Isolation of Limonene from Orange Rinds via Steam Distillation

The \((R)\)-enantiomer of limonene, \((R)-1\text{-methyl-4-(1-methylethenyl)}\text{-cyclohexene}\), a monoterpene, can be isolated from orange peels in a relatively pure form. While other citrus fruit may contain a larger total mass of limonene, it is present in those fruits along with other components; therefore isolating limonene from oranges is the most efficient. Limonene is used as a fragrance and flavoring agent in foods and cosmetics, as the solvent in some “green” cleaning agents, and is the “magic orange oil” purported to terminate termites.

Limonene will be steam distilled from orange rinds; the resulting mixture of water and limonene will be allowed to separate, and the limonene removed via Pasteur pipet. The isolated limonene will then be analyzed by polarimetry and the percent recovery from orange peels will be determined. The literature value for the specific rotation, \([\alpha]\), of pure \((R)\)-limonene is 125.6°; however, the limonene is typically not isolated in 100% enantiomeric excess. Percent recoveries vary with the type and freshness of the oranges used as well as experiment technique, but typically run about 2.0-2.4% (based on Winter 2015 class).

Techniques (from Techniques in Organic Chemistry by Mohrig, et al., 4th edition)
- Intermolecular Forces in Organic Chemistry (p 127)
  - Steam distillation (section 12.6; figure 12.21 but without the separatory funnel).
  - Drying of a liquid
- Use of a separatory funnel
- Polarimetry - Chapter 17, including using a volumetric flask to prepare a solution of known concentration

Equipment
- Mini (14/20) glassware kit, including separatory funnel, 500-mL and 50-mL round bottom flasks, condenser, still head, and Claisen adapter
- 500-mL heating mantle and variac
- 25-mL volumetric flask with stopper.

Safety
- Limonene. Irritant. Extremely hazardous in case of eye contact. Very hazardous in case of skin contact or inhalation. Gloves are probably not needed for the bulk of the lab, but should be worn when handling the isolated limonene.
- Ethanol. Hazardous in case of skin or eye contact or of inhalation. Flammable.

Procedure
1. Purchase two oranges and bring to class. Do not peel the orange before class; if you peel it immediately before class, put the peels in sealed plastic bag until you are ready to use them.
2. Separate orange rind from pith (white stuff) and pulp (the part you normally eat).
   a. Sometimes it works best to peel the oranges in the traditional manner and then scrape the pith off with a scoopula or plastic knife; with other orange types, it may work better to remove the peels along with some pulp and then scrape off the pulp plus the pith.
   b. If you peel them outside the lab and then place the edible portion of the orange in a plastic bag before returning to the lab, you may eat it later. Otherwise, dispose of the oranges in the large, plastic garbage bag provided. (donate to garden???)
3. Weigh the rinds.
4. Place the rinds in a blender (traditional or bullet), along with about 180 mL of water and blend until reasonably smooth. Pour the puree into a beaker, rinse the blender cup with about 50 mL of water, and add the water to the puree.
5. Transfer the puree to a 500-mL round bottomed flask. It is OK to use an additional 10-20 mL of water to aid the transfer. Add antifoaming agent (1-2 drops) and a boiling chip to the puree.
6. Assemble the apparatus as follows.
a. Put a 500-mL heating mantle on a lab jack or platform, and place the 500-mL flask in the mantle. Clamp the flask.

b. Put the Claisen adapter in the neck of the flask and then build the rest of the apparatus in Figure 12.21 (minus the separatory funnel), being sure to support the apparatus with clamps and Keck clips.

c. Draw a line on the receiving flask at the 35-mL mark. Do not use a graduated cylinder for the receiver.

d. Plug the heating mantle into the variac and the variac into the wall outlet.

e. Turn on the water flow to the condenser and check that it is exiting out to the sink at a slow, but steady, rate.

f. Turn on the variac and wait for boiling and then distillation to commence.

7. Record the temperature at the following points during the distillation: when the first drop of distillate is collected, after about 25 mL has been collected, and after 35 mL has been collected.

8. Avoid distilling the pot to dryness as the resulting burned orange slurry is a mess to clean up. If you are worried about the amount of water left in the pot at some point in the distillation just add more via the Claisen head. If it looks like your flask will be hard to clean, fill it with water and soap at the end of the distillation and let it sit that way until you are ready to clean up.

9. Once you’ve collected 35 mL of distillate check to see if limonene is still coming over, as follows. Carefully remove the receiver from the distillation apparatus, put it someplace safe, and stopper it. Meanwhile, place a small test tube under the vacuum adapter, collect about a mL of distillate in it, and agitate it to see if there is limonene (present as a second layer). If limonene is present in the fresh distillate then continue distilling into the test tube until you’ve collected about 5 mL more. Add the distillate from the test tube to that in the receiving flask. Turn off the heat. Distillate will probably continue to come over for at least a few minutes (maybe even 10-15): collect that in the test tube or flask with the rest.

10. Separate the limonene from the water as follows:
   a. Add 5 grams of NaCl to the distillate and swirl the mixture to dissolve the NaCl.
   b. Transfer the distillate to a separatory funnel. Let it stand for 5-10 minutes to allow the layers to separate well.
   c. Drain the lower aqueous layer off, leaving the upper limonene layer in the separatory funnel. Save a few mL of this lower layer, also known as the hydrosol, in a labeled and covered container.
   d. Use a disposable Pasteur pipet to remove the limonene from the separatory funnel and transfer it to a small test tube. Try not to transfer any water with the limonene, but don’t panic if you do.
   e. If the limonene is cloudy or you have reason to suspect that it still contains water, use a small amount of magnesium sulfate to dry it, and then remove the limonene from the solid drying agent by decanting it (if the solid dry agent has formed a clump), pipetting it off, or filtering through a small plug of cotton in a Pasteur pipet (Figure 9.3, page 136 of Techniques book).

9. Note the smells of both the limonene itself and the hydrosol. Once you have smelled the hydrosol you may dispose of it in the "Orange Stuff" beaker.

10. Weigh the limonene. If you are going to do polarimetry during a subsequent lab period weigh it now (for the yield) and again right before your do polarimetry.

11. Store the limonene in a small, labeled, stoppered test tube or a vial with a lid.

12. Polarimetry (may be done the following week):
   a. Obtain a 25-mL volumetric flask from the stockroom.
   b. Weigh the limonene (again, even if you weighed it during the previous lab period).
   c. Get about 30 mL of ethanol.
   d. Transfer the limonene to the volumetric flask, and rinse the container the limonene was poured from with about 15 mL of ethanol. Put the stopper on the flask and carefully swirl to mix the contents. Then carefully fill the volumetric flask with additional ethanol until the bottom of the meniscus is just at the line on the neck of the flask. You will want to add the last part drop wise using a Pasteur pipet. Mix again.
   e. If your solution is cloudy, transfer it to two clean, dry centrifuge tubes (it probably won’t all fit in one), centrifuge, and then pipet the now-clear liquid into a new clean, dry container leaving the cloudy residue behind. Centrifugation works better than the filtration suggested in the lab book and the YouTube video.
   f. Your instructor will help you with the polarimetry.
g. Don’t let the polarimetry tube roll off the lab bench to a certain death. Keep track of all its parts, including the ends and gaskets, and reassemble them when you are done. Rinse out the tube with water unless there is someone waiting to use it.

13. **Clean up.** The pith and any uneaten pulp from the oranges go in the large garbage bag at the front of the lab: it will be thrown out outside with the regular trash, or donated to the garden for their compost pile. The residue left in the pot and the hydrosol go in the large beaker in the hood labeled “Orange Stuff.” Once it settles, the liquid can be decanted off by the instructor or stockroom personnel. The polarimetry solutions will probably be poured into a flask or beaker as directed by the instructor.

**Practice Questions** (not to be turned in)

1. Why is it important to use rinds from freshly-peeled oranges?
2. Why does the limonene (boiling point 176°C) steam distill at about 97.4°C?
3. Calculate the fraction of limonene and the mass percent of limonene in the limonene-water mixture that would steam distill at 97.4°C. The vapor pressure of water at 97.4°C is 690. torr. Assume that the atmospheric pressure is 760 torr. Would the percent limonene be greater, the same or less in the mixture that steam-distilled at 99°C?
4. How would the presence of a small amount of water affect the experimental enantiomeric excess determined for the isolated limonene? How would the presence of a small amount of (S)-limonene affect the experimental enantiomeric excess determined for the isolated limonene? Would the same mass of water vs. (S)-limonene impurity have the same magnitude effect of the experimental enantiomeric excess?