Experiment B

Isolation of (+)-Limonene from Citrus

Reading: Handbook for Organic Chemistry Lab, Sections on Steam Distillation and IR Spectroscopy.

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 B.1). Many terpenes are responsible for the odors of plants like eucalyptus, pine, mint, lavender, rose, and others.

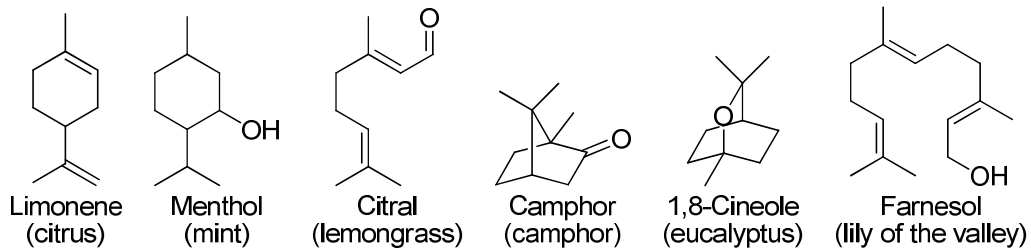

Figure B.1 » The structures of some naturally occurring terpenes, including limonene.

The distinguishing feature of terpenes is that they are made up of five-carbon isoprene units. The structure of isoprene contains double bonds (Figure B.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.

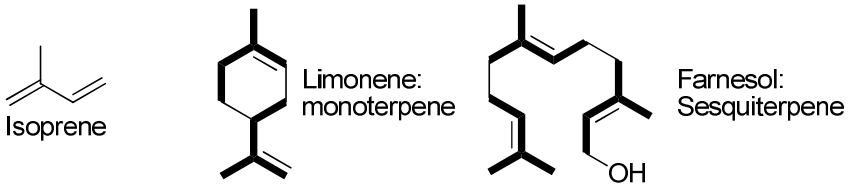

Figure B.2 » The structure of isoprene, and the isoprene units of limonene and farnesol.

Organic chemists use terpenes and other natural products as chiral starting materials for complex chemical syntheses or as inspirations for pharmaceuticals. Some natural products are attractive synthetic targets because of interesting or unusual structural features or medicinal applications.
Isolation of natural products typically involves multiple extractions and chromatographic steps, but certain organic oils can be freed of contamination by subjecting them to a process known as steam distillation. In today's lab, you will perform a steam distillation to isolate limonene from the peelings of citrus fruit. You will then use IR spectroscopy and polarimetry to analyze your isolated limonene.

Steam Distillation

Normally, a liquid boils when its vapor pressure is equal to the surrounding pressure. Generally, this is the same as the atmospheric pressure (1 atm or 760 mm Hg). A solution that is a homogeneous mixture of two or more miscible liquids will boil when the combined vapor pressures of its dissolved

components is equal to the surrounding pressure. The pressure of each component in a solution is related to its concentration in the mixture, and so the boiling point of a solution, or homogeneous mixture, is normally between the boiling points of the individual components. This was the case for the Simple and Fractional Distillation experiment performed previously in this class.

A heterogeneous mixture of two immiscible liquids will also boil when the combined vapor pressures of its components is equal to the surrounding pressure. However, because the liquids are immiscible, the vapor pressures of the individual components are independent of one another and not related to their concentrations. The two liquids independently exert vapor pressures against the external pressure, and when the sum of the partial pressures is equal to the external pressure, boiling occurs. Thus, the total vapor pressure of a heterogeneous mixture is given by the following equation:

$$P_{total} = P°_A + P°_B$$

where P_{total} is the total pressure of a system at a given temperature, and $P°_A$ and $P°_B$ are the individual pressures of components A and B at the given temperature. Generally, a heterogeneous mixture will boil at some temperature below the boiling point of either component. In Figure B.3, individual vapor pressures are plotted against temperature. Pure compound B, which has the lower boiling point, will boil at a temperature slightly above 125 °C. But when compound A is also present, the combined pressures add up to 760 mm—and the mixture boils—when the temperature is about 85 °C. This boiling temperature is lower than the boiling point of either compound alone, and it is the result of the combined effects of both compounds.

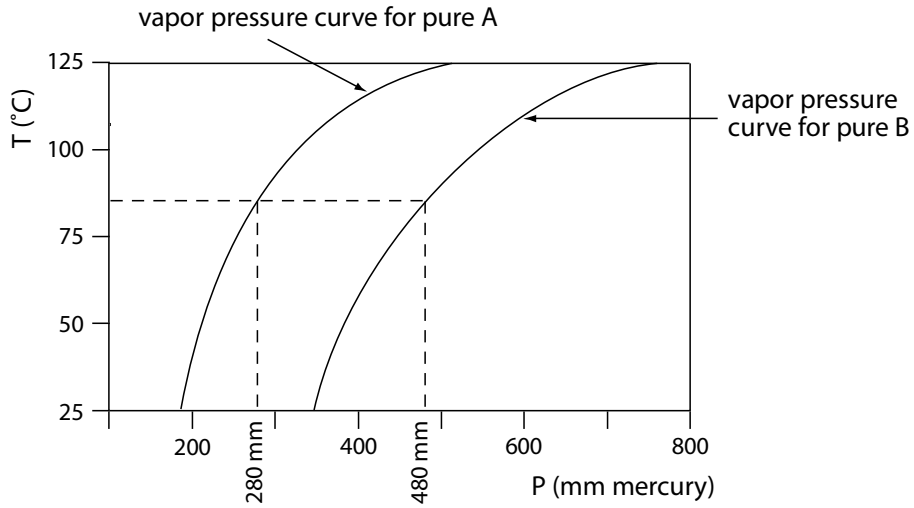

Figure B.3 » Effects of combined pressures in heterogeneous distillations.

We can take advantage of the fact that many water-insoluble liquids and solids behave in the manner described above for heterogeneous mixtures, volatilizing at temperatures below their boiling points. The effect described above is exploited in a technique called steam distillation, where an organic compound of moderate volatility and vapor pressure is distilled as part of a heterogeneous mixture with water. The boiling point of the mixture is slightly below 100°C, the boiling point of water. At this temperature, a fraction of the distillate will be the compound of interest. The greater the vapor pressure of the organic compound, the larger the fraction that will co-distill with the water. This technique is considerably gentler than regular distillation, since some organic compounds can decompose at temperatures approaching their true boiling points.

Since the amount of water is constantly decreasing during the procedure, a steam distillation is performed with the frequent addition of small amounts of water. Traditionally, this is done by using a setup similar to that shown in Figure B.4. A separatory funnel is placed above the round-bottom flask and is used to add water to it.

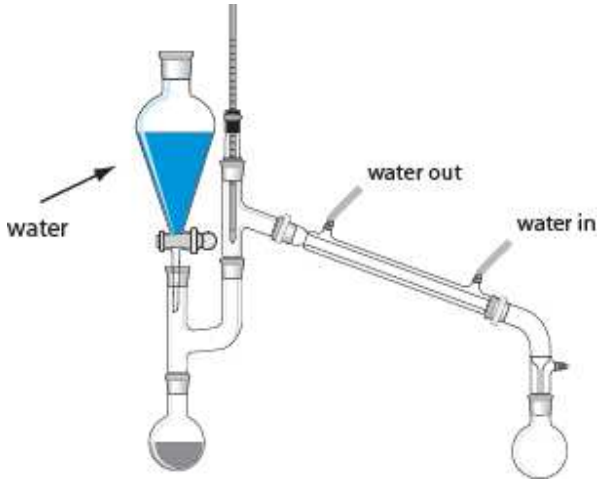

Figure B.4 » Traditional steam-distillation set-up. The use of a separatory funnel allows water to be added frequently.

In the interests of completing this lab within the allotted time, you will not be using this setup. The use of a Claisen adaptor makes the distillation path too long, and slows the distillation down enough that limonene collects very slowly in the receiving flask. Instead, you will use the same setup that you used for the Simple and Fractional Distillation lab, shown in Figure B.5.

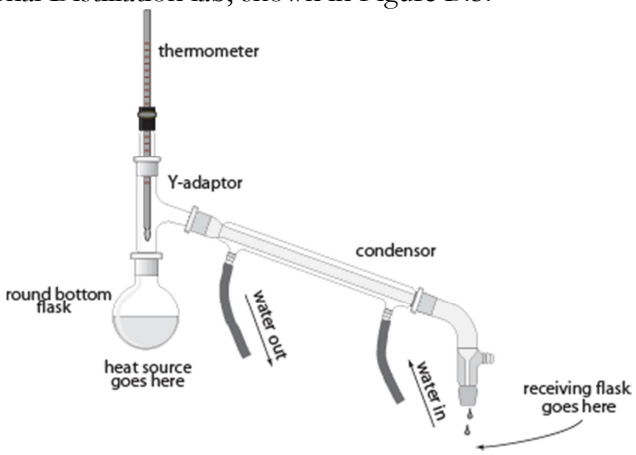

Figure B.5 » Glassware set-up for the steam distillation of limonene. Make sure that you clamp the apparatus securely to ring stands.

To add water to the flask, you will periodically remove the thermometer and thermometer adapter, and pour water in through the y-adaptor.

During the distillation, a process known as "bumping" may occur. This involves large bubbles of liquid and vapor erupting into the Y-adaptor and possibly flowing into the collection flask. Bumping is caused by the liquid superheating without becoming a vapor, due to overly fast heating. It can be prevented by heating more slowly and by not filling the distillation flask more than halfway. The use of boiling chips is also helpful in preventing boiling.

Polarimetry

Chiral, non-racemic compounds have the ability to rotate polarized light. This phenomenon is known as circular birefringence, and is where the phrase "optically active" comes from. The specific rotation of a material is an intrinsic, constant value at a given temperature and wavelength of light; specific rotation

can be found by using polarimetry. The polarimeter consists of a glass tube with flat ends to hold the sample, and a polarizing filter at each end of the tube. A light is shone onto the mirror at the bottom end of the polarimeter. As the light passes through the first filter it is polarized into a single direction, but this direction changes as it travels through the tube (if the sample is optically active). When it reaches the second filter, it may or may not pass through depending on whether it is polarized in the same direction as the second filter. The second filter is attached to an eyepiece which can be rotated, causing more or less light to pass through. At the darkest point, a reading can be made from the markings on the eyepiece which gives the observed rotation.

Specific rotation, [α], is then 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°. The polarimeter tubes that you will use have a path length of 12 cm, or 1.2 dm, and they contain a volume of 35 mL. Your TA will demonstrate how to take a reading on the polarimeters.

Safety Precautions

Pure limonene is an irritant, especially to the eyes, and it is flammable. Ethanol is flammable and toxic. Be careful that you do not cut yourself with the knives you use to remove the rind from the oranges.

Procedure

Peel an orange with a sharp knife or peeler. Remove just the exterior, brightly colored portion of the peel, called the "zest", as the white material underneath (the pith) contains little or no limonene. Determine the mass of the peelings, then place them in a blender. Add about 50 mL water, put the lid on the blender, and blend the mixture until a smooth puree is obtained.

Pour 50mL of this puree into your 100 mL round-bottom flask. (It is not necessary to get all the solids into the flask – much of the limonene will be in the liquid). Add several boiling chips. Attach the flask to the steam distillation system shown in Figure B.5. Use a 10mL graduated cylinder for the receiving flask.

Place a heating mantle under the distillation flask, connect the heating mantle to a Variac, and heat to boiling (Variac setting 60-70). Do not heat the flask too quickly, as this may cause the contents of the distillation flask to bump and contaminate the product. If this happens you will have to let the apparatus cool down, pour all the recovered distillate back into the distillation flask, clean the collection flask and condenser, and begin again.

Periodically add 5 to 10 mL portions of water to the distillation flask to replace the water that has been distilled over. Try to maintain the liquid level in the distillation flask at about half-full. You can add water easily by removing remove the thermometer and thermometer adapter, and pouring water in through the y-adaptor.

As the distillation proceeds, you should notice an upper layer of limonene forming in the 10 mL graduated cylinder. When the graduated cylinder is close to full, remove this layer with a Pasteur pipet and put it into a clean, tared vial. Pour off the remaining water into a beaker and set it aside; return the graduated cylinder to its place and continue distilling. Continue this process until there are about 45 minutes left in the lab period.

In the vial you may notice some droplets of immiscible water underneath your limonene layer, if you accidentally transferred some water to the vial along with your product. In this case, you can dry the limonene by adding a very small amount of sodium sulfate and then pipetting it to a new clean, tared vial.

Find the mass of the isolated limonene and obtain an IR spectrum. Pool your sample with the other students in the class and measure the optical rotation of the sample in ethanol using the polarimeter. If there is a large amount of water in the class pool of limonene, a separatory funnel may be useful to separate the layers.

Wastes

Place excess pulp into the containers marked "Waste Pulp". Do not pour pulp into the sink. Place any excess limonene and ethanol (from the polarimetry) in the recovery bottle in the main hood.

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) If you isolate 5 g of limonene and place it in a total volume of 35 mL:
    a) What would be the observed rotation, α?
    b) If the 5 g of limonene was actually not pure, what effect would this have on the observed rotation?

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