EXPERIMENT 5

ISOLATION OF LIMONENE FROM NATURAL PRODUCTS: STEAM DISTILLATION AND EXTRACTION OF CITRUS PEELS & MINT LEAVES

Part 1: Isolation of limonene from citrus peel by steam distillation  
Part 2: Isolation of limonene from mint leaves by extraction

Students will work in teams of two. Each team will do one steam distillation, and both students will do the mint leaf extraction. If there is an odd number of students, one student will do both steam distillation and extraction.

Reading Assignment: Pavia, Sections 18.1-18.4, 19.1-19.15

Pre-lab Questions:
1) Questions #1, 2, Pavia p. 284-285
2) In the spearmint leaf extraction, indicate which of the following solvents may or may not be suitable as a replacement for the hexane used in the experiment: acetone, diethyl ether, ethanol. Briefly explain your choices.
3) Questions #3, 4, 5, Pavia p. 311 – 312.

Part I. Isolation of (R)-(+) -Limonene from Grapefruit or Orange Peel:

\[
\text{\text{\text{\text{(R)-(+) -limonene}}}}
\]

Line and 3-D structures of (R)-(+) -limonene.

The major constituent of the steam-volatile oil of grapefruit or orange peel is (R)-(+) -limonene. It can be isolated as an essential oil of about 97% purity by a steam distillation of citrus fruit peels. Attempting to distill this essential oil directly from plant material is generally not feasible. Limonene is a high-boiling liquid (bp\text{\text{\text{763}}} = 175.5-176 \, \text{°C}) which decomposes under the high heat needed to bring it to a boil. Steam distillation is a much gentler
way to obtain essential oils, as it allows the distillation of co-mixture of oil and water at a boiling point less than 100 °C.

Limonene is responsible for the characteristic smell of citrus peel. The infrared, $^1$H NMR, and $^{13}$C NMR spectra of (R)-(+)–limonene are shown below.

IR spectrum of limonene.

Proton and Carbon NMR spectra for Limonene.

**CAUTION**

Wear gloves and safety goggles and conduct all aspects of this experiment in a hood, except for handling the oranges, grapefruit, or mint leaves. Hexanes and ethyl acetate are solvents of moderate toxicity, but should provide little hazard if handled in a fume hood and disposed of properly after use. Methylene chloride is a volatile solvent that is toxic and potentially carcinogenic. Work in a fume hood and wear disposable butyl nitrile gloves when using this solvent.
Procedure

Obtain the peel from a grapefruit or from two oranges. Cut the peel into pieces about 5-10 mm square using scissors, and put the peel into a 500 mL round bottom flask, and add 250 mL of water. **IMPORTANT:** The peel will swell during the distillation! If pieces are too large, it will impossible to get the peels out of the flask when the distillation is complete. Blenders will be available to break the peel into smaller pieces before distillation. Clamp the flask securely and then fit it with a simple distillation apparatus. Heat the flask over a sand bath, with stirring and vigorously distill the mixture until you have collected about 50 mL of distillate. During the distillation, you can begin Part II.

Transfer the distillate into a separatory funnel and extract the aqueous phase with 20 mL of dichloromethane. Dry the organic phase over anhydrous magnesium sulfate for a few minutes (Note 1). Isolate the limonene by filtering the extract through cotton into a tared flask and evaporate the solvent using a rotary evaporator. Weigh the crude limonene sample. Physical properties reported for purified (R)-(+) -limonene include:

\[
\begin{align*}
\text{bp}^{763} & = 175.5-176 \degree C \\
\text{d}^2_4 & = 0.8403 \\
\text{n}^2_5 & = 1.4743 \\
[a]^{19.5} & = +124.2 \degree
\end{align*}
\]

Note:

1. As alternative procedure for isolation of the limonene consists of collecting the distillate in two 20 x 150 mm test tubes. The limonene will separate to form a clear layer above the water and most of the limonene can be easily removed using a Pasteur pipette.

Reference

Part II. Isolation of Limonene from Spearmint Leaves

Mint oil is an essential oil derived from mint leaves. The composition of the oil is dependents on the particular mint variety. Oil of peppermint (from the species *Mentha x piperita*) is mainly composed of menthol, menthone, and menthyl acetate. Spearmint oil (from the species *Mentha spicata*) is mainly composed of carvone and limonene. Both essential oils contain dihydrocarveol.
Peppermint and spearmints leaves can be difficult to distinguish, so when you purchase “mint” from the supermarket, it may be either variety. The peppermint plant tends to grow taller and the leaf is a little larger. When blooming, it has a purplish blossom while spearmint has a pink or blue blossom. Peppermint often has a purplish hue to the stems and leaves and the leaves tend to have more teeth. In addition, spearmint stems, while lacking the purple hue, are distinctly square, while peppermint stems are not.

Procedure:

Weigh approximately 10 g mint leaves. Examine the leaves and determine whether they look more like spearmint leaves or peppermint leaves. Place your mint leaves in a mortar and pestle, add a pinch of sand, and grind the leaves to a pulp. Add approximately 25 mL hexane to the pulp, and continue to macerate the leaves until the liquid portion turns greenish-brown. Filter the mixture using a Buchner funnel.

TLC Procedure:

Spot your filtrate (concentrate on rotovap if necessary), your steam distillation isolate (make a dilute solution, approximately 1:100 limonene:solvent), and the standard limonene solution on an aluminum-backed TLC plate. Develop the plate using 9:1 hexane:ethyl acetate solution. After the plate is developed, examine the plate under the UV lamp, and record what you see. Some of the components in the mixture will not be visible under UV light. The non-UV active components can be visualized by immersing the plate in a potassium permanganate solution. Quickly dip the plate in the permanganate solution, less the excess solution drip into the container or onto a paper towel, then briefly heat the TLC plate on a hot plate. What new spots appear after the permanganate dip? Draw your TLC plate or take a picture with your cellphone. Any compound containing an alkene functional group will reduce the purple colored KMnO₄ to brown MnO₂ (Warning: KMnO₄ is a strong oxidant and will discolor skin and stain clothing.) If any of the spots are too faint to visualize using the KMnO₄ dip, concentrate your solutions on the rotovap and repeat the TLC procedure.

---


3 Procedure has been adapted from Davies, Don R.; Johnson, Todd M. J. Chem. Ed. 84, 318 (2007).
Analysis:

How does your limonene sample from the steam distillation compare with commercial limonene (standard sample of commercial limonene was provided for the TLC experiment)? Do the results of this TLC experiment confirm your prediction about whether you have spearmint oil or peppermint oil? Can you be sure that the limonene spot from your distillation and your spearmint leaf extraction on the TLC is indeed limonene? Why or why not?

Post-lab Questions:

1) Question #2, Pavia p. 326.

2) In the chromatography of spinach extract, if you changed the eluent to 7:3 Hexane:acetone, what effect would this have on the outcome of the TLC experiment? Would you expect limonene to come off sooner or later than it did with 9:1 Hexane:ethyl acetate? What effect would you expect if you used 100% hexane instead? Sketch TLC plates of the expected outcome from 7:3 hexane:ethyl acetate and 100% ethyl acetate.