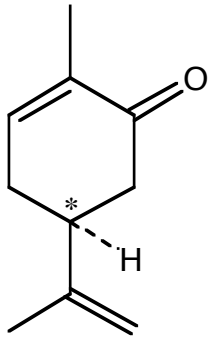
# MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Department of Chemistry
5.310 Laboratory Chemistry

## EXPERIMENT #3
## ESSENTIAL OILS[1]

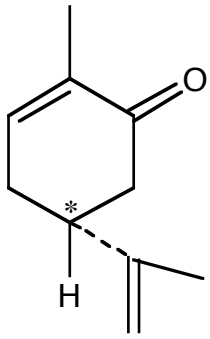
## I. INTRODUCTION

In this experiment[2] you will be working with oils prepared from caraway seeds and spearmint leaves. Each oil has a distinct and characteristic odor, yet carvone is the major component in both oils! It is amazing that the difference in odor is attributable solely to a difference in chirality of the carvone in the two oils. Due to chirality of odor receptors in the nose the R-carvone and S-carvone fit into different receptor sites, hence different odor. Can you distinguish between the odors? 8-10% of the population cannot.[3] Some physical data[4] are presented below.




*(S)* -(+)-Carvone

FW = 150.22; bp 98-100/10 mm
$n_D^{20}$ = 1.4970; d=0.9608 g/mL
$[\alpha]_D^{20}$=+61.7 $^o$ (neat 96%)
major component of caraway oil
*(Carum carvi)*




*(R)*-(-)-Carvone

Fw=150.22; bp 227-230 $^o$C
$n_D^{20}$ = 1.4990; d=0.9593 g/mL
$[\alpha]_D^{20}$=-62.5 $^o$ (neat 98%)
major component of spearmint oil
*(Mentha spicata)*

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[1] The experiment includes contributions from past instructors, course textbooks, and others affiliated with course 5.310 updated by John Dolhun Jun 2019.
[2] Adapted from: Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Engel, R. G. "*Introduction to Organic Laboratory Techniques*"; Saunders: Philadelphia, PA, 1990, pp. 96-107.
[3] *ibid*. p.103.
[4] Physical data is taken from Aldrich Chemical Catalog 1998-1999

All the physical properties should be identical except for the optical rotations of the two isomers (enantiomers), which are of opposite sign. Thus, for both (+)- and (-)-carvone, the infrared, nuclear magnetic resonance spectra, the gas chromatographic retention times, the refractive indexes, and the boiling points should be identical. Hence, the only difference in properties one should observe for the two carvones are the odors and the signs of rotation in a polarimeter. However, some of the physical properties presented above are not identical because of trace impurities.

The * in the formulas above denotes a chiral carbon center. Chiral or asymmetric compounds in nature exist only in living tissue or in matter that was once part of living tissue. Chirality plays a major role in the mechanisms of biological recognition. Yet it is a mystery why caraway plants, *Carum carvi*, produce *S*-(+)-carvone and spearmint plants produce its mirror image *(R)*-(-)-carvone. Other plants such as gingergrass produce racemic carvone. Nature goes one step further; some botanically indistinguishable plants grown in different countries can carry out complete metabolic sequences of mirror-image reactions. Presumably, the enzymes that catalyze the reactions also have a mirror-image relationship. Another example of chiral recognition[5] is found in the effect these two carvone isomers have on rates of reaction. The toxicity of *S*-(+)-carvone in rats is 400 times greater than that of *(R)*-(-)-carvone.

Essential oils are extracts from fragrant plants. They are used extensively in the perfume and flavoring industry. Most components of essential oils are terpenes that contain multiples of a five carbon structural unit, the isoprene unit (Fig. 1).

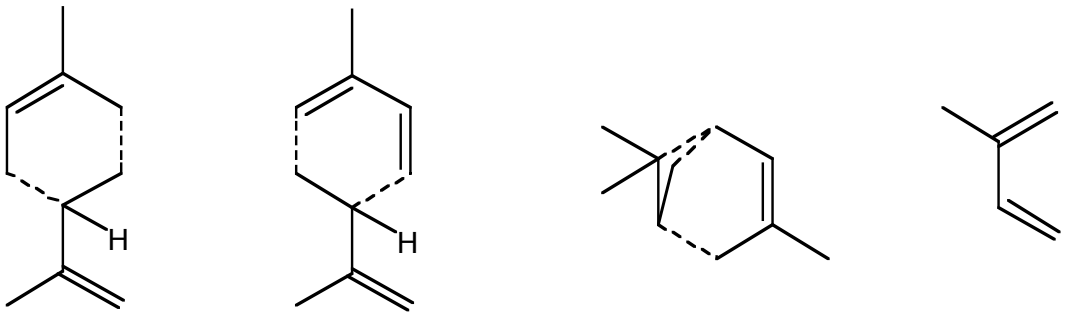



Figure 1. Representative monoterpenes. Isoprene units are shown to indicate the common structural features.

In addition to monoterpenes, compounds derived from two isoprene units, essential oils contain less volatile compounds derived from three and four isoprene units. These higher boiling components will be removed by vacuum distillation of the provided sample to permit facile gas chromatographic separation.

[5] The phenomenon in which a chiral receptor interacts differently with each of the enantiomers of a chiral compound is called **chiral recognition**.

## Overview of the Experiment

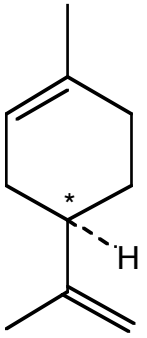
(A) You will be given a sample of either caraway oil or spearmint oil. The major component of these oils is carvone. You will separate the carvone from the higher-boiling and lower-boiling impurities (such as limonene), via vacuum distillation.

(B) You will use gas chromatography and refractometry to check the purity of your distillate and to estimate the relative concentrations of limonene and carvone in the oil.

(C) You will convert the carvone to its semicarbazone for use in a polarimetric analysis.

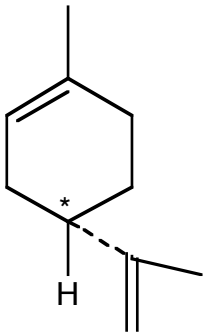
(D) You will obtain infrared spectra of the carvone and limonene fractions and interpret the results.

(E) You will also characterize the semicarbazone by melting point determination.




*(R)*-(+)-Limonene

FW = 136.24; bp 175.5-176$^{o}$C
$n_D^{20}$ = 1.4730; d=0.840 g/mL
$[\alpha]_D^{20}$=+123 $^{o}$ (neat )




*(S)* -(-)-Limonene

Fw=136.24; bp 175-177 $^{o}$C
$n_D^{20}$ = 1.4720; d=0.844 g/mL
$[\alpha]_D^{19}$=-94 $^{o}$ (c=10, ethanol)

(F) Visit the X-Ray Crystallography laboratory where spectra of selected crystals will be determined.

## II. SAFETY

1. **Carvone:** Ketone found in caraway, dill and spearmint oils. The oils are used for flavoring liqueurs, and in perfumes and soaps. Although both enantiomers occur naturally in consumer products, both should be handled with the usual care and not ingested under any circumstances.

2. **Limonene:** Occurs in various oils such as Levant wormseed oil, pine needle oil and other oils. It is used as a solvent, wetting and dispersing agent. It is not considered toxic, but is an irritant. Therefore, keep it off the skin.

3. **Semicarbazide hydrochloride:** Mutagen and cancer suspect agent. Do not inhale or ingest.

4. **Ethanol:** Flammable liquid. The denatured ethanol used in this laboratory is **NOT** safe to drink.

5. **Sodium acetate:** Irritant. Handle with usual caution.

## III. BACKGROUND FOR EXPERIMENTAL PROCEDURE

**General References**

| | |
|---|---|
| • Distillation | **MHS**, Chapter 12, pp 173-205 |
| • Vacuum Pumps | **TM(I)** Sec. 11C. |
| • Gas Chromatography | **MHS**, Chapter 20, pp 291-308 |
| • Polarimetry | **MHS**, Chapter 17, pp 240-251 |
| • Refractometry | **MHS**, Chapter 13, pp 206-211 |
| • Infrared Spectroscopy | **MHS**, Chapter 21, pp 311-344 |

**Videos: Digital Laboratory Techniques Manual**

**#7. Filtration**

**#11. Balances**

**#12. Melting Points**

**#15,16. Distillation I, II**

## Distillation

The difference between the boiling points of carvone (230 °C @ 760 torr) and limonene (177 °C @ 760 Torr) is sufficient to permit separation of the two compounds by distillation. However, carvone thermally decomposes at higher temperatures; therefore, a vacuum distillation is necessary.

Two problems are encountered in a vacuum distillation. The volume of vapor formed from a given amount of liquid is pressure dependent; i.e., the volume of vapor formed from one drop of liquid will be about 30 times as great at 25 torr as it was at 760 torr. As a result, serious bumping may occur. Boiling chips generally do not help much at the reduced pressures. Some of the bumping can be overcome with the use of a magnetic stir bar. The second problem is also related to the larger volume of vapor at lower pressure. The velocity of the vapor entering the column is greatly increased. This creates a greater pressure in the column than may be registered on the manometer. Maintaining a slow, steady rate of distillation can minimize this difference in pressure.

## Gas Chromatography and Refractometry

In Gas Liquid Chromatography a mixture of vapors is carried in a stream of helium (carrier gas) through a column. The vaporized sample components move through the column that is lined with a liquid stationary phase. The different components in the sample are retained on the stationary phase for different characteristic relative times. Each component ultimately reaches the Flame Ionization Detector, the most commonly used detector in GC (Air + Hydrogen gas, ratio 10:1). They are detected by their ability to form ions when they are burned in the $H_2$ / air mixture. The area under a peak in a gas chromatogram is proportional to the amount of that substance in the sample.

Among the factors that influence the separation of compounds by gas chromatography are selection of liquid phase, column temperature, and flow rate of carrier gas. Two common liquid (stationary) phases are silicone oil, which separates components on the basis of boiling point, and carbowax (polyethyleneglycols), which separates components on the basis of polarity. The effect of increased column temperature is to decrease the retention time of a component. As a rough approximation, a 10-15 °C decrease in column temperature corresponds to a doubling in the retention time. For most samples, the lower the column operating temperature, the higher the partition coefficient in the stationary phase, and hence, the better the separation. Too low a column temperature can lead to broad, asymmetric peak shapes. The criterion for resolution of the sample is simply achieving baseline separation of the components. Varying the column temperature and selecting the appropriate liquid phase will achieve resolution of the sample into its components. Identification of retention time can be accurately obtained using a pentane peak as a standard. There will always be enough pentane in the syringe to leave a small peak on the chromatogram. The retention time of the other peaks can be calculated using the pentane peak. The relative amounts of carvone and limonene in each fraction and the original oil may be calculated by using the area under the appropriate peaks.

By measuring the refractive index of the original oil, limonene and carvone fractions, you can estimate the purity of the respective fractions and the composition of the original oil. Assuming that the actual refractive index, $n$, measured for the two-

component mixture (limonene and carvone) is linear in the molar fraction, $x$, of any of the components, then one can write:

$$\mathbf{n = (1 - x_{carvone}) * n_{limonene} + x_{carvone} * n_{carvone}}$$

Plug in your data and determine the value of $x_{carvone}$ for the limonene and carvone distillation fractions and the oil itself. Compare these results with those obtained by GC.

## IV. EXPERIMENTAL PROCEDURE

**DAY 1: Distillation and Gas Chromatography: WORK in PAIRS. Split evenly the limonene and carvone fractions for the derivatization step where students work solo.**

### Part A. Distillation

Before setting up the glassware as shown in Fig. 2 have your teaching assistant demonstrate how to connect to the vacuum pumping manifold. You should be able to reduce the pressure to 1-2 Torr (or less) in a closed system. At the pressure achieved, calculate the temperature at which the limonene and carvone should distill over. Carefully assemble the glassware as shown in Fig. 2. **Lightly grease all joints as demonstrated by your TA**. Be sure to include a heating mantle, a stirring plate and an ice-bath in the setup. **Do not proceed until your TA has checked your setup. Test the vacuum on your system for any leaks without adding sample to the round bottom flask. Why is this important?**

TAs will give each pair of students 20 mL of unknown. Each student should **save** a small portion, about 5 drops, for gas chromatography and refractive index measurements when students will work solo. For the 20 mL unknown students should use a 50 mL round bottom flask, and add a 1/2" stir bar.

- Check if the water-in and water-out connections are set correctly (see Fig. 2) and secured with the wire tubing clamp. Make sure you are using water tubing not vacuum tubing.

- Turn the water on in the condenser.

- Immerse only the first receiving flask into a mixture of ice and water until you have collected the limonene then cool all four receiving flasks positioned under the outlet tubing of the cow in a mixture of ice and water. Why do you begin only cooling the one flask positioned to collect the first fraction?

- Put aluminum foil around the vigreux column and distilling flask, this will help raise the T of the system making the distillation proceed a bit faster.

- Turn on the magnetic stirrer and fully open the vacuum valve (note: pressure **cannot** be controlled with this valve. If an adjustment is necessary, please see the Instructor). The pressure should read less than 2 mm Hg (2 Torr) on the manometer. Determine the temperature the limonene and carvone fractions should begin to distill based on the pressure reading on the manometer, using the chart at the end of this experiment. Feel free to verify your answers with your TA.

- Use the Variac dial (starting at setting 40); increase the temperature slowly about 5 variac units every 5 minutes. Although some "explosive" bumping is expected, do not allow the heat to reach such a level that the column is flooded since this will dramatically decrease the efficiency of the separation.

**Note:** On a warm day the room temperature may be at or above the boiling point of limonene (ca 32 ºC) at reduced pressure. If the laboratory is warm be sure to check for limonene distillation before turning on the heat source.

When the oil begins to distill, the condensation can be seen inside the thermometer adapter, thus the progress of distillation can be monitored. The rate at which the temperature changes at the top of the column is significant. Be sure to record temperature information in your lab notebook.

To ensure an optimal separation, three fractions should be collected: **the limonene** fraction, **an intermediate fraction** that forms during the rapid increase in temperature after collection of the limonene fraction, and the **carvone fraction**.

**To collect each fraction, rotate (under vacuum) the cow adapter such that the end of the bent outlet sits above the next empty receiver flask.** Label flasks, for example, with letters **A, B,** and **C**.

When collecting the first fraction, chill only that one flask using a 100 mL beaker filled with ice. After collecting the first limonene fraction then cool ALL four collection flasks simultaneously with a large Pyrex crystalizing dish filled with ice. Why is it now important to cool all four flasks? At the end of the distillation use a glass Pasteur pipette to remove the material from your distillation flasks. Pouring it out will cause your product to be contaminated with stopcock grease, which may result in many extra peaks in your GC trace.

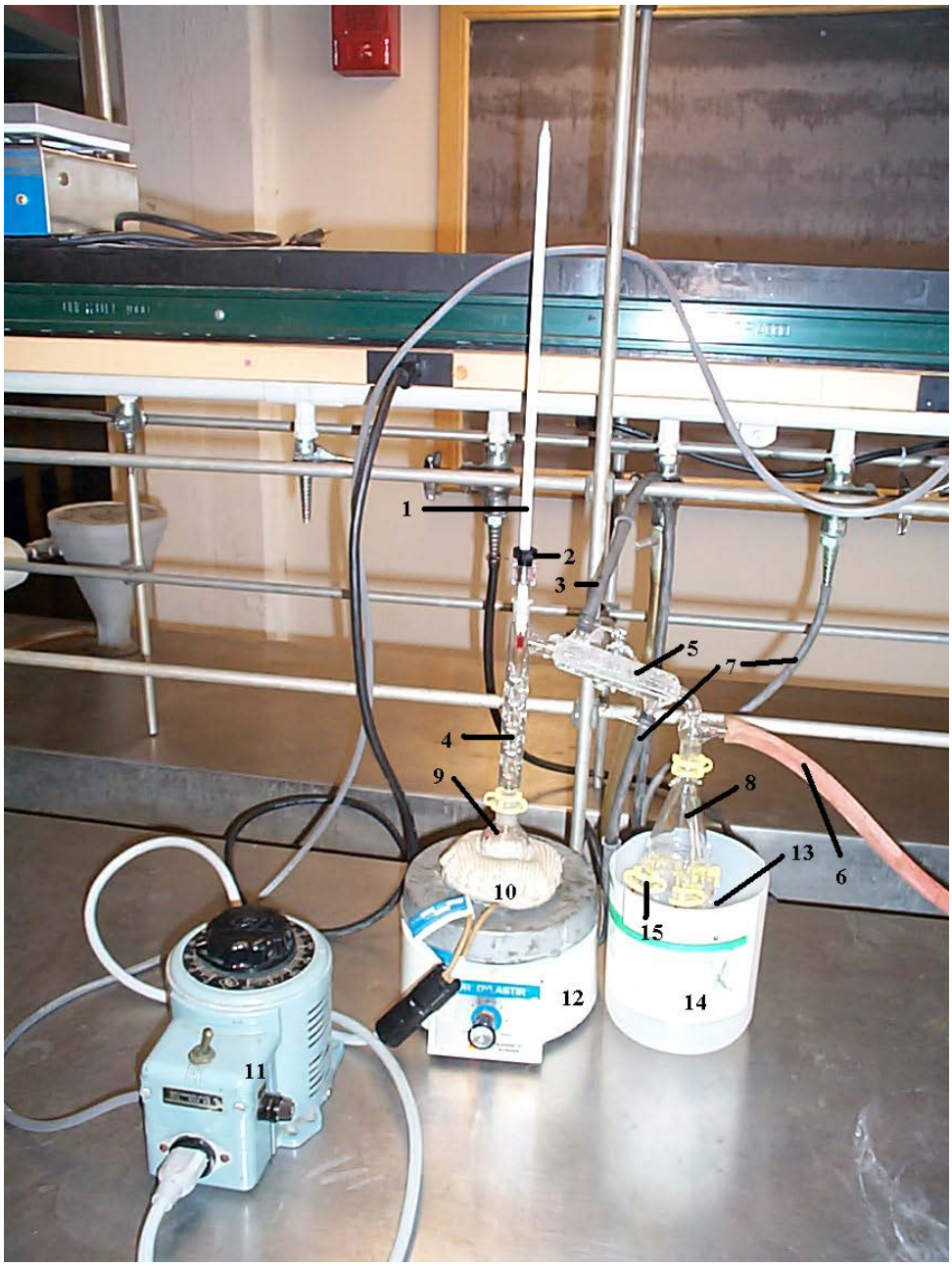



***Figure 2. Apparatus used for Vacuum Distillation***

1. Thermometer
2. Adaptor
3. Water-out
4. Vigreux column
5. Condenser
6. Vacuum connection
7. Water-in
8. Cow
9. 25 mL (10 mL of oil) or 50 mL (15 mL of oil) round bottom flask
10. Heating mantle
11. Variac
12. Stirring plate
13. 10 mL pear-shaped receiving flask
14. Bucket with ice and water
15. Keck clamps

## DAY 2 Part B. Gas Chromatography

Part B. Gas Chromatography: Each student should run the following three GC's:

(a) **the original oil**
(b) **fraction 1 (limonene).**
(c) **fraction 3 (carvone)**.

Read the general references on Gas Chromatography in *Techniques in Organic Chemistry* **(MHSM)**. Detailed instructions for use of the Agilent 7890B gas chromatographs are provided in the Appendix at the end of this experiment.

Save a small portion (5 drops) of your original essential oil sample and of the distilled limonene and carvone fractions for Gas Chromatography and Refractive Indexes measurements. The composition of these samples will be analyzed to determine the effectiveness of the separation. Follow the procedures in the appendix **carefully** for preparation of the gas chromatograph samples.

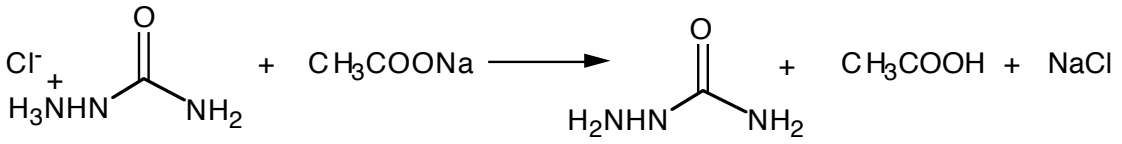
The biggest problems with sample preparation is:

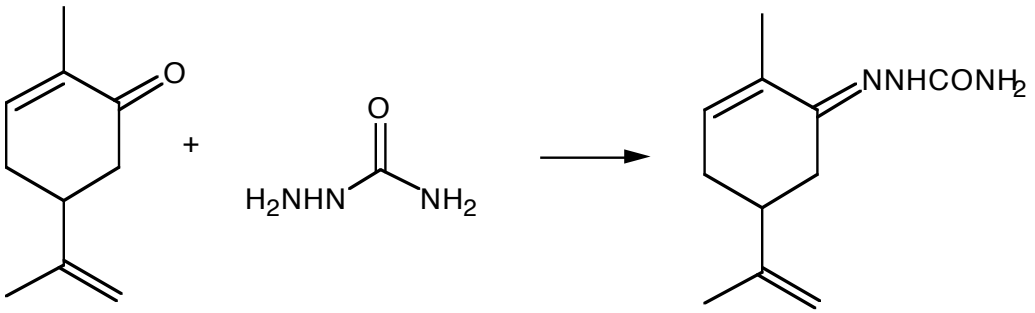
1) **Not carrying out the double dilution** (use disposable test tubes) for the GC analysis (thus the sample is too concentrated to get clean separation with minimal background noise and may overload the column)

**Procedure for the double dilution:**

Obtain a small test tube from the stockroom. Use a digital pipette to inject exactly one mL of pentane into a small test tube. At the same time, use the same digital pipette to inject one mL of pentane into a GC vial. Now, use a 200 microliter pipettor to inject 50 microliters of your sample into the test tube. Mix well, then draw out 50 microliters of that and inject into your GC vial. This is called a double dilution and the 50 microliters represents exactly one drop of sample. Immediately crimp seal the GC vial and label it on the side with a sharpie. Use this double dilution technique to make up three samples: the original oil, limonene and carvone sample that you distilled. Take the three vials to the GC room and place them into the correct positions in the auto sampler and enter the information on each vial into the sample run spreadsheet.

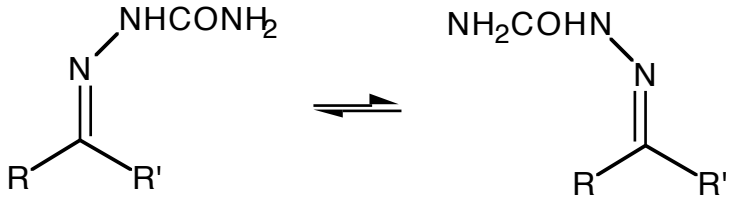
**DAY 2 (continued) Part C & D. Synthesis of the Semicarbazone, and Determination of the Infrared Spectrum of the Limonene and Carvone Distilled Samples**




Carvone | Semicarbazide | Carvone semicarbazone
mp 141-142 °C
FW 207.2

There are two diastereoisomers for the semicarbazone of (-)-carvone and (+)-carvone. They result from the restricted rotation about >C=N- bond. The α-isomer of (-)




or (+)-carvone melts at 162-3 °C, the β-isomer at 141-2 °C. Students will obtain either α-or the β-isomer depending on the actual conditions used. Because the limonene does not have a carbonyl group, the small amount of it, which remains in the carvone fraction, will not form a semicarbazone derivative. The limonene will remain in solution and be washed away during filtration.

In a large test tube, dissolve 0.5 g (4.5 mmole) of semicarbazide hydrochloride and 0.5 g of anhydrous sodium acetate (or 0.8 g of sodium acetate trihydrate) in 4 mL of distilled water and 7 mL of ethanol. Add 0.5 mL (0.48 g, 3.2 mmole,) of your carvone fraction. Stopper and shake your tube vigorously. Remove the stopper; place a boiling chip in the test tube and place in a beaker of warm water (ca. 80 - 90 °C) for 30 minutes. Careful! Contents of the test tube will boil. Beware of splashing!

To isolate the crystals of your semicarbazone derivative for x-ray analysis:

Remove the test tube from the water bath and slowly add 3ml of water down the side of the flask. You will likely see the product begin to immediately precipitate. Transfer the contents of the test tube to a 125 mL Erlenmeyer flask, decanting away from the boiling chips. Rinse out the test tube with water and ethanol as needed and add those washings to the flask. Place the Erlenmeyer flask into an 80 x 40 crystallizing dish half filled with water and heat on a hotplate. Continue to heat to dissolve the precipitate, add minimal amounts of ethanol as needed until the solution is clear. Begin to boil off some of the excess solvent by continuing to heat the flask indirectly in the shallow water bath. When about 15 mL of solution remains in the flask, stop heating and place the flask in the water bath into your drawer. The flask and water bath will remain in the desk drawer until the next lab period. This results in the formation of many fine needle-like crystals. The slow reheating and slow cooling steps promote recrystallization and the formation of single crystals for x-ray and optical activity studies.

## Part D. Infrared spectroscopy of limonene and carvone

Run infrared spectra following procedure demonstrated by your TA, of the limonene and carvone fractions obtained by distillation. The infrared spectrum may be run applying neat liquid directly to the diamond crystal on the Bruker IR. Apply several drops of liquid (disposable plastic pipet!) on the diamond crystal and follow the instruction in the Appendix II to run a spectrum.

## DAY 3 Instrumental Analysis of the Limonene and Carvone

At the start of Day 3 please filter your crystals that should now have grown from Day 2 in your desk drawers. Collect the crystals using vacuum filtration in a Hirsch funnel. Place the crystals in an open 20 mL scintillation vial (put your name on it) into a large crystallizing dish (provided by the TA) in a 100° C oven for at least 30 minutes before proceeding with Part E & F below.

Be sure to record details about the instruments used manufacturer model (number) for inclusion in your report.

## Part E. Melting Point of Semicarbazone Derivative

Take the melting point of the dry solid semicarbazone derivative and record the yield. If it is necessary to recrystallize the semicarbazone derivative, you may do so from an ethanol/water mixture. Repeat measurement of the melting point to verify the success of the recrystallization.

## Part F. Measurement of Optical Rotation by Polarimetry

Polarimetry measurements will be taken on the Rudolph Autopol 4 polarimeter (See instructions in Appendix III) and must be taken at 20° C. Check your report and make sure the temperature is relatively constant or re-do the analysis. Each student must do a polarimetric analysis of their sample. Make up 10 mL of a 1.5-3% (w/v) solution[6] of your semicarbazone in ethanol. Oftentimes, it is difficult to dissolve all of the crystals in the cold ethanol. Please set up a sand bath using a small crystalizing dish and heat the sand bath on a hot plate. Place your solution into a 25 mL Erlenmeyer flask and indirectly heat it by swirling it in concentric circles with your hand in the sand bath. This indirect heating should not boil your ethanol solution, but should dissolve your sample nicely in a few minutes. Add this homogeneous solution to a polarimeter tube obtained from your TA If there is any particulate matter or cloudiness to the solution filter it first. The instrument used to fill the tube is designed to prevent the formation of any air bubbles that could affect your results. Follow the instructions of your TA on the operation of the instrument used to fill the polarimeter tubes. Measure the optical rotation of the semicarbazone derivative solution in the polarimeter following the procedure demonstrated by your TA. This technique is very dependent on the interaction of light with a clean sample. Be sure the cell is clean afterwards by injecting acetone several times, then air drying the cell on the lab bench with the high pressure air outlet. There should be no smell left in the tube after cleaning. Make sure you do not throw out the caps on the polarimeter tube place the caps in the designated container in the polarimeter tube box. Also make sure you do not overtighten the caps these are similar to leur locks and operate on the principle that a gentle turn will lock the caps.

## Part G. Refractometry

Refractive index measurements will be taken on a Rudolph J357 refractometer (see Appendix IV). Measure the refractive indexes of pure limonene, pure carvone, original oil, limonene fraction and carvone fraction following the procedure demonstrated by your TA. Calculate $x_{carvone}$, and $X_{limonene}$ and compare with your GC results.

[6] Approximately 0.15 g to 0.3 g sample in 10 mL of solution

## DAY 4 X-Ray Crystallography Analysis

Lab sections will visit the MIT Chemistry Department X-Ray Crystallography Laboratory and the crystal structure of selected (+) and (-) carvone enantiomers will be determined.

## Crystal Structure Determination

Crystallography pertains to studying the structure and properties of crystals. More specifically, x-ray crystallography is a method of determining the three-dimensional structure of molecules on the atomic level by means of x-ray diffraction on crystal lattices. The diffraction pattern obtained from the interaction of a monochromatic x-ray beam with the lattice of a single crystal consists of hundreds or thousands of discrete reflections that form a lattice of their own, the reciprocal lattice. The individual reflections in this lattice can be understood as coefficients in a Fourier synthesis where the reflections' intensities correspond to the magnitudes and their reciprocal coordinates translate into the frequency. The result of the Fourier summation is the three-dimensional electron density function of the entire crystal. The determination of a crystal structure consists of several steps all of which pose their individual challenges.

A high quality single crystal is needed to determine a crystal structure and often, **crystal growth** is the bottleneck in structure determination. One of the best methods to grow quality crystals is vapor diffusion: an anti-solvent (also called precipitant) with a higher vapor pressure than the solvent is allowed to diffuse into a vial with a solution of the compound of interest and, over time, crystals form. It is important to keep crystals, once obtained, in their mother liquor as often solvent molecules are incorporated into the crystal lattice and drying the crystals might destroy them.

From the first diffraction images, one can usually judge the quality of the crystal and determine the **unit cell**. Depending on the crystal system, which corresponds to the symmetry group of reciprocal space, a data collection strategy can be devised. A good dataset is complete (>98%) and all data have been collected with a redundancy of 6 or better.

Once all data are collected, correction for polarization and other effects as well as absorption need to me made. This step is called **data reduction** and the result of it is a file containing a list of all reflections, each with a set of reciprocal coordinates $h$, $k$ and $l$, an intensity and a standard uncertainty.

Based on intensity statistics and systematic absences in reciprocal space, the symmetry group of the crystal, the **space group**, can be reconstructed (not always unequivocally). Knowing the space group is vital for correctly determining the crystal structure, as the

entire crystal is to be described by (usually) just one or two molecule(s). The crystal structure, therefore, is the spatial average of the entire crystal and space group symmetry operators plus translation expand the structure to the whole crystal.

For a Fourier synthesis one needs magnitude, frequency and phase. Unfortunately, only the first two can be derived directly from the diffraction experiment. Assigning a (preliminary) phase angle to each reflection is called solving the structure. For chemical crystallography, the **phase problem** is solved mostly with direct, dual-space, and Patterson methods; in protein crystallography other methods such as MAD/SAD phasing or molecular replacement are also used.

With the trial phases determined during structure solution, a first Fourier summation is performed and a preliminary model of the molecule can be obtained. In this model, some atom types may be assigned incorrectly and other details of the structure may still be missing. The way from the first solution to the final model is called **structure refinement**. This step can be easy at times (a matter of mere minutes) or difficult in non-routine cases when refinement may take days or sometimes even weeks.

Further Reading:

Müller, P., *Crystallography Reviews* **2009**, *15*, 57-83.

Clegg, W., *X-Ray Crystallography (Oxford Chemistry Primers)* 2nd Edition, Oxford University Press, **2015**.

## V. ANALYSIS & DISCUSSION

**Be sure to incorporate your answers into your written report (discussion section).**

1. Using the refractive index data determines the approximate composition of your oil, your carvone fraction and your limonene fraction with respect to limonene and carvone.

2. From the gas chromatography, report the percentages of limonene and carvone in your original sample and in the fractions following distillation. How effective was the distillation in separating these components? Are there any other (minor) components in any of the fractions? If so, how much of the sample do they constitute? Can you identify any of the minor components? Calculate the # of theoretical plates for each sample peak $n = 5.55\ (t_r/w_{1/2})^2$

3. Compare the results obtained from gas chromatography and refractive index measurements. How closely do they agree? If they are not in good agreement can you provide an explanation?

4. Which isomer of carvone do you have? Is it *R* or *S*? How do you know? What is the association between the chirality and the odor of the carvone isomers?

5. Calculate the **specific rotation** of the semicarbazone derived from carvone,

$$[\alpha]_D^{25} = \frac{\alpha}{C * l}$$

where $\alpha$ is the rotation angle read on the polarimeter, *C* is the concentration of the semicarbazone solution in grams per milliliter of solution, and *l* is the path length of the polarimeter tube in decimeters (these units are employed for historical, rather than rational reasons). Include a propagation of errors!

6. (a) Which features in the IR spectrum, can be assigned to limonene? To carvone? Identify as many functional groups as you can in each sample.

   (b) Do the spectra of the samples suggest the presence of a mixture of these two components, and if so, are they consistent with the GC data?

   (c) Can you use IR spectroscopy to distinguish between *(R)*- carvone and *(S)*-carvone?

7. Your well written discussion should address the following (within the text, NOT as a numerical series of answers to questions):

   1. Comparison of your distillation results (temperature ranges) with your chromatographic results.

   2. Explanation of how the separation of components could be improved.

   3. Comparison of your results (boiling points, compositions, m.p. of semicarbazones, optical rotations) with literature values.

4. Discussion of what information gas chromatography provides.

5. Discussion of what information infrared spectroscopy provides.

6. Discussion of what information X-Ray Crystallography provides, include data tables in your results section.

7. Discussion of what information these techniques do NOT provide.

8. Discussion of what the identity of the carvone enantiomer tells you (if anything) about the identity of the limonene in your sample.

## VI. ADDITIONAL READING

1. Ernest L. Eliel and Samuel H. Wilden, "*Stereochemistry of Organic Compounds*"; Wiley: New York, 1994, p 202.
2. S. Robinson and E.R. Gilliland, "*Elements of Fractional Distillation*,"4th ed.; McGraw-Hill: New York, 1950.
3. G. Guiochon, G; Guilleman, C. L. *Gas Chromatography Rev. Sci. Instrum.* **1990**, *61*, 3317.
4. D.L. Sayers and R. Eustace, "The Documents in the Case" (1930): a particularly nasty murder is solved by using polarimetry to distinguish between natural and synthetic muscarine [$C_9H_{18}NO_2$.HCl].
5. "Looking Glass Chemistry," *Science,* **1992**, *256*, 964.

# APPENDIX I

## Operation Instructions for GC 7890B

1. Turn on Hydrogen Generator switch on back takes 10 min to warm up.
2. Open the air valve on the wall and turn on the Air generator which sits on top of the hydrogen generator.
3. Fill the solvent vials B, B2, A, A2 with pentane on the robotic turntable, place back into their correct positions.
4. Turn on the Helium gas tank, this sits at the opposite end of the room please do not touch the regulator. Open the helium valve on the wall behind the instrument.
5. Turn on GC power button front bottom left
6. Turn on computer and printer. Password for computer *liquidbromine*.
7. Press Start on front of Hydrogen Generator make sure its warmed up and you press start before continuing with Step 8.
8. Double Click CP OPEN LAB Control Panel
9. Click Launch to start instrument
10. Go to Methods select Load & Run Method then double click Essential Oil_2
11. Click front detector on front of GC make sure H2 flow and air flow are turned on.
12. Go to Sequence click Sequence Table—Sequence table opens up
13. Append Lines third icon over click then insert # of samples you will run
    Fourth icon over allows you to delete lines if you make a mistake
14. In first line insert Method name Essential Oil_2 from pull down menu, then under injector location insert Front, Injection Source select As Method, Sample Type select Sample. Injection volume stays blank.
15. Now highlight Method Name, Injector Location, Injector source Injection Volume, Injection Location and Sample Type for all cells, then right click and select FILL DOWN—all the information from line 1 fills into all the sequence lines for your student runs. Injection Volume stays blank.
16. Now, have students place their samples into the GC starting at position #5. The first student should type in their sample location

under lines 1, 2 and 3 as 5, 6, and 7. Students should type the name of the sample which will appear on their spectra. The student should remember their sample location numbers.

17. Once all samples have been inserted press RUN.
18. You will get a message that the current sequence template has been modified. Do you want to save this as the sequence template file? **Answer NO.**
19. The machine says scheduling current sequence run, when GC is ready run starts automatically.
20. To Shut Down GC when all samples have run follow the procedure below.
21. Press Red X in top right corner of GC to Close Cartman GC-1 you will get a message that the Sequence has changed. Save current changes? **Answer NO.**
22. X off any remaining programs that are open.
23. Go to Start Menu then Shut Down Computer
24. Shut off Printer
25. Now Shut off GC
26. Turn Off Helium tank far end of the room, then shut the helium valve on the wall behind the instrument.
27. Shut the air valve on the wall, then, Turn Off Air using the on/off button top right corner behind the generator.
28. Press STOP on front of Hydrogen Generator then shut off at back of machine, on/off switch top right corner behind the generator.

# APPENDIX II
# BRUKER ALPHA II IR
# OPERATING INSTRUCTIONS

1. IR Stays on all the time do not turn off
2. Turn on Computer
3. Double click on OPUS 8.1 software
4. USER: Student PASSWORD: student
5. Click OK on message that comes up version of software
6. Look at computer screen make sure green light at lower right-hand corner is on indicates computer and IR are connected
7. To begin and take a measurement click on the green test tube
8. Routine Measurement dialogue box comes up—Fill in sample description
9. Click background single channel
10.. If Solid, place solid on the diamond ATR—then rotate arm over sample—then use pressure clamp lever all the way down onto the solid—click on sample single channel

Axis will appear as Absorbance v. wave number with peaks pointed up (this is what you want for a solid sample)—to change to transmittance v. wave number (liquid samples) click the button AB to TR on top of screen, you can toggle back and forth. There are two ways to label the spectrum, single peak pick which labels individual peaks and the standard peak pick which opens a dialogue box which can label all of them or some of them. Undo changes to file will undo what you just did. If you want to enlarge part of the spectrum use the Zoom button creating a box place on the area and click. You can always scale the Y axis to adjust and always click the undo button to get back to the original. Click the print icon to print the spectrum. Be sure to darken the color (black) is best for printing.

11. To run a new spectrum click on the test tube icon—run a background—then follow instructions above. The previous spectrum on the screen will automatically disappear.
12. All the data from the spectrum is located on C drive in the folder alpha FTIR data—you can open a file and insert a USB drive to copy the data.
13. **If running a Liquid sample**, move arm counterclockwise out of the way (no pressure necessary from the arm on the liquid). Place one drop of liquid on the diamond ATR and follow the steps in 10 above.
14. To clean diamond ATR use brush to collect solid sample into a weigh boat then for solids and liquids spray a little isopropanol alcohol on a kim wipe and clean the diamond ATR after you have ran the sample.

# APPENDIX III
# RUDOLPH POLARIMETER MODEL AUTOPOL 4
# OPERATING INSTRUCTIONS

1. Turn on instrument button located on back left side screen goes dark for 15s give it another minute to load before pressing anything—takes about 5 minutes to warm up
2. Click Temperature, Temp Trol, 20°C, then SET.
3. Zero the system on AIR with thermocouple in its reference position green light on.
4. Click Temperature, Temperature Correction, then Quartz, then press SET.
5. Take QCP (quartz control plate), insert thermocouple and place QCP in trough metal against metal. Live reading should match QCP 11.502±0.004. Keep QCP in machine until temperatures agree 20°C. If temperature does not match take out QCP and insert thermocouple into its reference holder and ZERO machine again. Reinsert the QCP, insert the thermocouple and try to verify the certified optical rotation of the QCP at 589nm which should be 11.502±0.004. Once verified remove QCP and place back in its wood box (return to stock room) place thermocouple back in its reference holder in the machine. Click Temperature, Temperature Correction, Unclick Quartz, then press SET.
6. Take a polarimeter cell and place into a sample loading module light should be turned on. Introduce the sample using a 10 mL syringe from the lower side, inject slowly and observe light will change to darker then as you inject it will get lighter and clear again. If light is clear white then it's a good sign you have no bubbles and your cell is ready to be capped. Inject sample until you see liquid rising at the higher end opening. Do not let liquid drip out. Cap the higher end while the syringe is still in place on the lower end, then remove syringe and cap the lower end. Click Temperature, Temp Trol, 20° C, then SET. Make sure you zero the machine with Air before inserting sample.
7. Insert thermocouple into sample holder and place holder in machine to the extreme right -side metal on metal. Make sure connecting wire to thermocouple does not block light beam.
8. Click main menu to get all icons to show up on machine.
9. Select Methods then Specific Rotation.
10. Now Press START and enter mass and volume of sample, then press CALCULATE and finally press SET.
11. Sample runs automatically after pressing SET.
12. Data prints with error analysis
13. Open machine door take out thermocouple put back into its resting place. Place sample holder into sample loading module. Remove both white caps and insert syringe in lower end and draw out as much sample as possible. Take sample holder and place over a waste container and flush with acetone using a clean syringe. Find an air outlet in lab and dry out sample holder thoroughly. Return sample cell to stock room.
14. When all samples have been run, click on exit Method, click Temperature and turn OFF Temp Trol, Turn off instrument in back.

# APPENDIX IV
# RUDOLPH J357 REFRACTOMETER
# OPERATING INSTRUCTIONS

***Always use soft disposable flexible pipettes to apply sample to prism. Never use glass pipettes which might scratch the prism.***

1. Turn on switch located on left front of instrument. Screen may appear dark for 15 seconds.
2. Wait 30 to 45 seconds for temperature to stabilize
3. Open the presser place deionized distilled water on the black prism until covered then close the presser. Make sure sample just covers black prism and stays within white circle.
4. Check sample quality blue bar should be at least 80% towards good if not add more sample.
5. Make sure the temperature of the refractometer is reading 20°C
6. Look at the live reading of water on the screen it should be 1.33294 to 1.33304. If its within that range prism is clean and you can continue with your sample. If not rinse prism again with water and dab off with tissue wipes.
7. Open the presser place sample on the black prism until covered then close the presser. Make sure sample just covers black prism and stays within white circle.
8. Press START—machine is set to take five readings each takes about 5s. When Start screen appears again measurement is complete.
9. Print results print key located bottom right—In the event printer does not print no worries data is stored and can be retrieved by date/time—from DATA and printed from there.
10. Clean off sample first using tissue dab off then put on few drops of methanol and dab off.

# 5.310 Laboratory Chemistry

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