EXPERIMENT 5

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

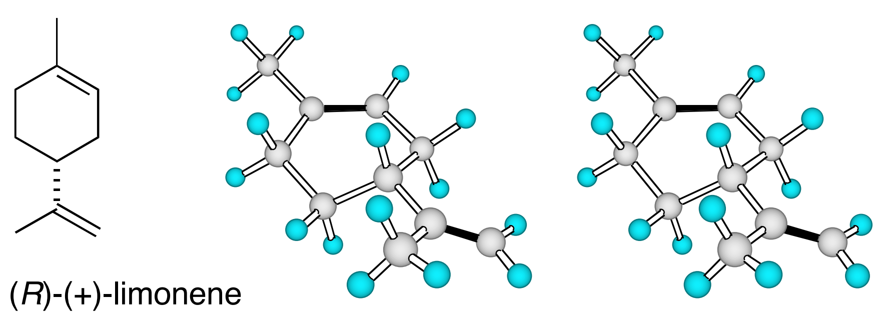
Part 1: Isolation of limonene from citrus peel by steam distillation
Part 2: Isolation of limonene from mint leaves by soxhlet extraction

Students will work in teams of two. Each team will do one steam distillation, and one soxhlet extraction. If there is an odd number of students, then the student will do either a steam distillation or extraction, depending on availability of glassware.

Reading Assignment: Pavia, Sections 18.1-18.4, 12.12

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.

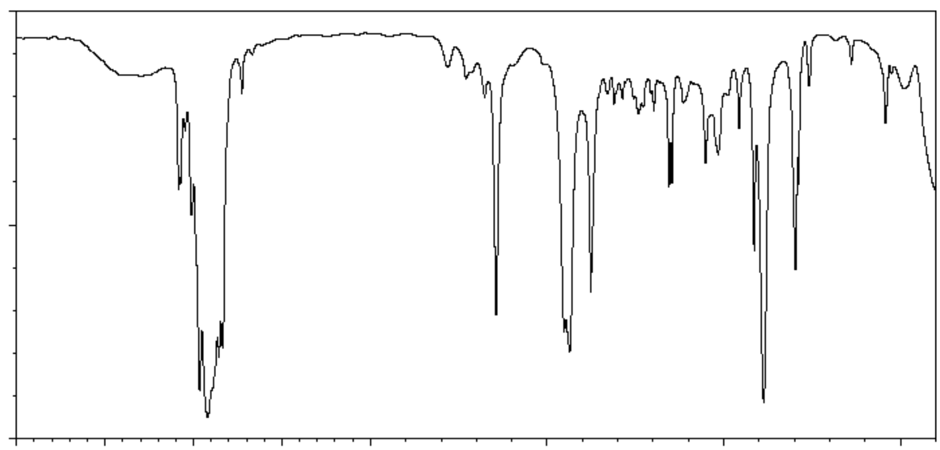
Part I. Isolation of (R)-(+)-Limonene from Grapefruit or Orange Peel:



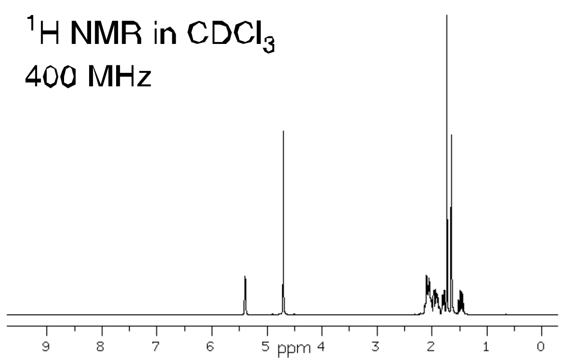
Line and 3-D structures of (*R*)-(+)-limonene.

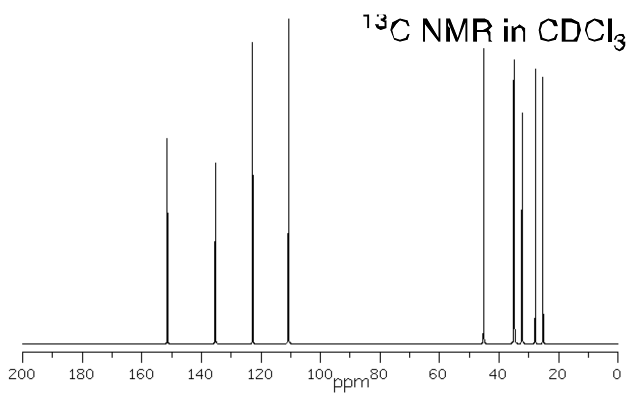
The major constituent of the steam-volatile oil of grapefruit or orange peel is (*R*)-(+)-limonene. It can be isolated as an esential oil of about 97% purity by a steam distillation of citrus fruit peels. Attempting to distill this essential oil directly from plant material is generally not feasible. Limonene is a high-boiling liquid (bp^{763} = 175.5-176 °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

Zest the peel from a grapefruit or from two oranges. The colored portion of the peel is the part that is rich in limonene, but the white pith swells up during the distillation and is difficult to remove during cleanup, so *minimize the amount of white pith* when you are zesting. *IMPORTANT: Once the fruit is brought into the lab, you can no longer eat it, so feel free to zest your peel the night before lab, and put the peel in a plastic bag*. Put the zested peel into a 500 mL round bottom flask, and add 250 mL of water. 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.

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:

$$bp^{763} = 175.5\text{-}176\ °C$$
$$d_4^{21} = 0.8403$$
$$n_D^{21} = 1.4743$$
$$[a]_D^{19.5} = +124.2°$$

Note:

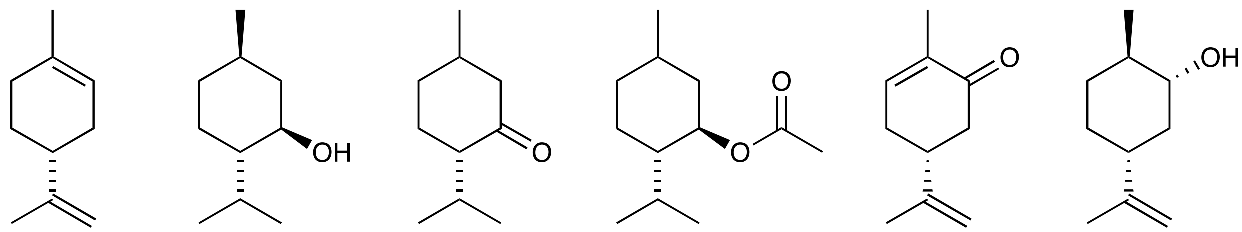
1. As alternative procedure for isolation of the limonene consists of collecting the distillate in two 20 x 150 mm test tubes. The limonene will separate to form a clear layer above the water and most of the limonene can be easily removed using a Pasteur pipette.

Reference

1) F. H. Greenberg, *J. Chem. Educ.* **45**, *537*, (1968).

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.



limonene menthol menthone menthyl Acetate R - carvone 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 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[2].

Procedure:

Weigh approximately 1.5 g dried mint leaves. Examine the leaves and determine whether they look more like spearmint leaves or peppermint leaves.

Heat a sand bath to 110 °C. Using a mortar and pestle crush the dried mint leaves, then transfer 1.5 g of ground leaves to a paper thimble. Place the thimble containing the ground mint leaves into the main chamber of the soxhlet extractor (see illustration on next page). To a 250 mL round bottom flask equipped with a stir bar, add hexanes until the flask is about ¾ full. Attach the soxhlet extractor on top of the round bottom flask using a small amount of grease on the ground glass joint and a keck clip. To the top of the soxhlet extractor, attach a condenser, again using a small amount of grease on the ground glass joint. Wrap distillation arm in foil, lower apparatus into oil bath, and let extraction proceed for 1.5 hours (you will need 4-5 siphoning cyles to obtain enough material for the TLC portion of the experiment).

Turn off the heat and lift the apparatus out of the sand bath to cool bath to cool. Remove hexanes using a rotary evaporator.

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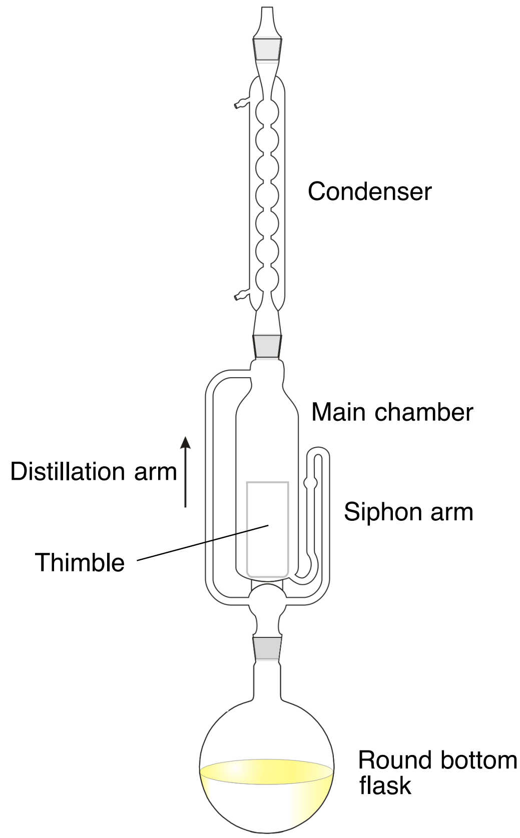
[2] http://www.livingspace360.com/index.php/a-look-at-spearmint-and-peppermint-28963/



Soxhlet Extractor Setup
http://glossary.periodni.com/glossary.php?en=Soxhlet+extractor

TLC Procedure[3]:

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 *(Draw TLC plate. Digital photography under a UV lamp requires a UV filter and the flash must be disabled)*. 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 $KMnO_4$ to

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

brown MnO_2 *(Warning: $KMnO_4$ is a strong oxidant and will discolor skin and stain clothing.)* If any of the spots are too faint to visualize using the $KMnO_4$ dip, concentrate your solutions on the rotovap and repeat the TLC procedure.

Analysis:

How does your limonene sample from the stean 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 TLC part of the experiment, if you changed the eluent to 7:3 Hexane:ethyl acetate, what effect would this have on the outcome of the TLC plate? 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% hexane.