Isolation Experiment

Part 1 Extraction of Caffeine from Tea
Part 2 Extraction of Carvone from Caraway Oil

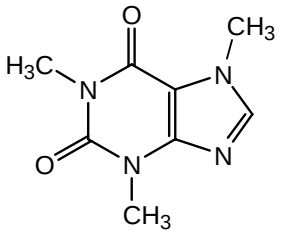
You should work in collaboration with a lab partner for the experimental part of this lab. Each of you should select one of the two parts to do, and share data. You must still write your reports individually. Each partner should be active in both parts of the lab. The partner that does the caffeine isolation should measure the optical rotation of carvone.

We have done some types of isolation already. Distillation, extraction, filtration, and column chromatography are certainly isolation procedures. But when chemists speak of entire experiments as isolations, they generally refer to a (possibly complex) set of steps that result in the isolation and purification of a natural product from a natural source. This is extremely important, since so many natural compounds are so important. Natural products provide medicines, materials, and starting compounds for other substances. Finding the *best* way to isolate natural products involves understanding the physical and chemical properties of the molecules in the source, adapting the published techniques of others to your situation, experience, and sometimes trial and error. It can, at times, be a somewhat tedious and repetitive process, but also very exciting because the chemist gets to play detective. Isolations involve many techniques, and test the logic of the chemist, as well as the familiarity of the chemist the properties of organic compounds (e.g., solubility, reactivity).

Part 1 Background (Isolation of Caffeine from Tea)

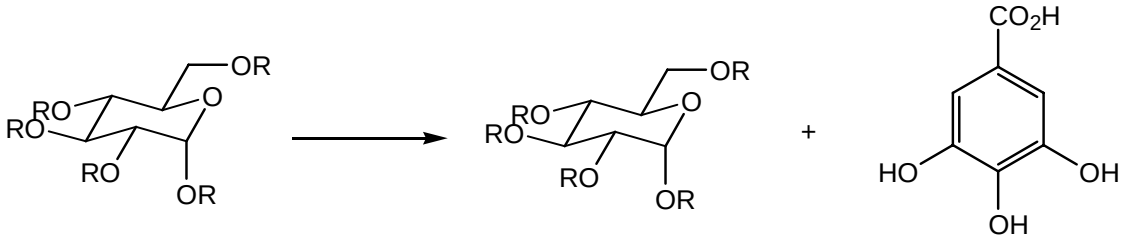
Legend has it that Daruma, the founder of Zen, fell asleep one day during his customary meditations. To ensure that this indiscretion would not recur, he cut off both of his eyelids. Where they fell to the ground, a new plant took root that had the power to keep people awake. Reports of the medical use of tea may go back nearly 5000 years.[1] Caffeine is an alkaloid and central nervous system stimulant. Alkaloids are (some) organic natural products that contain nitrogen. Since nitrogen is a basic element (lone pair), these compounds are given the name alkaloids (alkali-like). In one ounce of your average tea, there is 5-15 mg of caffeine. Coffee and Coca-Cola contain 12-25 and 3.5 mg per ounce of beverage, respectively. Caffeine addiction is real: an addict will experience lethargy, headache, and perhaps nausea after about 18 hours of abstinence.

[1] Pavia, D. L.; Lampman, G.M.; Kriz, G. S. *Organic Laboratory Techniques: A Contemporary Approach,* 3rd ed.; Saunders College Publishing: New York, 1988.



Caffeine

The separation of caffeine from tea is complicated because there is more in tea than just caffeine. Cellulose (a glucose polymer that makes up the structural material in plants) is the main ingredient in tea leaves, but fortunately is not soluble in hot water. Caffeine is soluble in hot water. However, so are tannins. Tannins are a group of compounds: many of them are esters of glucose and gallic acid. When tea is treated with water, the tannins are partially hydrolyzed. Tea also contains pigments, chlorophylls, and other minor components.



Tannins (any number of OH groups may be esterified)

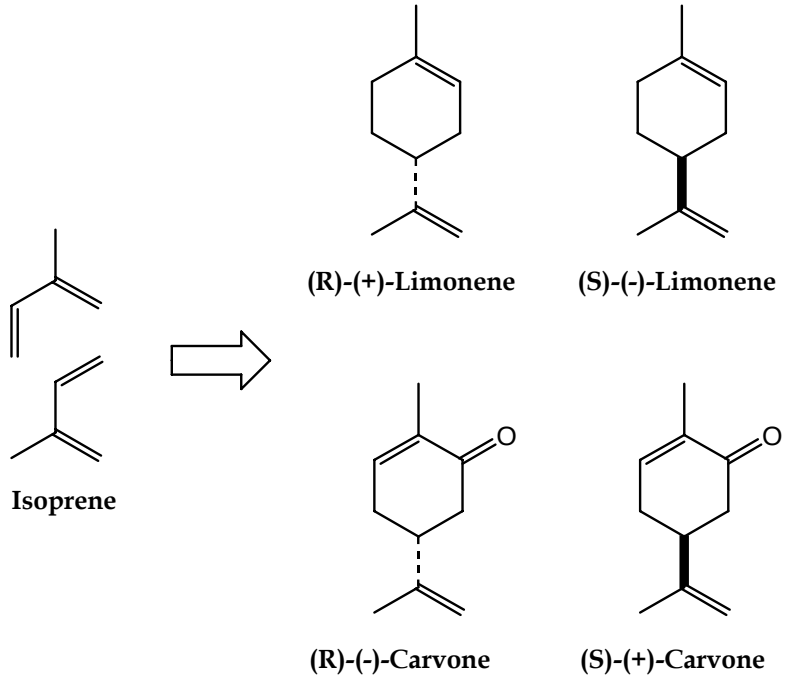
Some OR → OH in hot base

Gallic Acid

There is more than one way to get caffeine from tea, but we will follow a straightforward one using extraction of the tea leaves into water (brewing) and extraction. Imagine what is happening in each step. First we separate compounds based upon their ability to dissolve in hot water (as well as hydrolyzing some of the tannins, which makes *them* more soluble in hot water). Second, we separate compounds based upon their solubility differences in water versus an organic solvent (CH_2Cl_2).

Part 2 Background (Isolation of Carvone from Caraway)

Caraway seed is actually a dried fruit that is grown mainly in Holland. The essential oil of caraway, approximately 1-3% of the seed weight contains two main compounds, limonene and carvone. Both compounds are terpenes, compounds with carbon skeletons composed of isoprene units joined together. Isoprene is 2-methyl-1,3-butadiene. Many terpenes are natural products. They have a variety of roles in plants and animals: for instance, attractants or defense secretions. Some are known to be very potent cancer causing or cancer fighting agents.



Oddly enough, some organisms produce (R)-(+)-limonene while others produce (S)-(-)-limonene. Same thing for (R)-(-)-carvone and (S)-(+)-carvone. In this experiment we will isolate (+)-carvone from caraway oil using vacuum distillation.

Properties of Enantiomers

Identical Properties

Solubilities
BP, MP
Color
Density
Spectral properties (IR, NMR)

Different Properties

Interaction with plane polarized light (*specific rotation*: equal and opposite rotation)
Interaction with other chiral molecules

Important Terms

Chirality is equivalent to handedness. A **chiral molecule** is one that is not identical with its mirror image. An **achiral molecule** is one that can be superimposed on its mirror image. Any tetrahedral atom that has four different attached groups is a **stereocenter**, or **stereogenic center**. (Can you locate the stereocenter in carvone?) One point is that it is considered incorrect to use the term *chiral center*, or *chiral carbon*. This is because chirality is a property of a whole molecule, not an atom within the molecule.

A **plane of symmetry** bisects a molecule in such a way that the two halves of the molecule are mirror images of each other. Any molecule that has a plane of symmetry is achiral.

Enantiomers are stereoisomers that are related as an object and its mirror image. Enantiomers occur only with compounds that are chiral. Separate enantiomers rotate the plane of plane-polarized light and are said to be **optically active**. They have equal but opposite rotation.
Note: the subtle relationship between the term *chiral molecules* and *enantiomers*. *Chiral molecules give rise to enantiomers.* It is a sort of cause and effect relationship. Also note that chiral molecules, or enantiomers, must come in pairs.

Racemic mixtures (or **racemates**) are equimolar mixtures of enantiomers. Racemic mixtures are not optically active, because the rotation caused by one enantiomer is cancelled out by the rotation caused by the other enantiomer.

Scalemic mixtures are nonequimolar mixtures of enantiomers. Scalemic mixtures are optically active, but the actual rotation (direction and amount) will be determined by the amount of each enantiomer present.

Measuring Optical Activity

Using a **polarimeter**, we *could* measure the interaction of plane-polarized light of the carvone we isolate. In doing so, we would be able to discover which isomer is produced by caraway. Every chiral compound has, as a physical property, its own **specific rotation**, $\mathbf{[\alpha]_D}$, which is just a measure of the *direction* and *amount* of rotation caused by the compound as plane-polarized light passes through. The specific rotation is the rotation in degrees when the concentration is 1.0 g/mL, the path length (l) is 1 dm (10 cm), and the wavelength of light is 589 nm ("sodium D line"). When using a polarimeter, one reads an **observed rotation (α)**. Knowing the concentration of the sample and the path length allows the chemist to convert the observed rotation to the specific rotation.

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

It is enough to specify the specific rotation of one enantiomer. Thus, since (R)-limonene has a specific rotation of +125.6˚, we know that (S)-limonene must have a specific rotation of -125.6˚. Keep in mind that the *direction of rotation is in no way related to the* ***absolute configuration***. Thus, an R isomer could give rise to either positive or negative rotation. There is no correlation.

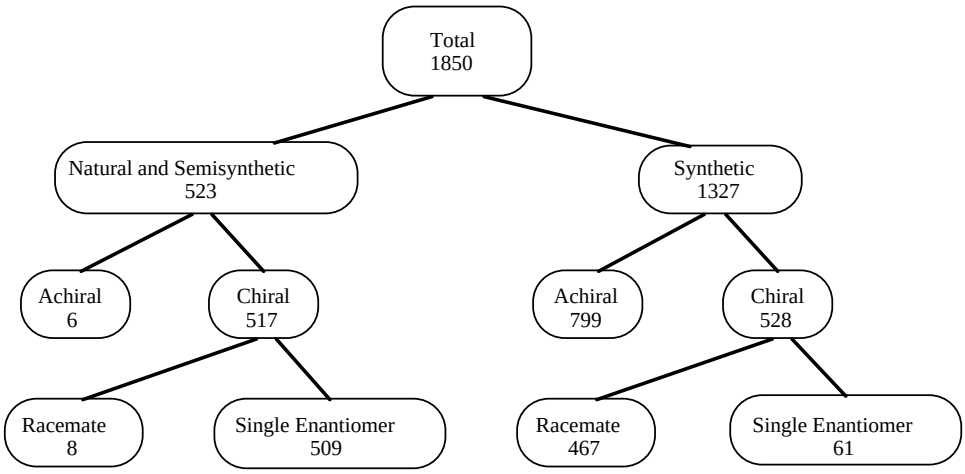
Once you have the specific rotation of your sample, you can compare it to the literature value. If you have a sample of (+)-limonene with a specific rotation of +100˚, then your sample is obviously not pure (+)-limonene, just as a low melting point means your sample is not pure.

More on stereochemistry.

The importance of stereochemistry cannot be overstated. Besides being a truly fascinating topic in its own right, the concept has huge implications in the world of biology, especially drugs.

Pharmaceuticals

It is interesting to consider the role of chirality in the pharmaceutical industry. There are approximately 1850 drugs marketed worldwide, which can be characterized as shown in the chart below (semisynthetic means that a compound from nature is modified in the laboratory).



There is a big push to market only one enantiomer of a drug (to avoid the undesirable side effects of the other enantiomer), so in the future there should be a shift from *racemates* to *single enantiomers* under the *synthetic* category.

Part 1 Procedure (Isolation of Caffeine from Tea)

Do not drink the tea. Work as partners for both days of the isolation experiments.

Remove the tea from three tea bags and weigh out the tea and record. Put the tea in a 250-mL three neck round-bottom flask along with 50 mL of saturated Na_2CO_3 solution and a few boiling stones. Attach a condenser. Stopper any open ports on the flask, but do not stopper the top of the condenser. Connect the water, and ***reflux*** (read Zubrick) the solution for about 15 minutes. While refluxing, set up for the upcoming vacuum filtration and heat about 30 mL saturated Na_2CO_3 solution (add a boiling stone to be safe) in an Erlenmeyer flask using a hot plate. You might also set up your separatory funnel for that upcoming step.

Decant the solution *while hot* **(careful!)** into an Erlenmeyer flask. *(Decant* means to slowly pour off the liquid while leaving the solid in the flask.) Rinse the tea leaves with 30 mL of hot saturated Na_2CO_3 and add this to the Erlenmeyer flask. *While still hot*, vacuum filter through about 1 cm of celite that covers the filter paper in the Hirsch funnel. Transfer the hot tea solution

to a separatory funnel, and then let it *cool.* Take a break here or wash your glassware and get ready for the next steps.

When the filtrate in the separatory funnel has reached room temperature, add 25 mL CH_2Cl_2, stopper, and *gently swirl the two layers together.* It is important that you **do not vigorously shake the funnel** like you normally do. You may invert the funnel from time to time and swirl for about one minute, but be gentle. You are mixing the layers, not beating the heck out of them. The reason for treatment is that, under certain conditions, CH_2Cl_2 will form emulsions (Zubrick, p.163), and we are concerned that this is one of those conditions! By not mixing too vigorously, you can sometimes avoid emulsion problems.

After swirling, place the separatory funnel on the ring, remove the stopper, wait, and hope that the layers separate. If they do not, add a small amount (10 mL) of water and wait. (Don't shake or anything, just add the water gently through the top.) If the layers still don't separate, see your TA. (Probably we will try saturated NaCl, which makes the polar water layer even *more* polar and sometimes breaks up emulsions.)

When the layers separate, drain the lower *organic* layer into an Erlenmeyer flask and extract the water with an additional (fresh) *25* mL of CH_2Cl_2. (Emulsion problems will be less likely this time, but still do the gentle swirling technique.) Again drain the lower organic layer into the *same* Erlenmeyer flask. Add anhydrous Na_2SO_4 (drying agent) to the combined organic extracts (until single crystals of drying agent are observed). Swirl the CH_2Cl_2/Na_2SO_4 solution for 20 seconds, and then let the solution sit 3-5 minutes. Next, do a simple gravity filtration into a *pre-weighed* 100 mL round-bottom flask (it is important that you do this drying technique well, or you will end up with water in your caffeine *after* the rotary evaporation step. if that were to happen, you would have to *redissolve* your caffeine in 25 mL of CH_2Cl_2, then again dry with Na_2SO_4, then again remove the CH_2Cl_2 via the rotary evaporator as described below).

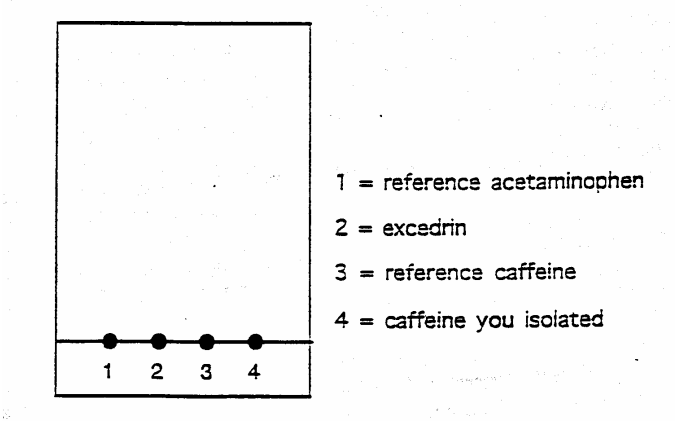
Remove the CH_2Cl_2 using rotary evaporation. After the rotary evaporation is complete (make sure you believe that all the CH_2Cl_2 is gone), reweigh the flask and determine the amount of caffeine isolated. If you have time, do the TLC experiment on this day, or you may do it later.

TLC Experiment

Get four small test tubes. To tube #1, add a sample of reference acetaminophen. To tube #2, add a small amount of crushed Excedrin. To tube #3, add a *reference sample* of caffeine. To tube #4, add a small amount of the caffeine you isolated. *How much to add to each of these tubes? Very little.* You need about 10 mg of sample in each tube. If you *loaded* your little spatula tip with some solid, that is probably 25-50 mg. Add about 1 mL of ethyl acetate to the four tubes and shake. It does not matter if the whole sample does not dissolve. Enough for the TLC will dissolve.

Prepare a developing chamber using ethyl acetate as the mobile phase. Draw a line (pencil!) about 0.5-1 cm from the bottom of the TLC plate and mark as shown below.

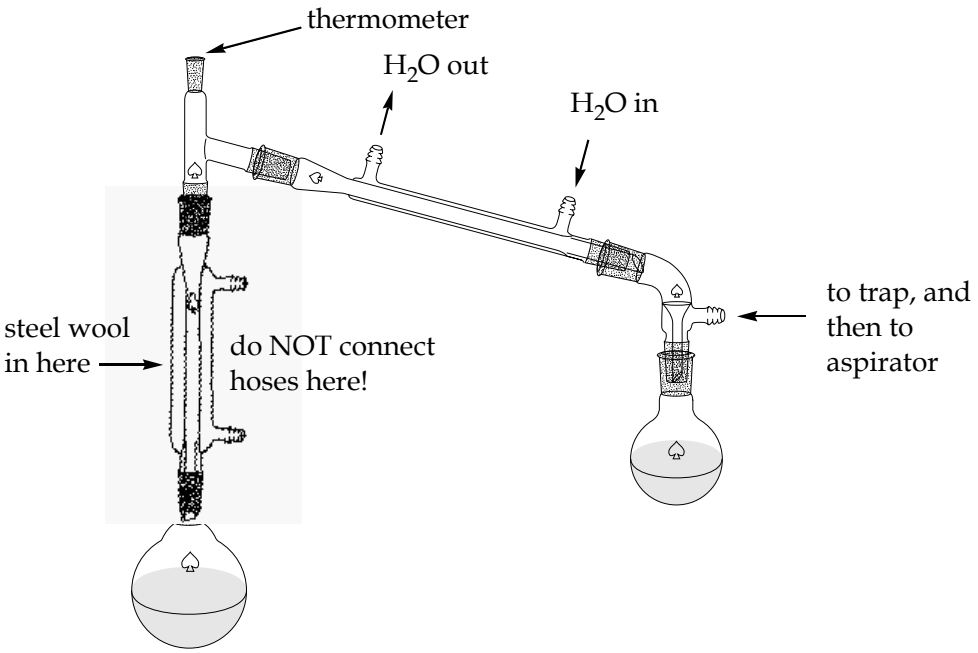
Spot all four solutions, making *very small spots.* You can touch each spot about 3-5 times. Dip the TLC pipette directly into the test tubes. You'll have to tip the test tubes to get the pipette to the liquid. You can the same pipette for all samples by sucking up some acetone and spotting onto a paper towel several times between each new sample.)



Elute the spots until the solvent front is about 0.5 cm from the top. Mark the solvent front with a pencil. Visualize the spots using UV light and then the I_2 chamber. Circle the spots with a pencil.

Part 2 Procedure (Isolation of Carvone from Caraway)

Place 4-5 boiling stones in a 50-mL round-bottom flask, followed by 15.0 mL of caraway oil. Assemble the apparatus as shown below, along with a heating mantle. You will need a Keck clamp at both ends of the condenser, and at the receiving flask. Make sure your Keck clamps work well! Do not put the distillation flask too close to the bench top, because you may need to lower the heat source at any time. A wooden block or a jack should be used for this purpose. Note that the hose connectors (labeled "H_2O out" and "H_2O in" in the figure below) function more effectively if they point down instead of up.



Before heating, do the following: Wrap the top of your distilling flask (not receiving flask) and the whole column loosely with aluminum foil. Try to leave a little opening so that you can peer into the distilling flask from time to time. At the trap, test that you can release the vacuum easily ("vacuum valve") at any time if you need to. Begin running water through the condenser. Close the vacuum valve and turn on the aspirator to begin creating a vacuum. Watch the distilling flask. Sometimes there is enough dissolved air in the caraway oil that you will see bubbles. When there are no more bubbles, begin heating. Begin with the thermostat around 40.

The temperatures noted below assume a vacuum of about 20 mmHg. However, once you set up your apparatus, you will not know the exact pressure in your system (there could be leaks), so the number given are only approximations. First you will collect the first component of caraway oil, limonene. Theoretically, we would expect the limonene to distill at about 75-80˚C. Once all of the limonene has distilled, increase the heat (if necessary) and watch the thermometer. When it hits about 100-110˚C, go to *Flask Change*.

***Flask Change*. You must do the following very carefully, or you may end up starting over.** First, remove the heat source completely. Slide it away from the flask. Then open the vacuum valve **slowly** to bring the pressure back up. Then **slowly** turn off the aspirator. Detach the receiving flask and attach a new one. Wait about 3 minutes for the distilling flask to cool a little.

At this point, turn the aspirator back on, and **slowly close the vacuum valve**. **This is the crucial step!** Keep one eye on the distillation flask and one eye on the top of the column (at the bend) and look for any signs of "bumping" (i.e., the carvone distills all at once). **If anything strange is happening, open the vacuum valve; do not turn off the aspirator quickly.** If your carvone bumped into the receiving flask, there is sometimes nothing you can do but start over. See your TA. If all goes well, you are now ready to collect carvone. Return the heat source and

collect any material that distills up to about 110-125˚C. **Do not distill to dryness.** Leave about 1-2 mL in the distilling flask. You should get around 5 mL of carvone.

When all done, open the vacuum valve **slowly** to bring the pressure back up. Then **slowly** turn off the aspirator. Remove the heat and remove your receiving flask. Allow the apparatus to cool before dismantling and cleaning.

Further Carvone Experimentation

1. Smell Test

Yes, it is rare that the organic chemist resorts to smelling things (unless you are employed as a "nose" in a perfume factory in southern France). However, we will make an exception in this case. Find the samples of (+) and (-)-carvone and smell them, and compare to the smell of the carvone you isolated. Can you tell which carvone you isolated?

2. IR

Record an IR spectrum.

3. Collection

Put your carvone in the appropriate container. Ask your TA or Monitor. We *may* get a class NMR and a class optical rotation of your material.

4. Optical Rotation

You will put your carvone sample into the polarimeter and measure the rotation. Your TA will show you how to operate the polarimeter. From the sign of the observed optical rotation, you should be able to confirm whether your sample is (+) or (-) carvone. Helpful hint: The (+)-Carvone will have an **observed rotation** between 0 and +180 degrees, while (-)-carvone will have an observed rotation between 0 and -180 degrees. The two observed rotations will not necessarily be equal in value. **Record the data written on the board regarding path length and concentration.**

Results and Discussion

Discuss whether your isolation of caffeine was successful in terms of the weight obtained (comparing to the amount and percentage recovery to what may be expected), as well as the TLC experiment that you conducted.
Each tea bag contains approximately 55 mg of caffeine (you used three of them), and for the carvone isolation, you may assume that the theoretical yield of carvone is 5mL. Also, make a statement about the composition of each spot on your TLC plate.

Discuss whether your isolation of carvone was successful in terms of the weight obtained. State whether you were able to identify the handedness of the carvone that you isolated according to the smell test, and on what basis you made that conclusion. Is the IR spectrum that you obtained consistent with the literature spectrum of carvone? Explain. Additionally, calculate the specific rotation of (+)-carvone and (-)-carvone from the optical

activities measured in the laboratory. For neat liquids (i.e. they are not dissolved in any solvent), you may assume that *c* (the concentration of the sample) is simply the density of the liquid (carvone). Are your calculated values consistent with the literature values? Why do you think the specific rotations obtained from the literature are not of identical magnitude? Explain.

Name: _____ Section: _____

_____

ID #: _____ Date: _____

Partner(s): _____

Part 1 Procedure (Isolation of Caffeine)

Weight of round-bottom flask (g): _____

Weight of round-bottom flask + caffeine (g): _____

Weight of caffeine (g): _____ % recovery _____

TLC Experiment:

	R_f value(s)
reference acetaminophen	_____
excedrin	_____
reference caffeine	_____
isolated caffeine	_____

Day 2 Procedure (Isolation of Carvone from Caraway)

Carvone from smell test (check one): (+)-carvone: _____ (-)-carvone: _____

Measured optical rotation: (+)-carvone: _____ (-)-carvone: _____

Calculated specific rotation: (+)-carvone: _____ (-)-carvone: _____

Literature values as follows:

$[\alpha]_D = +61^o$ for (+)-carvone (Merck Index) $[\alpha]_D = -62^o$ for (-)-carvone (Merck Index)