Fall Organic Chemistry Experiment #5
Resolution of Enantiomers by Diastereomeric Salt Formation

Suggested Reading

Lehman Operation #37 Nuclear Magnetic Resonance Spectroscopy p.244

Jones Section 4.9 Physical Properties of Diastereomers: Optical Resolution pages 176-178

Introduction

In this experiment, we will investigate the stereochemical nature of organic molecules. If you recall from class, isomers are compounds that have the same number and types of atoms, but they are arranged differently in each molecule. The two broadest categories are constitutional isomers and stereoisomers. The two structures shown in Figure 1 represent constitutional isomers (compounds with the same molecular formula, but different sequences of atoms). Clearly, these two compounds have different connectivity of atoms.

Figure 1

![Molecular Formula = C₅H₁₂](image)

2,2-dimethylpropane  n-pentane

The second category, stereoisomerism, is the focus of today's experiment. Stereoisomers have the SAME connectivity, but the atoms have a different arrangement in three-dimensional space. The study of stereoisomers and their effects on chemical reactivity is called stereochemistry. The two molecules shown in Figure 2 represent a common form of stereoisomers called enantiomers. Other examples of stereoisomerism include diastereomeric systems, the conformational analysis of cycloalkanes, and cis/trans isomerism of alkenes.

Figure 2

![mirror plane](image)

Enantiomers are nonsuperimposable mirror image structures. If you recall from Chapter 4 of your text, when a molecule contains a central carbon with four different colored groups (white, red, blue, and green), we say that the carbon is a stereogenic atom. We can configure the four groups around the stereogenic carbon to produce two different arrangements of atoms. The
resultant pair of structures are mirror images of one another that are non-superimposable. In fact, if you try to superimpose the two molecules you will only get any two of the four colored groups to coincide. This enantiomeric pair represents one example of a molecular system that is chiral (exhibits handedness). See Chapter 4 for further discussion about chirality and stereochemistry.

If you notice in Figure 2, I have labeled each isomer as R or S. These notations are commonly used to identify the absolute configuration at each chiral center in your molecule. The absolute configuration is used designate the precise relationship in three-dimensional space for each group attached to the asymmetric carbon. Each of the groups is assigned a priority based upon its atomic number. For example, in the enantiomeric pair shown above, the chlorine (Cl=17) is the highest priority group, followed by the hydoxyl group (O=8), methyl group (C=6), and hydrogen (H=1), respectively. In assigning the R or S designation, a model is made and the lowest priority group (in this case, the hydrogen) is placed 180° away from the observer. The other three groups in order of highest to lowest priority are assigned. The relative relationship is then determined: they are arranged either clockwise (R) or counterclockwise (S). This assignment is shown in Figure 3.

Figure 3

Now, remember that we said, "the study of stereoisomers and their effects on chemical reactivity is called stereochemistry". The second part of this statement is very important. There are many examples in organic chemistry where stereochemistry plays an integral part in the course of a chemical reaction. You will later learn that in the course of a chemical reaction a bridged intermediate may form. One such example is shown below. This bridge effectively blocks one face of the molecule resulting in the attack of the nucleophile (Br-) from the open face as shown in Figure 4. For more on this type of reaction, you can refer to pages 442-449 from your text.

Figure 4
As you can see, the product has two carbons, each with four nonequivalent groups attached. Thus, they are both stereogenic carbons and can be assigned the R or S configuration. In fact, this reaction proceeds with definite and expected stereochemical results. It is the structure of the starting material that ultimately determines which enantiomer(s) is/are formed during the course of the reaction. In this particular example, the cis-2-butene starting material will yield exclusively the (R,R) and (S,S) enantiomers. It is the bridged bromonium ion intermediate that ultimately controls how the second nucleophile attacks. This second nucleophile will attack from the least sterically hindered face promoting backside attack. This reaction is an excellent example of how stereochemistry can be predicted from the structure of the starting material and ultimately controlled by the structure of the intermediate in the mechanism of the reaction.

There are a number of common examples of how stereochemistry is involved in organic reactions (e.g. the SN2 reaction, epoxidation reactions, oxymercuration/reduction, etc.). In addition, there are numerous examples of stereochemistry in biological systems as well. Most enzyme-catalyzed processes involve the stereospecific binding of a substrate to the active site of an enzyme. Biological messenger molecules (e.g. neurotransmitters) bind to receptors with a high degree of stereospecificity. Indeed, chiral molecules exist widely throughout nature. All sugars, proteins (including enzymes), and nucleic acids (DNA and RNA) are chiral and occur in only one enantiomeric form. The opposite enantiomer to the naturally occurring one is not and usually cannot be utilized by biological systems.

Stereochemistry is prevalent in medicine, too. Over 50% of all pharmaceutical products on the market are chiral. In most cases, only a single enantiomer is biologically active while the opposite enantiomer is either inactive or toxic (see Section 4.14 on Thalidomide in your text, page 187). In recent years, the Food and Drug Administration (FDA) and the major pharmaceutical companies have become increasingly interested in producing enantiomerically pure products (see Chemical and Engineering News -- one issue per year is dedicated to the topic of chiral drugs -- click on link). Serious efforts in the laboratory over the past ten years have resulted in the production and marketing of chiral (single enantiomer) drugs. Continuing advancements in technology have now given scientists the resources to produce compounds that have very specific biological activity as a result of their stereospecificity (chirality). We are beginning to realize the achievement of the goal of developing drugs with decreased (or the complete absence) of side effects. In addition, agrochemical manufacturers have begun to utilize these advancements to produce pesticide replacements called biocides. These biocides are designed to specifically affect a single species of organism while leaving any other inhabitants in the vicinity (including humans) unaffected. There is a great deal of examples in the chemical literature on this topic.
How can we produce these enantiomerically pure compounds? There are actually a variety of techniques that range from producing mixtures of isomers and separating them to actually synthesizing the specific "pure" isomer. Some of the more common techniques are listed below:

1. **Asymmetric synthesis** --- using chiral reagents, catalysts, and fragments to produce molecules of a SINGLE enantiomeric configuration. Results in the total synthesis of an optically active compound. The preferred technique, but is difficult when dealing with biological molecules. Biomolecules tend to be extensive and contain multiple stereocenters.

2. **Chromatographic resolution of enantiomers** --- requires an optically active (chiral) stationary phase that allows passage of one enantiomer while the other is held up on the column. Generally applicable when dealing with a small amount of material.

3. **Enantiomeric enrichment** --- use a pool of small, optically active (chiral) starting materials to enrich the product percentage of a desired isomer. Usually results in the partial synthesis of an optically active compound.

4. **Enzymatic resolution** --- use of an enzyme to separate isomers.

5. **Enzymatic/microbial transformation** --- use of an entire biological system to synthesize the product of interest from a "fed" starting material.

6. **Resolution of diastereomeric salts** --- diastereomer formation followed by product enrichment by the selective crystallization of one diastereomer over the other.

7. **Diastereomeric derivatization** --- attachment of an enantiomerically pure extension (fragment) to both enantiomers to produce a diastereomeric pair. The pair can be physically separated usually by chromatography.

Each of these methods is commonly employed by scientists in industry, the government, and academia. We will be investigating the utility of the "the resolution of diastereomeric salts" as an example of a method to convert an enantiomeric mixture into a mixture of diastereomers. Recall, that enantiomers have identical physical properties that render them inseparable. Diastereomers, on the other hand, are separable because they have different physical properties.

In today's experiment, we are specifically interested in separating the enantiomers of racemic α-phenethylamine by conversion to a pair of diastereomeric salts using (+)-tartaric acid. We will be taking advantage of the fact that diastereomers (unlike enantiomers) have different physical properties. In this case, one of the diastereomeric salts, the (-)-amine-(+)-tartrate salt, has a lower solubility than its diastereomeric counterpart. We will fractionally crystallize the (-)-amine-(+)-tartrate salt from methanol. Once separated, we will treat the purified diastereomeric salt with base. This process will break apart the salt and regenerate the free unprotonated (-)-amine. In order to determine the enhanced purity of this enantiomer, we will form the (S)-(+)O-acetylmandelic acid salt and analyze by NMR spectroscopy.

**Procedure**
Place 7.8 g of (L)-(+)-tartaric acid and 125 mL of methanol in a 250 mL Erlenmeyer flask. Heat this mixture on a hot plate until the solution is nearly boiling. Slowly add 6.25 g of racemic α-phenethylamine to this hot solution. Stopper the flask and let stand overnight. The crystals that form should be prismatic. If needles form, they will not be optically pure enough to give a sufficient resolution of isomers -- they must be prisms! Needles should be redissolved (by careful heating) and cooled to slowly crystallize again. When you recrystallize in this fashion you can seed the reaction with a prismatic seed. If you have a mixture of prisms and needles, you can heat until most of the solid is dissolved. Usually the needles will dissolve faster than the prisms in this case.

Once you have obtained the correct type of crystals, during the next laboratory period you should isolate them by vacuum filtration. The crystals can be washed with a small portion of cold methanol. Partially dissolve the crystalline salt in 25 mL of water and treat with 4 mL of 50% NaOH. The resultant solution is extracted with 3 x 10 mL of dichloromethane. The organic layers are combined in a stoppered flask and dried with 1 gram of anhydrous sodium sulfate for about 10 minutes. Decant the dichloromethane into a preweighed 100 mL round bottom flask and remove the solvent on the rotary evaporator. Avoid transferring any of the white solid. Calculate the percent yield of the (S)-(−)-amine based upon the amount of racemate you started with.

Using a small test tube, weigh approximately 0.35 mmole of your resolved amine by adding it drop by drop from a Pasteur pipet. Cork the test tube. Weigh out approximately 0.40 mmole of (S)-(−)-O-acetylmandelic acid and add it to the amine in the test tube (Why are we doing this?). Using a clean Pasteur pipet, add about 250 mL of CDCl₃/TMS to dissolve everything. If the solid does not completely dissolve, you can mix the solution by drawing it several times into the pipet and redelivering back into the test tube. When everything is dissolved, transfer the mixture to an NMR tube (filter through a cotton-plugged Pasteur Pipet) and add enough CDCl₃/TMS to bring the total height in the tube up to 35 mm. Acquire a ¹H-NMR spectrum. Be sure to provide close-up views of the split peak clusters.