*Artemisia tridentata*)—three commercially available plants with a long history of use. Each isolated molecule has a common biosynthetic origin in the plant, and this is discussed to introduce the relationship between organic chemistry and biochemistry.

KEYWORDS: Second-Year Undergraduate, Natural Products, Organic Chemistry, Hands-On Learning/Manipulatives, Biosynthesis, Drugs/Pharmaceuticals, Plant Chemistry, Laboratory Instruction, Thin Layer Chromatography



It is common to associate the word “chemical” with the scientific laboratory, as something arising out of beakers and flasks at the hands of chemists wearing lab coats. Moreover, in the 21st century vernacular, the word “chemical” stands juxtaposed to the term “natural,” and, increasingly in public opinion, the two are considered mutually exclusive occurrences. While it is true that many manmade chemicals are toxic, the same can be said about many compounds produced by plants.¹ This experiment seeks to prove that a bioactive molecule, thymol, camphor, or citral, produced in the lab synthetically is spectroscopically identical to the same molecule produced by a plant, thyme, sagebrush, or lemongrass, respectively. By using thin-layer chromatography (TLC) and infrared (IR) spectroscopy to characterize a molecule that is both synthetically and biosynthetically produced, this experiment demonstrates that the synthetic and the natural compound are the same.

In choosing the plants for this experiment, certain criteria were considered. It was important that students already have some familiarity with the plant of interest. All three plants have an extensive history of use, which, as well as being interesting, enriches the context for learning and understanding the significance of the science being performed (see Supporting Information for detailed information about each natural product and the proposed biosynthetic pathway from the common intermediate geranyl pyrophosphate).^{2,3} Constraints were placed on each isolation due to its intent as a first

semester, introductory organic chemistry lab experiment. It was required that the plants yield a molecule through steam-distillation, without chromatographic purification, in large enough quantities and with high enough purity to be characterized solely using IR spectroscopy and TLC (the purity of the isolated molecules was additionally verified using ¹H NMR spectroscopy by the instructors, see Supporting Information for representative spectra). To this end, thymol constitutes up to 83% of the essential oil obtained from thyme; sagebrush essential oil contains up to 40% camphor; and lemongrass essential oil contains up to 85% citral.^{4–7}

In principle, the technique utilized in this experiment, steam-distillation, works by separating volatile, water-insoluble compounds from nonvolatile compounds through codistillation. Water and the nonvolatile compounds present in the plant are immiscible and, as such, form a two-phase system called a heterogeneous azeotrope. The combined vapor pressures of the two compounds result in a lowered boiling point for the entire system. As a result, the water and volatile compounds codistill, which preserves the volatiles that could potentially decompose when exposed to higher temperatures.^{8–10} This technique has been applied to several undergraduate isolation experiments including natural product isolation from spices,¹¹ anise,^{12,13} cloves,¹⁴ citrus fruits,^{15,16} as well as others.^{17–20} Teaching

steam-distillation introduces a lab technique not often taught in undergraduate organic chemistry courses and extends the concept of simple distillation (often taught in the first semester of an organic lab course) to natural product isolation.

■ EXPERIMENT

Students work individually. One, 3 h laboratory period is required to complete the experiment. Dried plant material (10 g) or fresh plant material (20 g) is placed in a high-speed blender with distilled water (200 mL) and blended to a fine slurry. The slurry is transferred to a round-bottom flask (500 mL) with a distillation apparatus attached and a stir bar, and it is placed directly on a hot plate turned to high heat. A beaker (200 mL) is used as the receiving vessel to collect approximately 100 mL of distillate. The bioactive molecule is extracted from the distillate with an appropriate organic solvent (30 mL). The organic phase is dried with magnesium sulfate, and the organic solvent is removed on a hot plate at medium heat. The residue is analyzed using TLC and IR spectroscopy and compared to commercially available synthetic standards. For details concerning specific solvent types used for each isolation, preparation of standards, samples for analysis, and spectroscopic details, see the Supporting Information.

■ HAZARDS

Hexanes, pentanes, ethyl acetate, dichloromethane, diethyl ether, deuteriochloroform, thymol, camphor, and citral are skin, eye, and lung irritants. Ethyl acetate, diethyl ether, hexanes, and pentanes are highly volatile flammable liquids. Thymol, camphor, and citral are potential allergens. *n*-Hexane is a neurotoxin. Hexanes, pentanes, ethyl acetate, dichloromethane, deuteriochloroform, and diethyl ether should be placed in a flammable waste container.

■ RESULTS AND DISCUSSION

In practice, six sections of 16 students (96 total students) worked individually to isolate their natural product of choice. The exact quantity of the isolated molecule varied depending upon the plant and harvesting conditions, but it was generally more than sufficient for analysis. From our experience, near universal success was observed in this experiment both in isolating the desired molecule within 3 h as well as successfully comparing the isolated molecule by TLC and IR spectroscopy to the synthetic standard. The purity of the distillate product was established by observing a single TLC spot with identical R_f values to the authentic sample, and in almost all cases, additional impurity spots were absent on the TLC plate. Furthermore, students who isolated their natural product were able to correlate successfully functionality found in their target molecules with the corresponding peaks in the IR spectra (e.g., an aldehyde peak in citral, a broad alcohol peak in thymol, and a ketone peak in camphor; see the Supporting Information's Student Handout for more detail). Overall, student comments on this lab module were overwhelmingly positive, citing it as one of the most enjoyable and engaging laboratories of the term.

Perhaps more importantly, this experiment was able to emphasize to students that there is a continuing dialogue between the "laboratory of evolution" and the "laboratory of the scientist," a dialogue that uses chemicals as its language. For future scientists interested in pharmacology, an understanding of this relationship and the role played by plants in drug

discovery is very important. This experiment helped to demonstrate the diversity that arises from common origins; the building block geranyl pyrophosphate is a proposed biosynthetic precursor common to the monoterpenes citral, thymol, and camphor. This experiment acquainted students with a simple natural product isolation and characterization techniques. TLC and IR spectroscopy were used to characterize the compounds, and commercially available synthetic standards were shown to be equivalent to the same molecule produced by and isolated from a plant. In so doing, compounds produced by plants and those synthesized in the lab were shown to be analogous.

■ ASSOCIATED CONTENT

Supporting Information

Student handout; instructor's notes; spectral data. 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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