Experiment 9

Isolation of Limonene: Using Liquid Carbon Dioxide as a Solvent

Reading: Handbook for Organic Chemistry Lab, sections on Green Chemistry (Ch. 4) and Optical Activity and Polarimetry (Ch. 22). Organic Chemistry by Marc Loudon, 6th ed., pp. 237-241 (6.3).

Limonene, the chief component of orange oil, is widely used as a fragrance and flavoring, as well as a cleaning solvent. Limonene is an example of a terpene, a class of natural products biosynthesized by the assembly of isoprene units into various structures (Figure 9-1). Many terpenes are responsible for the odors of plants like eucalyptus, pine, mint, lavender, rose, and others.

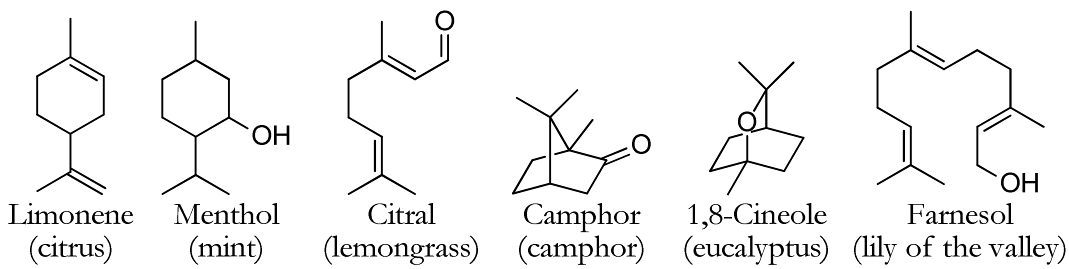

Figure 9-1: The structures of some naturally occurring terpenes, including limonene.

The distinguishing feature of terpenes is that they are made up of five-carbon isoprene units. The structure of isoprene contains double bonds (Figure 9-2), but these may or may not be present in the terpene. Terpenes, often referred to as isoprenoid compounds, are classified according to the number of carbon atoms that they contain: 10 carbons (2 isoprene units) is a monoterpene, 15 is a sesquiterpene, 20 is a diterpene, 25 is a sesterpene, 30 is a triterpene, and 40 is a tetraterpene.

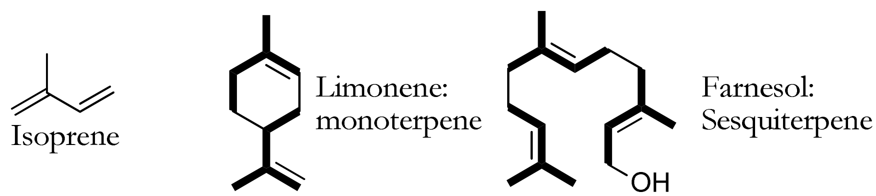

Figure 9-2: The structure of isoprene, and the isoprene units of limonene and farnesol.

Isolation of natural products typically involves multiple extractions and chromatographic steps, but certain organic oils can be freed of contamination through a variety of isolation methods such as solvent extraction, steam distillation, or liquid or supercritical CO_2 extraction. However, solvent extraction and steam distillation have deleterious environmental consequences, driving the use of more environmentally benign techniques to achieve the same end. Supercritical or liquid carbon dioxide is useful as a green alternative to solvent isolation because it is nonflammable, relatively nontoxic, readily available and environmentally benign. CO_2 is captured from the atmosphere, and the resulting sublimation or evaporation is carbon neutral, resulting in limited greenhouse gas emissions. Industrial-scale CO_2 processing has had commercial success in many separation and extraction processes. The tunable solubility properties, low toxicity, and ease of removal of CO_2 have led to well established CO_2 technology for the extraction of various food products, including essential oils and hops, for the decaffeination of coffee and tea, and for dry-cleaning of clothes.

Carbon dioxide is well suited for this application due to the relatively low temperatures and pressures used to form liquid CO_2. As shown in Figure 9-3, CO_2 sublimes (goes directly from a solid to a gas) at normal atmospheric pressure of 101 kPa. The triple point of CO_2, where solid, liquid, and gas phases coexist in equilibrium, is achieved at 520 kPa and -56.6 ºC. At this point, dry ice melts, forming liquid carbon dioxide. If the temperature and pressure are increased to the critical point (7380 kPa and 31.0 ºC), the CO_2 exists as a supercritical fluid, having no distinct liquid or vapor phase, but properties that are similar to both.

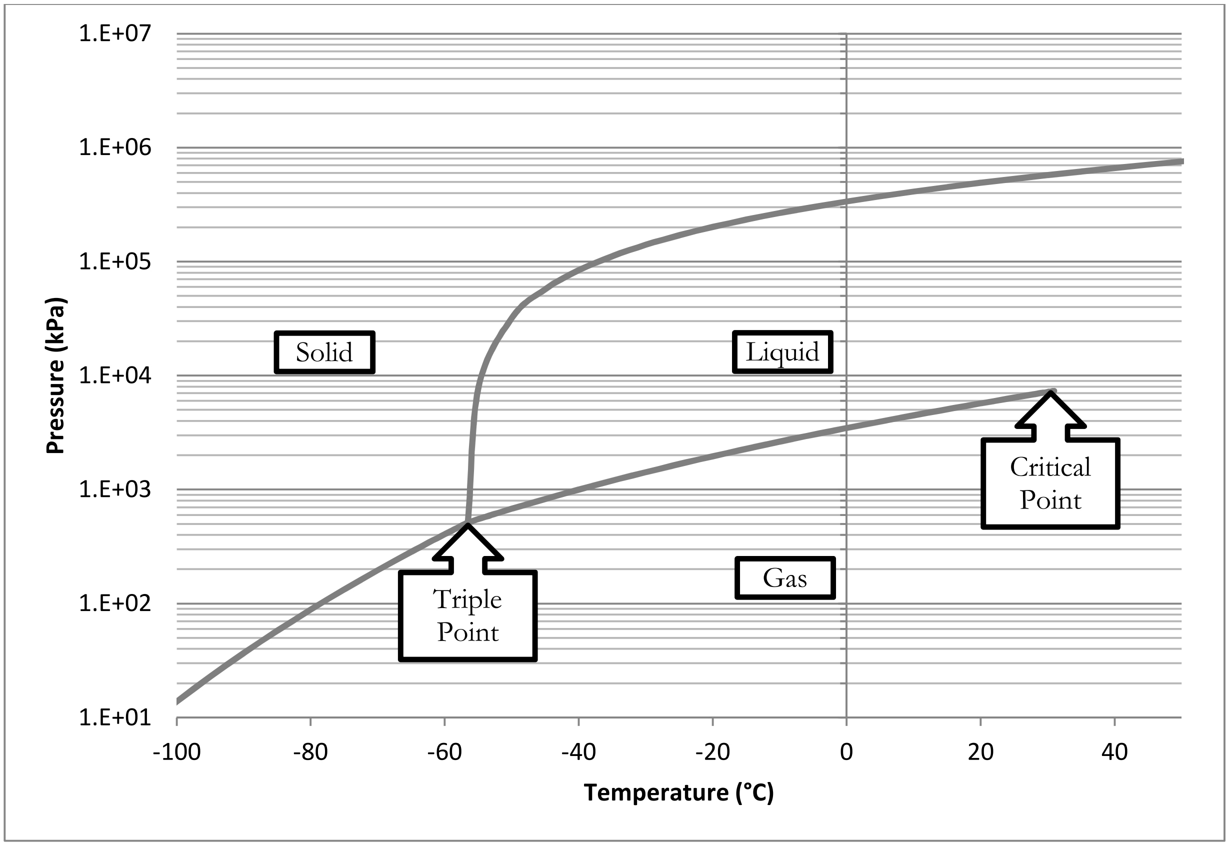

Figure 9-3: The temperature-pressure diagram for carbon dioxide.

At normal atmospheric pressures, dry ice will sublime at temperatures above -78°C. However, when placed into a sealed container that limits the rate the gas can escape, pressures and temperatures can build sufficiently to result in a solid to liquid transition. In today's lab, you will capitalize on this physical characteristic of CO_2 to perform liquid extraction to isolate limonene from orange peel. You will then use polarimetry to analyze your isolated limonene.

Polarimetry

Since limonene is a chiral molecule, it is possible to use polarimetry to measure its yield and purity. Specific rotation, [α], is given by the following formula:

$$[\alpha] = \frac{\alpha}{l \cdot c}$$

where α is the observed rotation in degrees, l is the path length of the polarimeter tube in decimeters, and c is the concentration in g/mL. The literature value for the specific rotation of (+)-limonene is +124°. Since two enantiomers always have the same magnitude but opposite signs of specific rotation, this means that the specific rotation of (-)-limonene is -124°.

A 1:1 mixture of two enantiomers is called a **racemic mixture**, and does not rotate polarized light at all. Since one compound is trying to rotate light clockwise and the other is trying to rotate light counterclockwise, they cancel out each other's effects. If two enantiomers are mixed together in non-equal amounts, they will partially cancel each other out but the more prevalent enantiomer will still win out. The specific rotation of this mixture is given by the specific rotation of each enantiomer multiplied by what fraction of the sample it makes up (abbreviated as X). For example, a sample containing 60% of (+)-limonene and 40% of (-)-limonene would have an observed rotation of

$$[\alpha]_{sample} = \left(X_{(+)} \cdot [\alpha]_{(+)}\right) + \left(X_{(-)} \cdot [\alpha]_{(-)}\right) = (.60 \cdot 124°) + (.40 \cdot -124°) = 24.8°$$

Enantiomeric excess or **ee** can be used to describe how much more there is of one enantiomer than the other. This is the percent of the more prevalent enantiomer, minus the less prevalent enantiomer. For example, the mixture containing 60% of (+)-limonene and 40% of (-)-limonene would have an ee of 20% (R), as shown in Figure 9-4.

60% (+)-limonene   40% (-)-limonene   =   20% ee of (+)-limonene

Figure 9-4: A limonene sample with 20% ee of (+)-limonene.

The specific rotation of the mixture can be calculated by the specific rotation of the dominant enantiomer, multiplied by the ee. Note that the rotation of the sample will have the same sign as the rotation of the dominant enantiomer.

$$[\alpha]_{sample} = (ee \cdot [\alpha]_{dominant\ enantiomer}) = (.20 \cdot 124°) = 24.8°$$

In this experiment, you will isolate limonene from orange peels, but it may contain both (+) and (-) limonene. You will need to calculate the relative amounts of both.

Safety Precautions

The use of high pressure vessels poses risks from rupture; flying projectiles or shards are a possibility. The experiment is designed to mitigate that risk. It is essential that you protect yourself and other students by reading all safety notes and the entire procedure before starting the lab.

- Pure limonene is an irritant, especially to the eyes, and it is flammable.
- Use the provided centrifuge tubes and cylinders. You should not use any glassware during the extraction steps.
- Always wear eye protection to prevent foreign material from becoming lodged in your eye.
- Use gloves, especially when working with the dry ice. In the event of contact with the skin it may cause tissue damage.
- Use a brand-new centrifuge tube for the experiment, and discard the tube once you are finished with the lab. Using a tube more than five times will result in structural degradation and increase the risk of rupture while pressurized.
- Zesters are very sharp and can easily cut your skin. Use caution when zesting the orange.

Procedure

You will work individually for most of this lab, but you will work in groups of 4-5 to zest an orange.

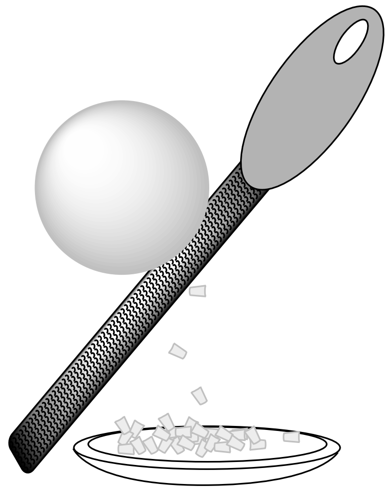

Figure 9-5: Zesting an orange.

Together in a group of four or five students, collect an orange and a zester from your TA. Use the zester to remove all of the exterior, brightly colored portion of the peel, called the "zest", by putting a watch glass or crystallizing dish on the bench, then holding the zester up over it, as shown in Figure 9-5. The easiest way to zest the orange is to hold the zester still while you move the orange back and forth.

Once the orange is off-white all over, you can stop zesting, as the white material (the pith) contains little or no limonene. Divide the zest equally between you, and place the orange into the trashcan – do not eat it, as it may be chemically contaminated. Wash the zester clean and return it to your TA.

To prepare the extraction tube, you will follow the steps shown in Figure 9-6. Obtain an unused 15 mL centrifuge tube and record its mass. Collect a wire trap and insert it into the tube so that it is held in place by friction and will not fall out after gentle shaking. Record the mass of the tube plus the wire trap. Add the zest collected earlier, making sure it is packed loosely. Record the total weight of the tube, and then calculate the mass of zest added to the tube.

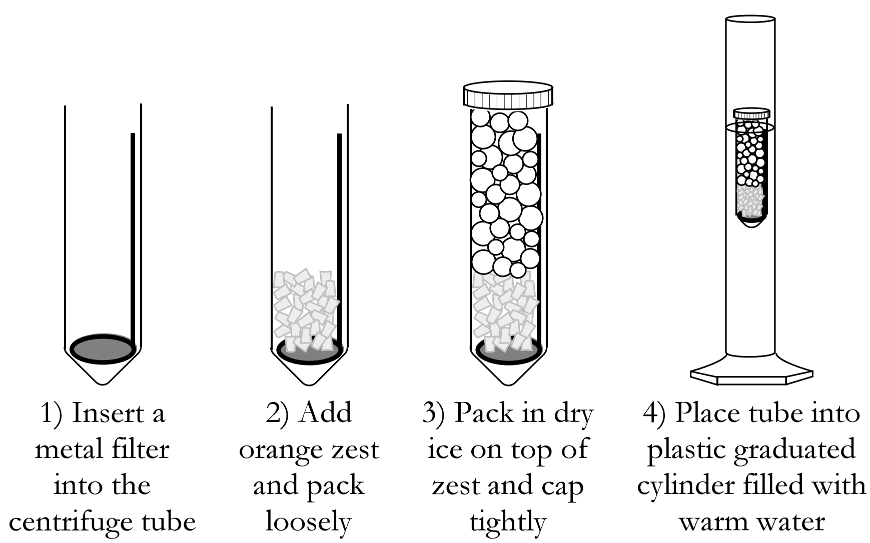

Figure 9-6: The extraction apparatus you will use for this lab.

Fill a plastic graduated cylinder half full with warm tap water (40-50°C) to create a water bath. (It is important to fill it no more than halfway, to direct any ruptures upwards instead of outwards.) Set it in your fume hood and remove any water-sensitive materials from the hood because splashing may occur if the cap shoots off the tube. You can share the water bath with another student, but you should write your initials on the top of your tube so you can identify it. One of you can use the bath while the other is packing dry ice into their tube.

Fill the rest of the centrifuge tube completely full with crushed dry ice. Twist the cap tightly until it stops turning; if it does not stop the lid is likely stripped and needs to be replaced.

Transfer the capped tube, tapered end down, into the warm water in the bath. Do not place anything above the bath while the extraction is proceeding, including your face. After 15 seconds the solid-to-liquid phase transition should be evident. If after 1 minute no liquid is present, the seal is not tight enough and needs to be reset. If multiple tries still do not produce any visible liquid, the cap may be stripped and you will have to start over with a new tube and cap. Do not use Parafilm or tape to seal the tube – this may prevent the slow release of CO_2 and increase the risk of an explosion.

After the liquid appears, it should pass through the peel to the bottom of the tube. If no liquid is observed at the base of the tube, the peel was packed too tightly. During this time the liquid should gradually boil off. Wait until all of the liquid has evaporated, remove the tube and open the cap carefully since the contents may still be under pressure. If necessary, rearrange the orange peel to allow permeation of the liquid CO_2 throughout. Add more dry ice to perform a second and then a third extraction in the same manner.

Your extract will collect in the conical portion at the bottom of the centrifuge tube. This isolate will be approximately 0.1 mL, and pale yellow in color. Carefully remove the solid peel and trap by pulling the wire handle. Any remaining solids on the walls of the tube can be removed with a spatula. Tap the orange peel into the regular trash can, and return the wire trap so it can be reused.

Dry the outside of the tube with a paper towel, weigh the tube and calculate the mass of the extract. Pool your limonene with the other students in the class by adding 1-2 mL of ethanol to the plastic tube, swirling to dissolve the limonene, and then pouring it into the polarimeter tube. Discard the centrifuge tube afterwards.

Tell your TA the mass of limonene you collected, so that they can give the class the combined mass of limonene. One student from your class should measure the optical rotation of the sample with the polarimeter. To do this, top up the cell with ethanol to a path length of 10 cm, or 1.0 dm, and a volume of 25 mL. The polarimeter is located in the instrument room; instructions for using it are included in the Handbook.

Once the limonene sample for your class has been measured in the polarimeter, transfer it to a round-bottom flask, rotovap it to remove the ethanol, add $CDCl_3$ and transfer the sample into an NMR tube. This NMR spectrum should be sent to the TA's email, so that they can forward it to the entire class.

Wastes

All waste from this lab can be placed into the regular, non-chemical trash. **Do not throw away the wire traps, and do not rinse the plastic graduated cylinder with acetone (it may partially dissolve).**

Lab Report

Your conclusions should include:

- What specific rotation did you calculate for your class's sample?
- Based on your answer to the previous question, what is the enantiomeric excess of the sample? Which is the dominant enantiomer of limonene? What percent of each enantiomer makes up this

sample?

- How pure is the sample, according to NMR? What impurities are present, if any?

Study Questions

1) (+)-Limonene is the (R)-enantiomer. Draw the structure of this enantiomer indicating the proper stereochemistry at the stereocenter of the molecule.

2) You isolate 5 g of limonene and place it in a polarimeter cell with a total volume of 30 mL and a path length of 1.2 dm. You observe a rotation of 2.48º. The sample's NMR spectrum shows pure limonene with no other contaminants.
   a) What is the specific rotation of this sample?
   b) What is the enantiomeric excess of this sample, and which enantiomer is in excess? What percent of each enantiomer makes up this sample?

3) What are the major diagnostic IR bands that you would expect to see in limonene?