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Laboratory Experiment

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Nature's Treatment for Irritable Bowel Syndrome: Studies on the Isolation of (−)-Menthol from Peppermint Oil and Its Conversion to (−)-Menthyl Acetate

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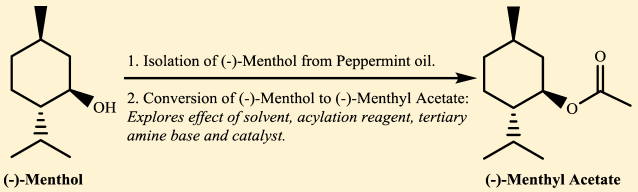
Supporting Information

ABSTRACT: A simple, robust, and reproducible method was developed for the isolation of (−)-menthol from peppermint oil and to study the effect of different types of leaving groups, catalysts, solvents, and tertiary base on the extent of esterification of (−)-menthol to (−)-menthyl acetate. In this experiment, students compare leaving group properties of acetate and chloride ions from the acylating reagents acetic anhydride and acetyl chloride, respectively. The extent of conversion is compared when pyridine and 4-(dimethylamino)pyridine are used as catalysts, when *N*,*N*-diisopropylethylamine is used as tertiary base, and when the solvent is changed from dichloromethane to diethyl ether to *N*,*N*-dimethylformamide. Students are assessed on the chromatographic/spectroscopic purity and yield of (−)-menthol isolated and on their understanding of the factors that affect its extent of conversion to (−)-menthyl acetate. Full spectral characterization of both compounds is also conducted. They also complete a series of answers to questions based on lecture material presented on this topic, complete a crossword as a formative assessment tool, and are required to present a PowerPoint slide to their peers on a particular aspect of the bench to bedside development of peppermint oil for the treatment of irritable bowel syndrome.



KEYWORDS: *Upper-Division Undergraduate, Laboratory Instruction, Organic Chemistry, Collaborative/Cooperative Learning, Hands-On Learning/Manipulatives, Chromatography, Esters, Medicinal Chemistry, Natural Products, NMR Spectroscopy*

INTRODUCTION

Many natural plant products have been used in the treatment of a diverse selection of ailments and diseases since ancient times. This experiment provides a valuable learning experience to students in the use of peppermint oil (PO) as an herbal medicine and the application of key analytical techniques frequently used in the laboratory. The primary isolation technique utilized in this experiment is flash column chromatography, allowing students the opportunity to master the skills required to achieve precise isolation of a substance of interest from a complex mixture. In converting (−)-menthol to (−)-menthyl acetate, students gain an understanding of the esterification process and the factors affecting its transformation. Students interpret IR, HRMS, and NMR spectra as a means to compare and contrast structural similarities and differences between (−)-menthol and (−)-menthyl acetate. This experiment complements many other laboratory based experiments on the isolation of Nature's medicine including cinchonine and quinine from *Cinchona calisaya*,[1] valtrate from *Centranthus ruber*,[2] galantamine from *Leucojum aestivum*,[3] lovastatin from red yeast rice,[4] parthenolide from *Tanacetum parthenium*,[5] and hyperforin from *Hypericum perforatum*.[6] This experiment offers an interesting comparison to the chromatographic separation of (−)-menthol from consumer products[7] and also provides a new insight into a variety of chromatographic, spectroscopic and polarimetric analyses[8,9] previously conducted on PO and (−)-menthol.

BACKGROUND

Peppermint (*Mentha* × *piperita L.*) (Figure 1) is a perennial herb first described in 1696 by John Ray and is cultivated in many parts of the world.[10,11] Background information on *Mentha* × *piperita L.*, quality, efficacy, and safety standards of peppermint oil and, in particular, the significance of peppermint in the treatment of irritable bowel syndrome (IBS) and other therapeutic applications is highlighted using a cyclical model (Figure 2).

EXPERIMENTAL OVERVIEW

The aim of this experiment is to isolate (−)-menthol from PO, to explore the esterification of (−)-menthol to (−)-menthyl acetate, and to confirm the identity of both compounds. The experiment and workshop are intended to complement the more theoretical aspects of the PO course delivered to our students. A comprehensive overview of the learning outcomes for the PO course, teaching methods and assessment criteria are shown in Table 1. The experimental component can be conducted over two, 3 h laboratory periods with a follow-on workshop on spectral assignment. It has been completed by 30

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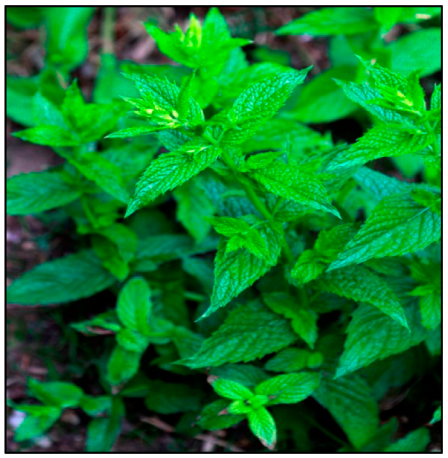

Figure 1. *Mentha* × *piperita L.* photograph taken from author's (E.M.C.) garden.

students divided into two groups in an undergraduate pharmacy degree program. The first part of the experiment involves the individual isolation of (−)-menthol. The conversion of (−)-menthol to (−)-menthyl acetate (Figure 3) is performed as a group session where students are assigned a given method of esterification and are required to monitor the extent of formation of (−)-menthyl acetate by TLC. Results are then compared at different time points with a detailed debate taking place among the group on the factors that affect the extent of conversion of (−)-menthol to (−)-menthyl acetate. Topics for discussion include: which catalyst is the most efficient and why? What effects do the solvent and leaving group have on the reaction speed? Is the tertiary amine base, *N*,*N*-diisopropylethylamine (DIPEA), required for the reaction? Together with the instructor, students also interpret IR, 1-D, and 2-D NMR spectra obtained on both compounds.

EXPERIMENTAL DETAILS

This experiment can be divided into three individual components: (I) isolation, (II) esterification, and (III) characterization of (−)-menthol and (−)-menthyl acetate. In part I, students work individually. PO (25 μL) is loaded on a preprepared flash column. Pure fractions containing (−)-menthol are identified following application of each solution, including a reference and test solution, onto a TLC plate (20 × 20 cm). After development, the plate is visualized, pure fractions are identified and the yield of (−)-menthol is recorded. In part II, students work individually to study the factors that affect the conversion of (−)-menthol to (−)-menthyl acetate following one of 11 procedures (Table 2). A mini-workup of each reaction is carried out at 5, 10, 20, and 40 min time points. At each time point, 10 μL of the quenched reaction mixtures (upper layer) is applied onto the TLC plate, containing (−)-menthol and (−)-menthyl acetate as reference compounds. After development, the plates are visualized with vanillin/H_2SO_4 or anisaldehyde/H_2SO_4 and the extent of esterification at each time point documented. As 6 reactions of 11 will have gone to completion in 20 min, students isolate (−)-menthyl acetate in pairs for subsequent spectroscopic analysis. Full experimental details are in the Supporting Information. The follow-on workshop (part III), using spectral data obtained on student samples, explores in detail spectral characterization of both compounds.

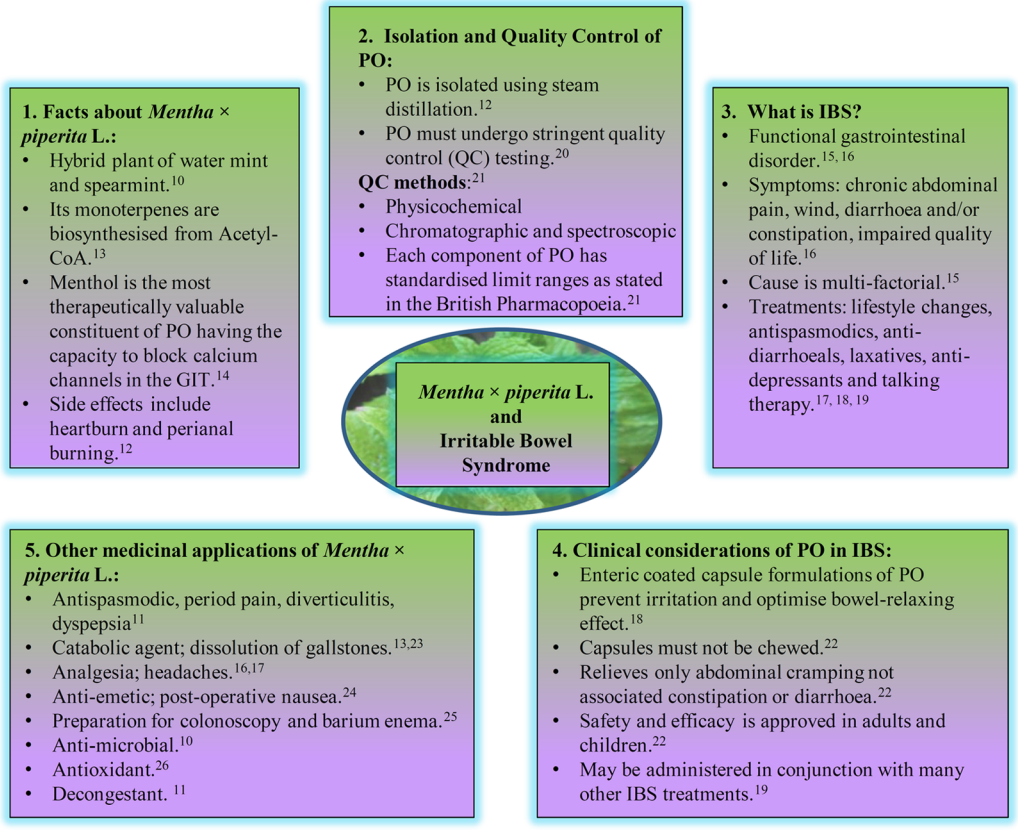

Figure 2. Overview of *Mentha* × *piperita L.* and its use in the treatment of IBS.

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J. Chem. Educ. XXXX, XXX, XXX−XXX

Table 1. Overview of Learning Outcomes, Teaching Methods and Assessment

Overall learning outcomes	Delivery methods	Assessment
The students should be able to	·Lecture series	Crossword puzzle (formative)
1. Describe the biosynthetic pathways employed by*Mentha* × *piperita* L. to produce therapeutically valuable compounds		Annual examination:
2. Discuss the regulatory framework for quality control of PO		·Biosynthesis of (−)-menthol. ·Quality control of PO
3. Recognize and explain methods for the isolation of PO from *Mentha* × *piperita* L.and, in turn, of (−)-menthol from PO		
4. Demonstrate an elegant method for the isolation of (−)-menthol from PO using Flash Column Chromatography and Thin Layer Chromatography.	·Laboratory practicals ·Laboratory workshop on structure elucidation	Laboratory report:
5. Convert (−)-menthol to (−)-menthyl acetate via an esterification reaction		·How to prepare the flash column correctly ·Purity of (−)-menthol spots on TLC ·Percentage yield of (−)-menthol isolated
6. Discuss other methods used to convert (−)-menthol to (−)-menthyl acetate		
7. Confirm that extent of reaction is dependent upon base, solvent and catalyst used		
8. Perform structure elucidation studies on (−)-menthol and (−)-menthyl acetate using spectroscopic techniques		Method and factors affecting conversion of (−)-menthol to (−)-menthyl acetate. Annual examination: ·Structure elucidation ·Related spectroscopic and chromatographic techniques
9. Understand the causes and consequences of IBS and discuss treatment options	·Lectures ·Student-led seminar	Annual examination:
10. Gain insight into the clinical use of PO in treating IBS		·Clinical evidence in support of PO for treatment of IBS

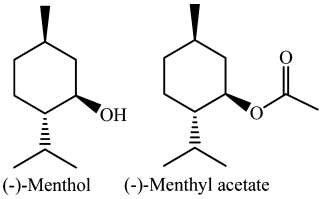

Figure 3. (−)-Menthol and (−)-menthyl acetate.

HAZARDS

Laboratory coats, protective gloves and eye protection must be worn during this experiment, especially when applying the anisaldehyde/H_2SO_4 or vanillin/H_2SO_4 visualization reagents. DCM, DMF, ether, ethyl acetate, pentane, $CDCl_3$, sulfuric acid, DMAP, DIPEA, acetic anhydride, acetyl chloride, vanillin, anisaldehyde, (−)-menthol, and peppermint oil are toxic reagents if inhaled, swallowed, or absorbed transdermally. Contact of such agents with skin or clothing should be prevented. They must be used within the fume hood and inhalation of vapors avoided.

RESULTS AND DISCUSSION

All aspects of the experiment were conducted with upper-division sophister pharmacy students. The isolation of (−)-menthol from PO proved quite reproducible and robust once the "tips for success" contained in the Student Handout were followed. In general, the yield of (−)-menthol ranged from 7 to 13 mg. In the second part of this experiment, students studied the extent of esterification of (−)-menthol to (−)-menthyl acetate when different leaving groups (chloride and acetate ions), acylation catalysts, solvents, and tertiary base were employed. As expected with test solution 2, in the absence of a catalyst, the reaction failed to form (−)-menthyl acetate over the time course of the experiment (Figure 4). In general, as a leaving group, chloride ion is better than acetate.[27] Thus, in the reaction of (−)-menthol with commercially available AcCl, using pyridine as a catalyst and DIPEA as the tertiary base, the reaction proceeded slowly with AcCl and not at all when Ac_2O

Table 2. Reaction Conditions to Follow for the Conversion of (−)-Menthol, 10 mg, to (−)-Menthyl Acetate and Outcome of Each Reaction at Different Time Points[a]

Acylation conditions	1	2	3	4	5	6	7	8	9	10	11
Solvent	DCM[b]	DCM	DCM	Et_2O[c]	DMF[d]	Et_2O	Et_2O	DCM	Et_2O	DMF	Et_2O
	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Catalyst	DMAP[e]		DMAP	Py[f]	DMAP	DMAP	Py	DMAP	Py	DMAP	DMAP
	7.8 mg		7.8 mg	5 μL	7.8 mg	7.8 mg	5 μL	7.8 mg	5 μL	7.8 mg	7.8 mg
Tertiary amine base	DIPEA	DIPEA	DIPEA	DIPEA	DIPEA	DIPEA	DIPEA[g]				
	28 μL	28 μL	28 μL	28 μL	28 μL	28 μL	28 μL				
Acylating reagent	Ac_2O[h]	Ac_2O	AcCl[i]	AcCl	Ac_2O	Ac_2O	Ac_2O	Ac_2O	AcCl	Ac_2O	Ac_2O
	14 μL	14 μL	11 μL	11 μL	14 μL	14 μL	14 μL	14 μL	11 μL	14 μL	14 μL
Product 5 min	Good	None	Mixture	Poor	Fair	Good	None	Good	Poor	Good	Good
Product 10 min	Very good	None	Mixture	Poor	Good	Very good	None	Very good	Poor	Good	Very good
Product 20 min	Complete	None	Mixture	Poor	Very good	Complete	None	Complete	Poor	Very good	Complete
Product 40 min	Complete	None	Mixture	Poor	Complete	Complete	None	Complete	Poor	Complete	Complete

[a]The progress of the reaction was monitored after 5, 10, 20, and 40 min time points to investigate the effect of solvent, catalyst, acylation reagent and tertiary base on extent of transformation of (−)-menthol to (−)-menthyl acetate. [b]DCM = dichloromethane. [c]Et_2O = diethyl ether. [d]DMF = *N*,*N*-dimethylformamide. [e]DMAP = 4-(dimethylamino)pyridine. [f]Py = pyridine. [g]DIPEA = *N*,*N*-diisopropylethylamine. [h]Ac_2O = acetic anhydride. [i]AcCl = acetyl chloride. Et_3N may also be used instead of DIPEA.

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J. Chem. Educ. XXXX, XXX, XXX−XXX

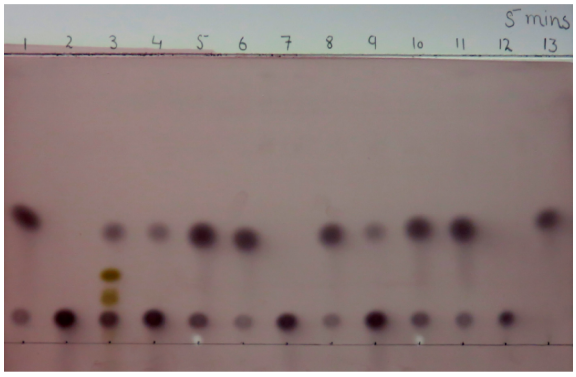

Figure 4. TLC results at the 5 min time point showing clear differences in the extent of esterification of (−)-menthol under the experimental conditions employed. Spots 12 and 13 are (−)-menthol and (−)-menthyl acetate reference standards. Aliquots (10 μL) of each solution were applied onto the TLC plate with 3 μL of 1 mg/mL reference solutions. Plate was visualized with vanillin/H_2SO_4. (Note: Anisaldehyde/H_2SO_4 is also equally effective as a visualization reagent.)

was used as acylation reagent (spots 4 and 7 in Figure 4). However, extent of reaction is reversed when DMAP is used as a catalyst. This is possibly because the acetate counterion generated is more basic than chloride and facilitates attack of the alcohol onto the DMAP-acylium ion pair complex **1** (Figure 5) (spots 1 and 3 in Figure 4).[28] Even in the presence of DIPEA, to sequester HCl or acetic acid, DMAP-catalyzed esterifications are usually faster with Ac_2O than with AcCl.[28]

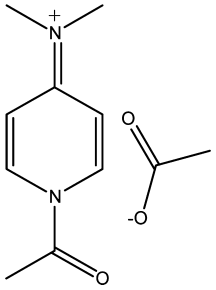

Figure 5. DMAP-acylium ion pair complex **1**.

As anticipated, the acylation reaction proceeded slowly when AcCl was used with DMAP, having only proceeded to give approximately 15% (−)-menthyl acetate after 5 min (spot 3 in Figure 4). This was also the only set of conditions to produce impurities repeatedly, highlighting to students that synthetic transformations may be problematic if consideration is not given to the conditions employed to conduct transformations. The extent of the acylation of (−)-menthol by Ac_2O in the presence of DMAP (Figure 4) was also studied when solvents of different polarities were employed: Et_2O, DCM and DMF. It is well known that nonpolar solvents facilitate breakdown of **1** (Figure 5) with subsequent rapid ester formation, whereas polar solvents, such as DMF, stabilize this intermediate and, thus, the reaction tends to be slower in this solvent.[28] Another contributing factor is, following nucleophilic attack by (−)-menthol onto the carbonyl of **1** (Figure 5), the resulting charged transition state complex rapidly collapses into noncharged products in nonpolar solvents. There was little difference in the extent of reaction between DCM and Et_2O, whereas in DMF, as expected, the extent of the reaction was slower (compare spots 1, 6, vs 5, respectively, in Figure 4). Complete conversion to (−)-menthyl acetate was observed after 20 min in Et_2O and DCM, whereas in DMF it was only observed at the 40 min time point (see Student Handout). The effect of the tertiary base on the reaction speed in Et_2O and DCM was slight as evidenced by comparison of spots 1 with 8 and 6 with 11 in Figure 4.

Full spectra characterization is provided in the Supporting Information. In brief, the IR spectrum was useful to distinguish both compounds by the presence of an OH stretch at 3272 cm^{-1} for (−)-menthol and the ester functionality at 1737 cm^{-1} for (−)-menthyl acetate. A cursory inspection of the ^1H NMR and ^{13}C NMR spectra showed the presence of the three hydrogen singlet at 2.06 ppm and carbonyl signal at 170.6 ppm, respectively, for the acetate group of (−)-menthyl acetate, and the downfield shift in the ^1H NMR spectra of the bridging proton at position 1 (CHOH) from 3.44 ppm in (−)-menthol to 4.70 ppm in (−)-menthyl acetate (CHOAc).

PRACTICAL REPORT/ASSESSMENT

Students were provided with the crossword clues as a formative assessment tool at the start of the teaching term and were required to complete it as the course on peppermint oil was delivered. They were assessed on their practical performance and on their submitted reports where marks were awarded for their flash column preparation, purity of the (−)-menthol sample isolated, understanding of the factors that affect its extent of conversion to (−)-menthyl acetate and spectral assignments on both (−)-menthol and (−)-menthyl acetate. The annual end-of-year examination on this topic contained questions based on structure elucidation, biosynthesis of (−)-menthol, quality control of PO and clinical evidence to support the use of PO in the treatment of IBS. It was apparent from their reports and the quality of answers provided that students in general had a firm understanding of the peppermint oil course.

ASSOCIATED CONTENT

Supporting Information

A student handout, including detailed experimental procedures and questions, notes for the instructor, answers to the student questions, spectra, tabulated spectral data and the crossword for student assessment is provided. 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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