

## CHEM 321: OPTICAL RESOLUTION OF 2-PHENYLSUCCINIC ACID

From R. Stephani and V. Cesare, *Journal of Chemical Education* 74, 1226 (1997)

The purposes of this experiment are to show how enantiomers can be separated if they are converted into a mixture of diastereomers and to understand how careful choice of solvent can permit selective precipitation of one compound from a mixture of compounds. In addition, the following techniques will be introduced: 1) reflux to maintain a constant temperature, 2) recrystallization to purify a compound, 3) determination of melting range to test the purity of a compound, and 4) measurement of optical rotation to further characterize an optically active compound.

*Chirality* is a necessary condition for the existence of optical activity (the ability to cause rotation of the plane of polarized light). This property is a direct consequence of the configuration, or orientation of bonded atoms in space, around the chiral center. Think of a chiral center as an atom to which four different groups are covalently attached. If you study models of chiral molecules, it soon becomes apparent that their mirror images are not superposable on each other. Such mirror images represent different *stereoisomers* — they are different molecules. The difference, however, is subtle. Pure samples of mirror image molecules behave identically toward physical tests like melting and boiling points and solubility in given solvents. The only way that these isomers differ physically is the direction in which they rotate plane-polarized light — their optical activity. Nonsuperposable mirror image molecules are called *enantiomers*. Enantiomers are distinguished from each other by designations like "R" or "S", "D" or "L", or by the direction of the rotation they cause ("+" or "-").

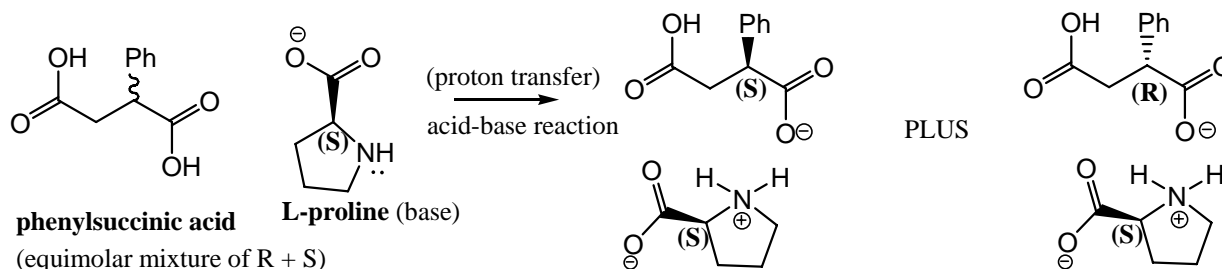
Since the presence of optical activity is an indication of a particular configuration of atoms, any change in optical activity that occurs during a chemical reaction offers a way to study the kind of configurational change that accompanies the reaction. Optically active reagents have long been important in studies of reaction mechanisms because they may offer glimpses at the molecular details of the process as the reaction proceeds.

How does one obtain optically active compounds? The first source was natural products — chemicals produced by living organisms. These chemicals are invariably synthesized by catalysts called enzymes, which are large chiral molecules specially designed to carry out a particular chemical transformation. Part of their specificity lies in how they bind, or attract and hold, the molecular pieces that are to be fashioned into the product. Most often, these pieces can attach to the enzyme only in one particular orientation. This specificity of attachment means that the atoms of the product always end up being connected to each other in only one of several possible ways. For this reason, if the biologically produced product contains one or more chiral centers, it is optically active. Examples include table sugar, honey, monosodium glutamate (MSG), vitamins, paper (if we could measure it!), . . . — in short, most biologically produced substances are optically active.

But the existence of chirality is not by itself a sufficient condition for optical activity. Consider a *racemic* chiral compound (a sample in which there are exactly equal populations of both enantiomers). Any rotational effect that one enantiomer has on plane-polarized light will be exactly canceled somewhere else in the mixture by its mirror image. As a consequence, the mixture causes no net rotation, and appears optically inactive. So, for optical activity to be evident, there must be an excess of one enantiomer over its mirror image. Given that enantiomers have identical physical properties, how can the mirror-image molecules become separated without the aid of some living thing?

The answer lies in converting the mixture of enantiomers into different compounds — a pair of *diastereomers* (stereoisomers that are not mirror images of each other). Diastereomers are not chemically identical, and so they differ in common physical properties. They are potentially separable by ordinary physical methods like different solubility in a given solvent, which allows recovery of a single diastereomer. An ideal solvent would completely precipitate one diastereomer while the other one remained in the solution. A suitable solvent is found by trial and error. In this experiment, 2-propanol is the suitable solvent that will selectively precipitate most of one diastereomer and leave most of the other diastereomer in the solution. The recovered diastereomer must be converted back to the original compound from which it was made — now a single enantiomer. Its optical activity can be measured to verify that one enantiomer was isolated in excess over the other.

Living organisms produce many reactive substances as single enantiomers. Such compounds can be employed to make diastereomer pairs by chemical reaction with a racemic mixture of a desired compound. In this experiment, racemic (R + S)-phenylsuccinic acid is separated (*resolved*) into optically active enantiomers in a sequence of two steps. First we exploit its chemical nature (it is an acid) by treating it with an optically active biologically produced base, L-proline. This is an ordinary acid - base reaction that produces a salt. The acid and base are structurally more complex than HCl and NH<sub>3</sub>, but the concept is the same. The phenylsuccinic acid acts as the proton donor, while proline is the proton acceptor.



The two salts shown above are stereoisomers that are not mirror images, so are a pair of diastereomers. Note that the two diastereomeric salts (S,S) and (R,S) differ in their anions (phenylsuccinate), which are nonsuper-posable mirror images, but have identical cations (L-proline). In this case, the salt that contains the dextrorotatory acid ("+") is the less soluble one, and precipitates. Filtration permits discard of the more soluble salt, which carries away one enantiomer ("-") of the phenylsuccinic acid.

Once the less soluble diastereomeric salt has been isolated, only a single enantiomer of phenylsuccinic acid remains. It now must be recovered from the salt (the second step). This is simple since neutral phenylsuccinic acid is poorly soluble in water. A strong acid (6 M HCl), added in excess to the salt, is used to protonate the water-soluble anion. As the anion binds the H<sup>+</sup>, it loses its negative charge and becomes poorly soluble in water. The resulting solid acid can be recovered by filtration. [*Proline likewise gains H<sup>+</sup>, but this gives it a net + charge, making it very soluble in water. It washes away in the acidic water, leaving nearly pure phenylsuccinic acid as the solid.*]

Recrystallization is a technique that can be used to remove contaminants. One chooses a chemically inert solvent or pair of solvents in which the desired compound is poorly soluble at or below room temperature, but quite soluble at the boiling temperature of the solvent or pair of solvents. The crude substance should be dissolved in the smallest possible volume of solvent. This volume is found by trial and error, occasionally stirring a suspension of the solid in a few mL of solvent while heating the covered mixture. Ideally, the last of the solid dissolves just as the liquid begins to boil. If some solid remains when the solvent gently boils, one adds a little more solvent while maintaining a gentle boil (beaker covered all the time). [If after this no further dissolving occurs, the remaining insoluble matter must be filtered out by another technique (called "hot filtration"). Your TA or instructor can help you with this technique, if you need to do this.]

The now clear, hot, covered solution is allowed to cool slowly to room temperature. As the temperature falls, the desired compound becomes less soluble and begins to form a crystal lattice. Slow cooling is essential to permit the growing lattice to reject impurity molecules which do not fit properly into the lattice. Rapid cooling results in capture of impurities in the lattice, or prevents lattice formation all together, resulting in an oil.

Once near room temperature and not forming more solid, the mixture is chilled using an ice bath to maximize recovery of the solid. When the temperature of the mixture is approx. 5 °C, the mixture is agitated to suspend the solids and immediately vacuum filtered. The impurities remain soluble and pass through the filter. The crystals can be rinsed with a small amount of clean ice cold solvent to remove traces of impurities dissolved in the original solvent. The solid on the filter is a more or less a pure substance.

**Polarimetry** The success of this experiment is evaluated by computing the apparent specific rotation of the product. Specific rotation is defined as  $[\alpha]_D^{20} = r/lc$  where

- $[\alpha]_D^{20}$  = the specific rotation (at 20°C using the "D line" of a sodium lamp; units are [deg•mL]/[g•dm])  
r = the observed angular rotation of plane-polarized light in degrees  
l = cell path length in decimeters (1 dm = 10 cm; use 1 dm in equation)  
c = concentration of compound in g/mL of solution

Normally, the yellow "D" line (589.0 nm) of a sodium vapor lamp is used. Our polarimeter approximates this with a filter. (Specific rotation has been found to depend on the structure of the molecule, solvent, temperature, wavelength of light, and other factors.)

**Before coming to lab, complete the following prelab exercise in your lab notebook.** You will need to do something similar after you measure your product's optical rotation.

The published specific rotation of (+)-phenylsuccinic acid is +173.3° (Shiraiwa, T., Sado, Y., Fujii, S., Nakamura, M., Kurokawa, H., *Bull. Chem. Soc. Japan* (1987), **60**, 824-827). Our polarimeter has 1 dm cells that contain 8 mL of solution. Assume that we want to see about 10° of rotation. What mass of product should be dissolved in the 8 mL? Outline a procedure to make the needed solution using your product.

### EXPERIMENTAL SECTION (work in pairs)

In what follows, mole ratios are important. Record the mass of phenylsuccinic acid that you actually use (it may be different from the recommended value below), compute the number of moles, and accurately weigh the correct mass of proline to give the same number of moles. Formula weights are printed on bottle labels.

**Synthesis of diastereomeric salts:** Weigh racemic phenylsuccinic acid (1.94 g, 0.01 mole) into a 100 mL boiling flask. Add 2-propanol (50 mL) and swirl to dissolve the solid. Add L-proline (1.15 g, 0.01 mole). Swirl for a minute to mix, then set up for reflux (boiling chip!) and boil gently for 30 min. During this time, all the proline should dissolve. The less soluble salt may precipitate. At the end of 30 minutes, turn off and remove the heating mantle. Allow the flask to air-cool about 10 min. (If there is no precipitate, use a Pasteur pipet to remove about 1 mL to a small test tube. Scratch inside this test tube several minutes until the contents begin to get cloudy. Immediately return the contents to the boiling flask and swirl. Cloudiness and then crystals should gradually form.) Once the contents of the flask are near room temperature, take it to a sink and run cold water over the outside for one minute while swirling (NO WATER INSIDE!).

**Recovery of less soluble salt:** Vacuum filter the contents of the flask using a medium Buchner funnel. To get rid of 2-propanol and any unreacted phenylsuccinic acid, resuspend the crystals in 10 mL of acetone in a small beaker. Change to a Hirsch funnel (small, conical; use proper paper!) and filter again. Repeat a second time with 15 mL of acetone. Pull air through the solid for a couple of minutes after the last wash to evaporate most of the acetone. The recovered solid is the less-soluble diastereomeric salt of the pair that you made.

**Recovery of resolved acid:** Add all of the solid to 10 mL of ice-cold 6 M HCl in a small beaker in an ice bath. Stir for 5 min to allow complete protonation. The solid that results is the now-resolved phenylsuccinic acid in its protonated form. The proline, its former partner in the salt, remains soluble in the aqueous phase.

Remove the beaker from the ice bath and quickly dry off the outside with a paper towel to prevent water from contaminating the crystals. Swirl and pour as fast as possible into a clean Hirsch funnel. Use a Pasteur pipet to drizzle 1-2 mL of **ice-cold** water over the entire surface of the solid to wash away as much 6 M HCl as possible. Don't use too much water, as the product is somewhat soluble and will slowly dissolve.

**Recrystallization:** The recovered acid contains contaminants – possibly some left-over proline and some HCl. These must be removed to get a good optical activity measurement because proline has optical activity of its own, and any HCl would add mass but not optical rotation. Water turns out to be a good recrystallization solvent for this product. Put the entire crude product into a small beaker, and add approx. 5 mL of DI water. Cover the beaker with a watch glass, and **keep the beaker covered at all times unless you need to access its contents!** Heat and gently

swirl the mixture on the hot plate until all the solid dissolves (add a little more water if the entire solid is not in solution just as the solution begins to gently boil). Once everything has dissolved, take the beaker off the heat and leave it covered. Let it cool slowly to room temperature; then (and only then) move it into an ice bath. Swirl occasionally. Prepare a clean Hirsch setup, remove the beaker from the ice bath and dry its outside, swirl, and dump everything into the funnel. If a lot of product remains in the beaker, you can return the filtrate to the beaker, cool again, dry, swirl, and dump on top of the funnel contents. Pull air through for a couple of minutes, then spread the crystals onto a clean paper towel (folded for safety!) so they can dry for at least a day.

**Characterization:** Tare an empty analytical balance, and record the mass of a small weighing boat. Put your dried product in the boat and weigh again to obtain the mass of product you recovered. Prepare a melting point tube containing the some of the product, and keep it in a safe place until you have access to a melting point apparatus.

**Optical rotation:** reweigh the boat so that you know the remaining mass of product. Transfer all the remaining crystals to a clean, dry 10 mL graduated cylinder and add about 7.0 mL of acetone (record the exact volume in your graduated cylinder, and do all transfers with a Pasteur pipet – do not pour). Mix by using a Pasteur pipet to suck up and then expel the liquid in the cylinder. Do this until all solid has dissolved and you see no Schlieren lines as you expel liquid. Adjust the volume to 8.0 mL with more acetone (Pasteur pipet; don't contaminate acetone supply – pipet has product in it) and mix again as before. Stand the 1 dm polarimeter cell on end so that the bulge in the glass is at the top. Use the Pasteur pipet to transfer the solution from cylinder to cell. Fill the cell brim-full – it won't take all 8 mL. Position the top window so that its metal projection mates with the slot in the metal top of the cell. Then secure it with the large ring nut. Tip so that the bubble is trapped in the bulge and mount the cell in the polarimeter. Measure the optical rotation: take at least three "zero" readings with the polarimeter empty plus three readings of your solution. After you have finished, empty the cell into a mixed organics bottle and wash the top window (both sides) and the cell with several very small rinses of acetone.

**Melting range:** catalogs, web sites and the reagent bottle all list the melting range of phenylsuccinic acid as about 167° C. However, this melting range pertains to the racemic mixture of both enantiomers. Since R and S molecules have (subtly) different shapes, they interfere with each other's deposition into the crystal lattice, which depresses the melting range of the mixture.

After separation, each enantiomer is in the company only of molecules identical in shape to itself, so formation of a regular crystal lattice is easier than it is for the racemic mixture. The melting range of pure (+)-phenylsuccinic acid (and of course of pure (-) as well) reported in the source for this protocol is 185-186° C.

**REPORT:** Follow the Lab Syllabus Guidelines for Introduction and Experimental Sections.

For results, present your findings either as a small table or short paragraph. Include specific rotation of your product, its optical purity (defined in question 4 below), and the yield (be careful – what would 100% be?). Compare your results to published values together with citations of the source(s) of those values.

Also, discuss the significance of the results in light of the objectives stated in the introduction.

In an Appendix, show pertinent sample calculations. In addition, answer the following enrichment questions there:

1. You have some racemic 2-aminopentane (structure below). Could the phenylsuccinic acid recovered from this experiment be used to resolve the amine into its pure enantiomers? Explain. (*Hint: consider acid or base character of the suggested reactants.*)  
$$\text{CH}_3\text{CH}(\text{NH}_2)\text{CH}_2\text{CH}_2\text{CH}_3$$
2. Would the mother liquor (the liquid that came through the filter after the first precipitation) have had optical activity? Explain.
3. **Two chemical factors** can cause the observed (computed) specific rotation magnitude to be lower than expected. This probably happened in your experiment. Name these chemical factors and tell why each would lower the computed value of  $[\alpha]$ .
4. You have a solution of a single optically active compound. If the concentration of the compound is doubled,
  - a. does magnitude of the observed optical rotation of that solution change? How/Why?
  - b. does the  $[\alpha]$  of the compound change? How/Why?
5. A solution at a certain concentration of (+)-alanine from a fossil of a fern leaf exhibits an  $[\alpha]$  of  $+27.0^\circ$ . At the same concentration, pure (+)-alanine has an  $[\alpha]$  value twice as large. Optical Purity (or Enantiomeric Excess) is defined as follows:

$$\% \text{ Optical Purity} = \left( \frac{[\alpha]_{\text{sample}}}{[\alpha]_{\text{pure}}} * 100 \right)$$

- a. What are the values of  $[\alpha]$  for pure (+)-alanine and pure (-)-alanine?
- b. What is the percent optical purity of (+)-alanine from this fossil sample?
- c. The  $[\alpha]$  value of the fossil sample is lower than that of pure (+)-alanine because the fossil sample contains some (-)-alanine. What percentages of (+) and (-)-alanine enantiomers are in this sample?