

# Reduction by Biological Reagents and Determination of Optical Purity

Adapted from Pavia, Lampman, Kriz, and Engel, *Introduction to Organic Laboratory Techniques*, 3<sup>rd</sup> Edition, Saunders College Publishing, Orlando, FL, 1990.

## Experiment 29

### *Chiral Reduction of Ethyl Acetoacetate; Optical Determination Using a Chiral Shift Reagent*

Fermentation

Stereochemistry

Reduction with yeast

Use of a separatory funnel

Nuclear magnetic resonance

Chemical shift reagents

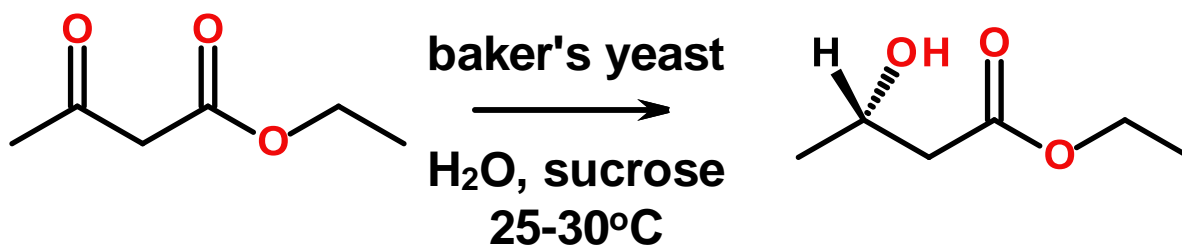
Optical purity determination

The experiment described in Experiment 29A uses common baker's yeast as a chiral reducing agent to transform an achiral starting material, ethyl acetoacetate, into a chiral product, (S)-(+)-ethyl 3-hydroxybutanoate. In Experiment 29B, you will use nuclear magnetic resonance spectroscopy to determine the optical purity (enantiomeric excess) of the product. This experiment requires the use of a chiral shift reagent.

## Experiment 29A

### *Chiral Reduction of Ethyl Acetoacetate*

In this experiment, you will use baker's yeast as a chiral reducing agent to convert ethyl acetoacetate into a chiral product (S)-(+)-ethyl 3-hydroxybutanoate. The chiral product is used as an important building block in the laboratory synthesis of natural products.



The product ethyl 3-hydroxybutanoate is formed principally as the enantiomer with the (S) configuration. The reaction does produce a small amount (generally less than 10%) of the opposite enantiomer (R)-(-)-ethyl 3-hydroxybutanoate.

### SPECIAL INSTRUCTIONS

The fermentation requires at least 3 days; the experiment should be begun in advance of the time set aside for product isolation and polarimetry. If the fermentation is allowed to continue for longer than 3 days, the optical purity (enantiomeric excess) of the product will increase. Under these conditions, however, it may become difficult to identify the minor enantiomer in Experiment 29B.

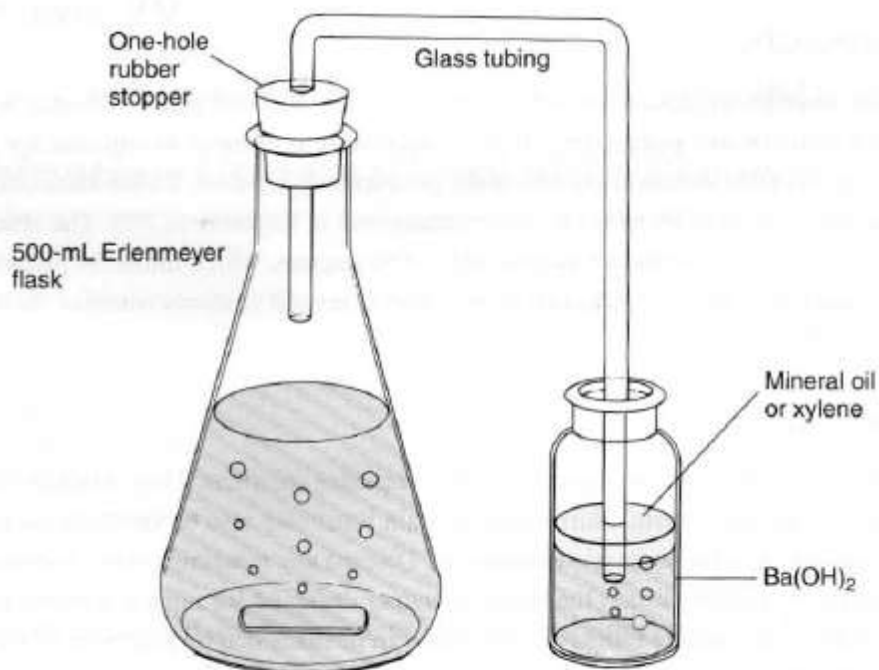
### WASTE DISPOSAL

Most of the solutions used in this experiment are dilute aqueous solutions. They should be discarded into a waste container for aqueous waste. Slurries that contain yeast may also be safely discarded by diluting them and pouring them into the same waste container. Discard any remaining ethyl acetoacetate or ethyl 3-hydroxybutanoate by pouring it into the waste container designed for nonhalogenated organic waste. The filtration residue that contains Filter Aid and yeast can be discarded by placing it into a trash can.

### PROCEDURE

**Fermentation Apparatus.** Equip a 500-mL Erlenmeyer flask with a magnetic stirring bar and a one-hole rubber stopper with a glass tube leading to a beaker or a test tube containing a solution of barium hydroxide. Protect the barium hydroxide from air by adding some mineral oil or xylene to form a layer above the barium hydroxide. The figure below shows the apparatus for this experiment. A precipitate of barium carbonate will form, indicating that carbon dioxide is being evolved during the course of the reaction. Oxygen from the atmosphere is excluded through the use of the trap.

Add 100 mL of tap water, 30 g of sucrose, and about 3.5 g (one package) of dry baker's yeast to the flask. Add these materials, while stirring, in the order indicated. Attach the trap to the fermentation flask. Stir this mixture for about 1 hour, preferably in a warm location. Add 4.0 grams of ethyl acetoacetate, and allow the fermenting mixture to stand at room temperature until the next laboratory period, stirring vigorously. If your laboratory is equipped with a shaker, place your flask in the shaker until the next laboratory period.



Apparatus for the fermentation of ethyl acetoacetate.

After this time, prepare a second warm (about 40 °C) solution of 30 g sucrose in 100-mL tap water. Add this solution, along with 3.5 g (one package) of dry baker's yeast to the fermenting mixture and allow it to stir for 48 hours (with the trap attached) at room temperature.

**Isolation of Product.** Place about 8 g of Filter Aid (Johns-Manville Celite) in a beaker with about 20 mL of water. Stir the mixture vigorously, and then pour the contents into a small Büchner funnel (with filter paper) while applying a *gentle* vacuum, as in a vacuum filtration. Be careful not to let the Filter Aid dry completely. This procedure will cause a thin layer of Filter Aid to be deposited on the filter paper. Discard the water that passes through this filter. Decant as much of the clear supernatant fluid as possible and pass it through this filter, using *very gentle* suction. Filter the residue through the same filter. The extremely tiny yeast particles are trapped in the pores of the Filter Aid. Wash the residue with 20 mL of water, allowing the water to pass into the flask containing the filtered reaction mixture. Add 30 g of sodium chloride, and stir the mixture vigorously for 5 minutes. Extract the aqueous solution with three separate 30-mL portions of diethyl ether using a 250-mL separatory funnel. Be careful not to shake the separatory funnel too vigorously to prevent the formation of emulsions. If an emulsion should develop, drain the aqueous solution from the separatory funnel up to the level of the emulsion. Add 2-3 mL of water to the separatory funnel and swirl the mixture to break up the emulsion. Drain the remaining water from the separatory funnel.

Collect the ether extracts in a 125-mL Erlenmeyer flask, add 1 gram of anhydrous magnesium sulfate, stopper the flask, and allow the solution to dry for at least 5 minutes. Decant the liquid into a beaker, add a boiling stone, and evaporate the ether using a warm water bath in the hood and a stream of air or nitrogen to recover the liquid ester. You should recover about 2-3 mL of liquid .

**Column Chromatography.** Prepare a small chromatography column in the following manner. Place a small plug of cotton in a 5 ¼ inch Pasteur pipet. Tamp the cotton to form a loose plug. Add alumina on top of the cotton plug to form a column 1 cm high. Tap the pipet with your finger to pack the alumina. Using a second Pasteur pipet , add the crude hydroxyester to the column. Rinse the remaining crude product onto the column using 1-2 mL of methylene chloride. Collect the eluted product in a 10-mL Erlenmeyer flask. Use a dropper bulb to force the liquid material through the chromatography column . Dry the organic layer over anhydrous magnesium sulfate for about 10 minutes. Decant the dried solution into a preweighed 10-mL beaker. Evaporate the solvent in a warm water bath (at about 60°C) using a gentle stream of air or nitrogen . Weigh the beaker again in order to determine the weight of the pure hydroxyester obtained.

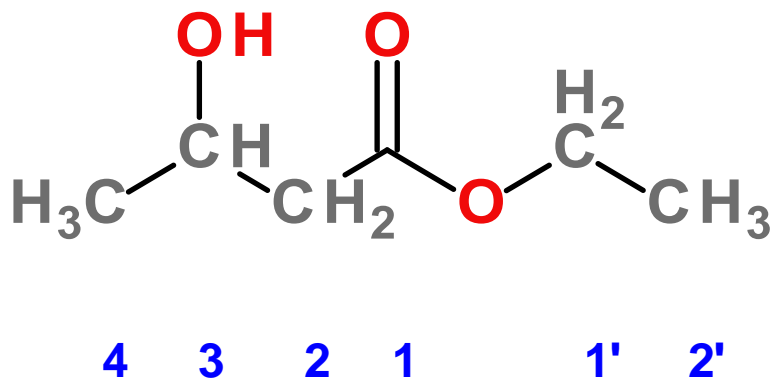
When you have isolated your product , it is important that you check it for purity before proceeding to complete the experiment. Obtain an infrared spectrum of your product. Make sure that you observe an O-H stretching peak at around 3200-3500  $\text{cm}^{-1}$  and that the C-O stretching peak of the ketone functional group (at about 1715  $\text{cm}^{-1}$ ) has disappeared.' If the C O stretching peak remains, or if the O- H stretching peak is absent, your reduction did not take place, and you will have to repeat the fermentation procedure.

If you are going to perform Experiment 29B, remove a 0.030-g portion of your product, place it in an NMR tube.

## Experiment 29B

### ***NMR Determination of the Optical Purity of (S)-(+)-Ethyl 3-Hydroxybutanoate***

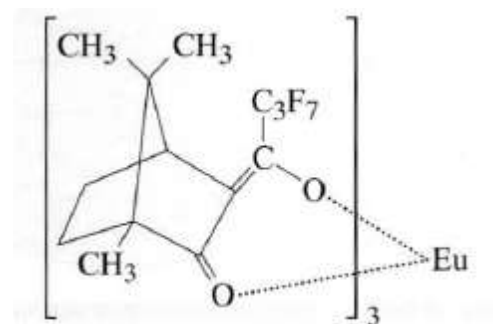
Experiment 29A gave a method for the chiral reduction of ethyl acetoacetate. This reduction produces a product that is predominantly the (S)-(+) enantiomer of ethyl 3-hydroxybutanoate. In this procedure, we will use NMR to determine the actual optical purity of the product. An NMR spectrum of racemic ethyl 3-hydroxybutanoate is shown on page 283. In this spectrum there is no discernible difference between the two enantiomers. The methyl hydrogens on carbons 2' and 4 appear together at about 1.25 ppm, the methylene hydrogens on carbon 2 appear at 2.4 ppm, the hydroxyl proton appears at 3.6 ppm, and the methylene hydrogens on carbon 1' and the methine hydrogen on carbon 3 appear together at about 4.2 ppm.



Although the normal spectrum shows no visible difference for the two enantiomers, there is a method that will allow the spectra of the two enantiomers to be distinguished. This method uses a chiral shift reagent. These reagents "spread out" the resonances of the compound with which they are used, increasing the chemical shifts of the protons that are nearest the center of the metal complex by the largest amount. Since the spectra of both (+)- and (-)-ethyl 3-hydroxybutanoate are identical, the usual chemical shift reagent would not help our analysis. However, if one uses a chemical shift reagent that is itself chiral, one can begin to distinguish the two enantiomers by their NMR spectra. The two enantiomers, which are chiral, will interact differently with the chiral shift reagent. The complexes formed from the (R) and (S) isomers and with (+)-camphor-containing shift reagent will be diastereomers. Diastereomers usually have different physical properties, and the NMR spectra are no exception. The two complexes will be formed with slightly differing geometries. Although the effect is small, it is large enough to begin to see differences in the NMR spectra of the two enantiomers. In particular, the originally superimposed methylene and methine multiplets will begin to be resolved

The chiral shift reagent used in this experiment is tris[3-(heptafluoropropyl-hydroxymethylene)-(+)-camphorato]europium(III), or  $\text{Eu}(\text{hfc})_3$ . In this complex, the europium is in a chiral environment because it is complexed to camphor, which is a chiral molecule.

$\text{Eu}(\text{hfc})_3$  has the following structure:



## SPECIAL INSTRUCTIONS

This experiment requires use of an NMR spectrometer.

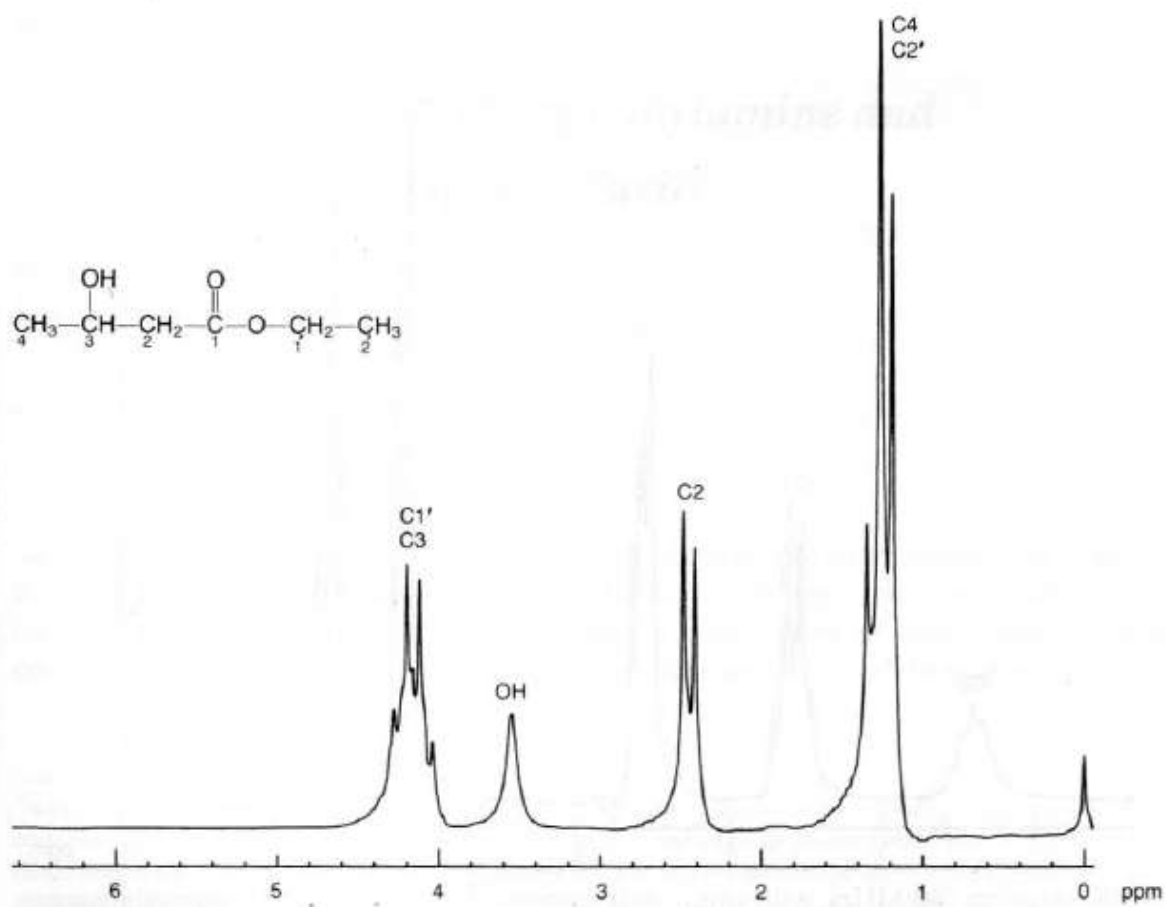
## **WASTE DISPOSAL**

Discard the remaining solution from your NMR tube into the container designated for the disposal of halogenated organic waste.

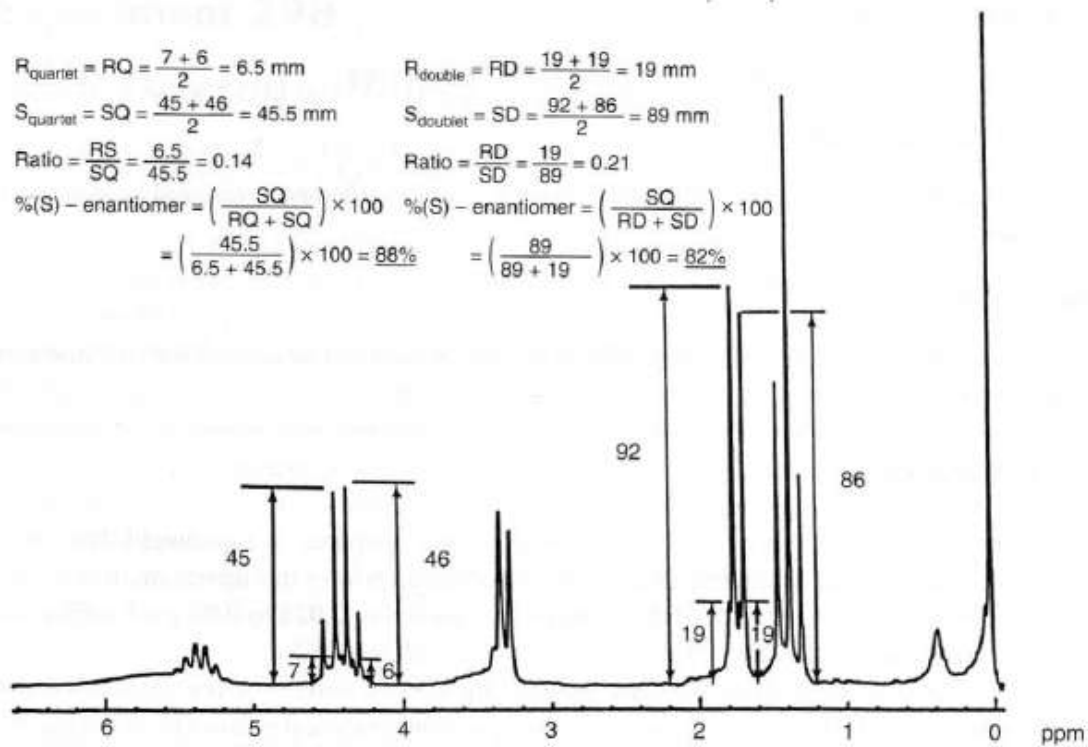
## **PROCEDURE**

Place approximately 0.030 g of ethyl 3-hydroxybutanoate (prepared in Experiment 29A) in an NMR tube. Use a Pasteur pipet and an analytical balance to perform this operation. It is not important to weigh an exact quantity of the ester; any amount from 0.025 to 0.05 g will suffice, but you must know the exact weight.

Divide the quantity of ester that you weighed by 1.35 to determine the amount of shift reagent you will need. Using smooth weighing paper, use the analytical balance to weigh out this quantity of shift reagent. Again, it is not necessary to be perfectly exact, but you must record the amount. Carefully add this shift reagent to the NMR sample. Add a small quantity of  $\text{CDCl}_3$

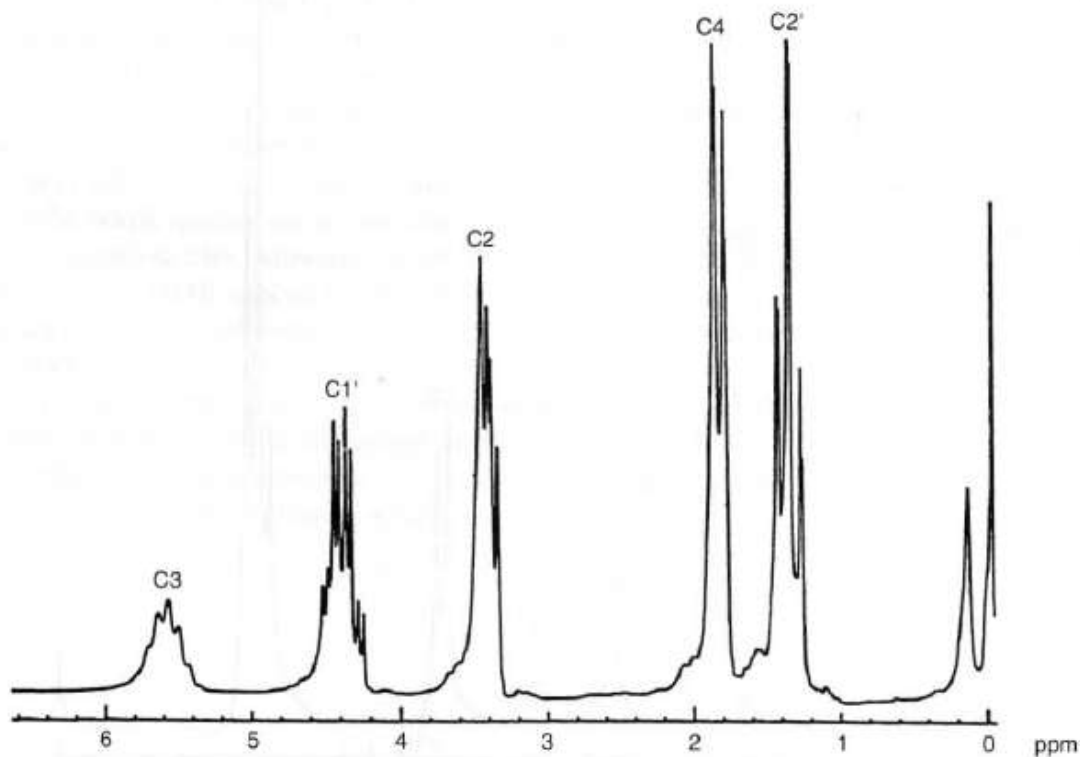


NMR spectrum (90 MHz), of racemic ethyl 3-hydroxybutanoate.



NMR spectrum (90 MHz), with chiral shift reagent, of chiral reduction product of ethyl acetoacetate.





NMR spectrum (90 MHz), with chiral shift reagent, of racemic ethyl 3-hydroxybutanoate. (Note: The OH resonance is off scale.)

solvent, which contains tetramethylsilane (TMS), but do not add more than double the initial volume of the sample of the ester. Allow the sample to stand for 20 minutes. Determine the NMR spectrum of the sample.<sup>1</sup> The peaks of interest are the methyl hydrogens on carbon 4 and the methylene hydrogens on carbon 1'. You should be able to see two sets of overlapping multiplets. If you do not see this pattern, you may not have added enough shift reagent, the amount of one of the enantiomers may be too small, or it may just appear as shoulders on the base of the peaks from the larger multiplet. If you wish, add a second portion of shift reagent, similar to the portion added originally.

Determine the percentage of each isomer in both of your samples in the following manner. Compare the heights of the two inner peaks of the quartets that correspond to the methylene hydrogens on carbon 1'. Determine the ratios of the heights of these peaks. Repeat the comparison, using the two peaks of the doublets that correspond to the methyl hydrogens on carbon 4. Refer to the sample determination illustrated on the NMR spectrum. Average all the

<sup>1</sup> Note to the Instructor: It is a good idea to test the ability of your instrumentation by preparing a reference sample containing equal quantities of racemic ethyl 3-hydroxybutanoate and a sample obtained from Experiment 29A. This mixed sample should contain about 75% (S)-(+)-isomer and 25% (R)-(-)-isomer. Use about 0.030 g of this mixed sample. The results from this mixed sample can be used to tell how well the method is working and also to help students assign the upfield and downfield peaks to the correct enantiomers.

ratios that you have determined and calculate the percentages of each enantiomer from this average ratio. On a typical NMR spectrometer, this comparison-of-peak-heights method gives results that are accurate to within 2-3 percentage points when measuring an accurately prepared reference sample.

## REFERENCES

Seebach, D., Sutter, M. A., Weber, R. H., and Zuger, M. F. "Yeast Reduction of Ethyl Acetoacetate: (S)-(+)-Ethyl 3-Hydroxybutanoate." *Organic Syntheses*, 63 (1984): 1.

## Experiment 30

### *Resolution of (±)-α-Phenylethylamine and Determination of Optical Purity*

Resolution of enantiomers

Use of a separatory funnel

NMR spectroscopy

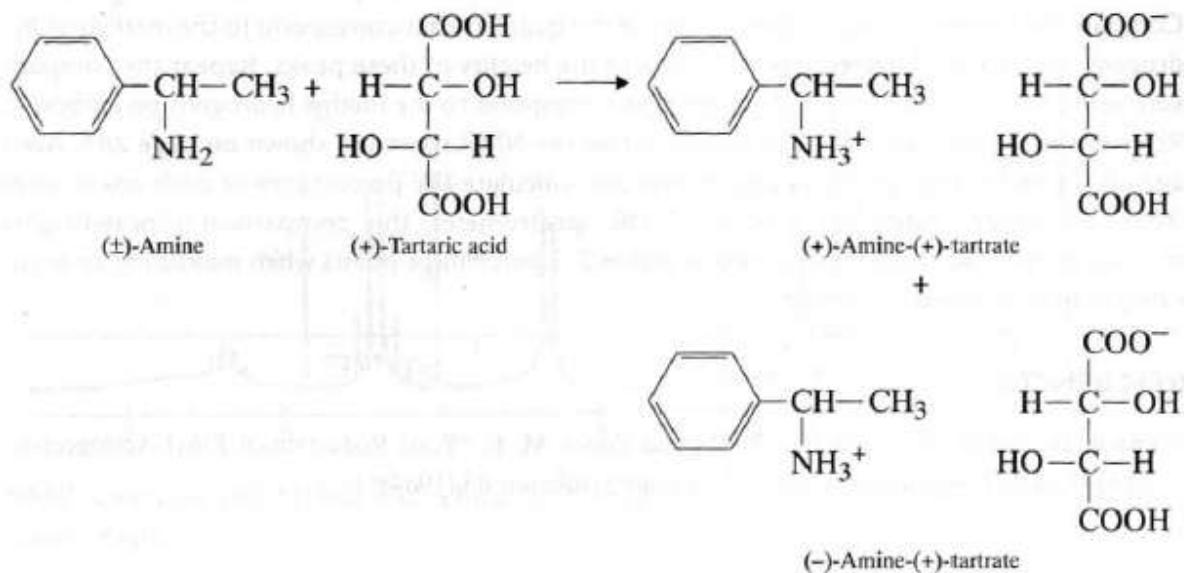
Chiral resolving agent

Diastereomeric methyl groups

Although racemic (+)-α-phenylethylamine is readily available from commercial sources, the pure enantiomers are more difficult to obtain. In this experiment, you will isolate one of the enantiomers, the levorotatory one, in a high state of optical purity (large enantiomeric excess). A resolution, or separation, of enantiomers will be performed, using (+)-tartaric acid as the resolving agent. After obtaining the product, you will test its purity using NMR spectroscopy and a chiral resolving agent.

## RESOLUTION OF ENANTIOMERS

The resolving agent to be used is (+)-tartaric acid, which forms diastereomeric salts with racemic α-phenylethylamine. The important reactions for this experiment follow.

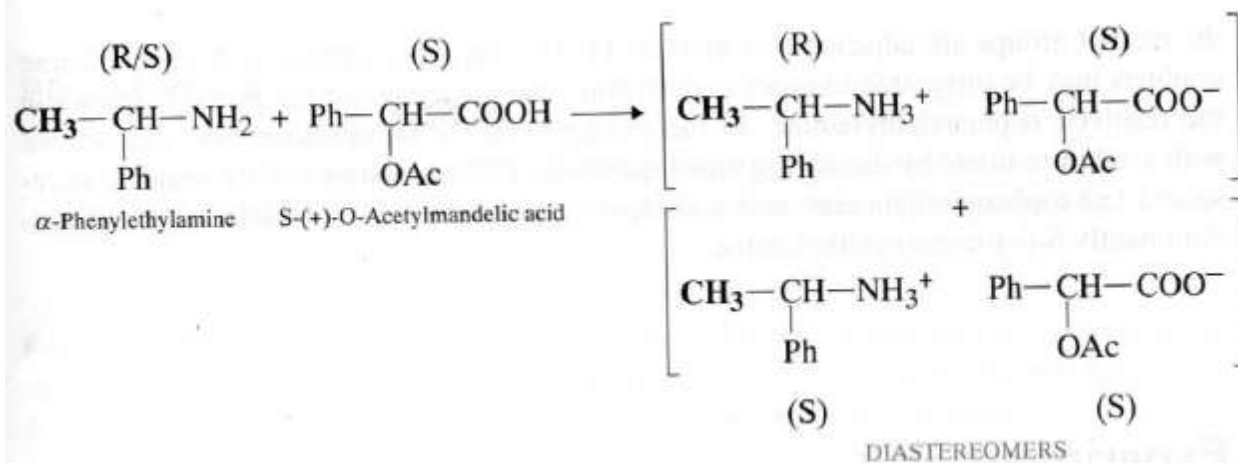


Optically pure (+)-tartaric acid is quite abundant in nature. It is frequently obtained as a by-product of winemaking. The separation depends on the fact that diastereomers usually have different physical and chemical properties. The (-)-amine-(+)-tartrate salt has a lower solubility than its diastereomeric counterpart, the (+)-amine-(+)-tartrate salt. With some care, the (-)-amine-(+)-tartrate salt can be induced to crystallize, leaving (+)-amine-(+)-tartrate in solution. The crystals are removed by filtration and purified. The (-)-amine can be obtained from the crystals by treating them with base. This breaks apart the salt by removing the proton, and it regenerates the free, unprotonated (-)-amine.

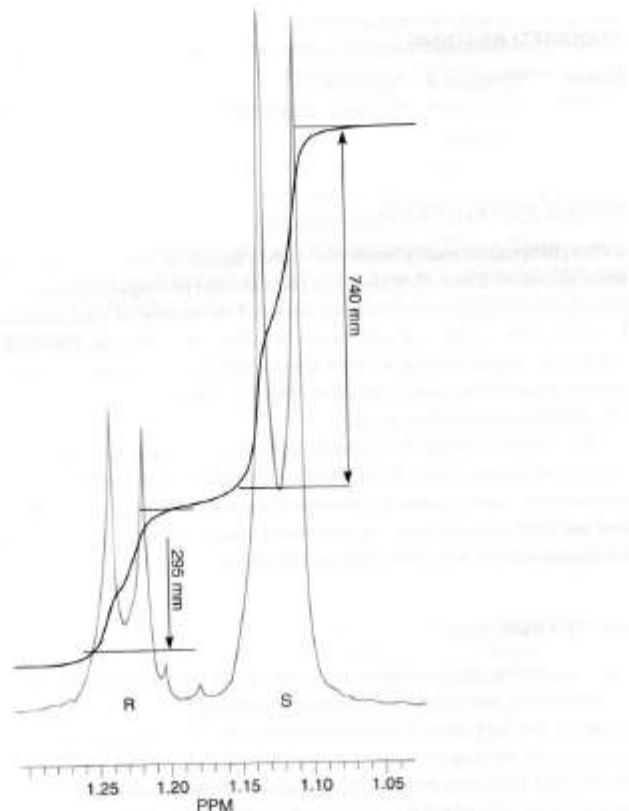
## NMR DETERMINATION OF OPTICAL PURITY

A polarimeter can be used to measure the observed rotation  $\alpha$  of the resolved amine sample. From this value, you can calculate the specific rotation  $[\alpha]_D$  and the optical purity of the amine. An alternate, and perhaps more accurate, means of determining the optical purity of the sample makes use of NMR spectroscopy. A group attached to a stereogenic (chiral) carbon normally has the same chemical shift whether that carbon has either R or S configuration. However, that group can be made diastereomeric in the NMR spectrum (have different chemical shifts) when the racemic parent compound is treated with an optically pure chiral resolving agent to produce diastereomers. In this case, the group is no longer found in two enantiomers, but rather in two different diastereomers, and its chemical shift will be different in each environment.

In this experiment, the partly resolved amine (containing both R and S enantiomers) is mixed with optically pure S-(+)-O-acetylmandelic acid in an NMR tube containing  $\text{CDCl}_3$ . Two diastereomers are formed:



The methyl groups in the amine portions of the two diastereomeric salts are attached to a chiral center, S in one case and R in the other. As a result, the methyl groups themselves become diastereomeric, and they have different chemical shifts. In this case, the R isomer is down field, and the S isomer is upfield. These methyl groups appear at approximately (varies) 1.1 and 1.2 ppm, respectively, in the proton NMR spectrum of the mixture. Since the methyl groups are adjacent to a methine (CH) group, they appear as doublets. These doublets may be integrated in order to determine the percentage of the R and S amines in the resolved  $\alpha$ -phenylethylamine. In the example, the NMR spectrum was determined with a mixture made by dissolving equal quantities (50/50 mixture) of the original unresolved ( $\pm$ )- $\alpha$ -phenylethylamine and a student's resolved product, which contained predominantly S(-)- $\alpha$ -phenylethylamine.



300-MHz spectrum of a 50-50 mixture of resolved and unresolved  $\alpha$ -phenylethylamine in  $\text{CDCl}_3$ . The chiral resolving agent *S*-(+)-*O*-acetylmandelic acid was added.

## Experiment 30A

### *Resolution of ( $\pm$ )- $\alpha$ -Phenylethylamine*

In this procedure, you will resolve racemic ( $\pm$ )- $\alpha$ -phenylethylamine, using (+)-tartaric acid as the resolving agent.

### SPECIAL INSTRUCTIONS

$\alpha$ -Phenylethylamine readily reacts with carbon dioxide in the air to form a white solid, the *N*-carboxyl amine derivative. Every effort should be taken to avoid prolonged exposure of the amine to air. Be sure to close the bottle tightly after you have measured the rotation of your amine, and be sure to place your sample quickly into the flask where you will perform the resolution. This flask should also be stoppered. Use a cork stopper, because a rubber stopper will dissolve somewhat and discolor your solution. The crystalline salt will not react with carbon dioxide until you decompose it to recover the resolved amine. Then you must be careful once again.

## WASTE DISPOSAL

Place the mother liquor solution from the crystallization, which contains (+)- $\alpha$ -phenylethylamine, (+)-tartaric acid, and methanol, in the special container provided for this purpose. Aqueous extracts will contain tartaric acid, dilute base, and water, they should be placed in the container designated for aqueous wastes.

## PROCEDURE

**Preparations.** 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  $\alpha$ -phenylethylamine ( $\alpha$ -methylbenzylamine) to this hot solution .

**CAUTION:** At this seep. the mixture is very likely to froth and boil over.

**Crystallization.** Stopper the flask, and let it stand overnight. The crystals that form should be prismatic. If needles form, they are not optically pure enough to give a complete resolution of the enantiomers-prisms *must form*. Needles should be dissolved (by careful heating) and cooled slowly to crystallize once again. When you recrystallize, you can "seed" the mixture with a prismatic crystal, if one is available. If it appears that you have prisms, but that they are overgrown (covered ) with needles, the mixture may be heated until *most* of the solid has dissolved. The needle crystals dissolve easily, and usually a small amount of the prismatic crystals remains to seed the solution. After dissolving the needles, allow the solution to cool slowly and form prismatic crystals from the seeds.

**Workup.** Filter the crystals, using a Buchner funnel, and rinse them with a few portions of cold methanol. Partially dissolve the crystal line amine-tartrate 'salt in 25 mL of water, add 4 mL of 50% sodium hydroxide, and extract this mixture with three 10-mL portions of methylene chloride using a separatory funnel. Combine the organic layers from each extraction in a stoppered flask and dry them over about 1 gram of anhydrous sodium sulfate for about 10 minutes. During this waiting period , preweigh a clean, dry 25-mL Erlenmeyer flask, stoppered with a cork. You will need this preweighed , stoppered flask in the next section. Decant the dried solution into a 50-mL beaker, and evaporate the methylene chloride on a hot plate (about 60°C) in the hood . A stream of nitrogen or air can be directed into a beaker to increase the rate of evaporation. The product is a liquid. Some solid amine carbonate will form on the sides of the beaker during this operation. If you prolong this step, a large amount of the white solid will form.

**Yield Calculation and Storage.** Carefully transfer the liquid amine to the preweighed 25-mL Erlenmeyer flask. If possible, avoid transferring any of the white solid . Stopper the flask and

weigh it to determine the yield . Also calculate the percentage yield of the S-(-)-amine based on the amount you started with.

## Experiment 30B

### *Determination of Optical Purity Using NMR and a Chiral Resolving Agent*

In this procedure, you will use NMR spectroscopy with the chiral resolving agent S-(+)-O-acetylmandelic acid to determine the optical purity of the S-(-)- $\alpha$ -phenylethylamine you isolated in Experiment 30A.

## SPECIAL INSTRUCTIONS

Be sure to use a clean Pasteur pipet or syringe whenever you remove  $\text{CDCl}_3$  from its supply bottle. Avoid contaminating the stock of NMR solvent! Also be sure to fill and empty the pipet or syringe several times before attempting to remove the solvent from the bottle, If you bypass this equilibration technique, the volatile solvent may squirt out of the tube before you can transfer it successfully to another container.

## WASTE DISPOSAL

When you dispose of your NMR sample, which contains  $\text{CDCl}_3$ , place it in the container designated for halogenated wastes.

## PROCEDURE

Using a small test tube , weigh approximately 0.05 mmole (0.006 g, *MW*; 121 ) of your resolved amine by adding it drop by drop from a Pasteur pipet. Cork the test tube to protect it from atmospheric carbon dioxide. Carbon dioxide reacts with the amine to form an amine carbonate (white solid ). Using a weighing paper, weigh approximately 0 .06 mmole (0.012 g, *MW*; 194) of S-( + )-O-acetylmandelic acid and add it to the amine in the test tube. Using a clean Pasteur pipet, add about 0.25 mL of  $\text{CDCl}_3$  to dissolve everything. If the solid does not completely dissolve, you can mix the solution by drawing it several times into your Pasteur pipet and redelivering it back into the test tube. When everything is dissolved , transfer the mixture to an NMR tube using a Pasteur pipet. Using a clean Pasteur pipet, add enough  $\text{CDCl}_3$  to bring the total height of the solut ion in the NMR cube to 35 mm.

Determine the proton NMR spectrum, preferably at 300 MHz, using a method that expands and integrates the peaks of interest. Using the integrals, calculate the percentages of the R and S

isomers in the sample, and its optical purity.<sup>2</sup> Compare your results from this NMR determination to those you obtained by polarimetry.

## REFERENCES

Ault, A. "Resolution of D,L- $\alpha$ -Phenylethylamine." *Journal of Chemical Education*, 42 (1965): 269.

Jacobus, J., and Raban, M. "An NMR Determination of Optical Purity," *Journal of Chemical Education*, 46 (1969) 351.

Parker, D., and Taylor, R.J. "Direct <sup>1</sup>H NMR Assay of the Enantiomeric Composition of Amines and p-Amino Alcohols Using O-Acetyl Mandelic Acid as a Chiral Solvating Agent." *Tetrahedron*, 43, No. 22 (1987) 5451.

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<sup>2</sup> Note to the Instructor: In some cases, the resolution is so successful that it is very difficult to detect the doublet arising from the R-(+) $\alpha$ -phenylethylamine + S-(+) O-acetylmandelic acid diastereomer. If this occurs, it is useful to have the students add a single drop of racemic  $\alpha$ -phenylethylamine to the NMR tube and redetermine the spectrum. In this way, both diastereomers can be clearly seen.