Use of Enzymes in Organic Synthesis: Reduction of Ketones by Baker’s Yeast Revisited

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Important and interesting additions to the undergraduate organic laboratory are biological reaction systems that can enhance stereochemical outcomes of common organic reactions. Stereochemistry is a vital part of the organic chemistry curriculum and is often one of the most challenging for the organic student. Current organic chemistry laboratory textbooks touch lightly on this subject, usually having one or two experiments pertaining to stereochemical control and analysis. Yeast reduction of ethyl acetooacetate and resolution of amines with chiral resolving agents are such examples, found in today’s laboratory texts (1).

Another important topic for the organic undergraduate is 1H NMR spectroscopy. Most texts cover the straightforward and fundamental principles but there is little attention given to the more advanced problems pertaining to enantiotopic and diastereotopic protons. The undergraduate experiment discussed in this article entails both the elements of stereochemistry and in-depth 1H NMR analysis of diastereotopic proton systems.

The reduction of ethyl acetooacetate using common baker’s yeast is a traditional experiment that shows the stereoselective power of biochemical systems (2). Two primary problems have kept this reaction from being a mainstay in our undergraduate organic laboratory curriculum. First, students have routinely obtained low yields of the alcohol product and in many cases, often up to 50% of the students could not isolate any alcohol. Second, difficulties were frequently encountered in the analysis of the product mixture when determining the stereoselectivity of the reduction reaction. We found that the results using chiral shift reagents in conjunction with 1H NMR analysis were unreliable.

Two other articles in this Journal have addressed separately some of these problems. North (3) presented a laboratory experiment where organic solvents were used for a non-fermenting system of yeast reduction. The use of these solvents increased the yield of the alcohol. A major drawback to this protocol is the large volume of organic solvent used. At the University of Washington our classes range between 60 and 400 students and volume of solvent use is always a concern. In another article by Lee and Huntington (4), yeast was used for the reduction of acetophenone and the enantiomeric excess was successfully ascertained by formation and 1H NMR analysis of Mosher’s esters. However, this protocol required chromatographic purification of the alcohol, giving low yields, and the preparation of the esters required a 12-h reaction time.

Our Experiment

In our method of reducing ethyl acetooacetate we used an aqueous fermenting yeast system with a small quantity of hexane added (Scheme I). Another improvement in the experimental protocol was the use of micropore filtration before extraction of the yeast mixture, which reduced the emulsions that have been problematic during isolation of the alcohol in the past. The alcohol was recovered in good yield, usually in the 30–70% range. The alcohol product was analyzed using GC–MS to determine the extent of ketone reduction, which was consistently above 90%. The alcohol product was converted with (+)-α-methoxyphenylacetic acid using dicyclohexylcarbodiimide (DCC) to form a diastereomeric ester pair (Scheme II) that was analyzed by 1H NMR to determine relative quantities of SS and SR diastereomers.

The chiral reduction developed for our undergraduate laboratories eliminates the drawbacks to the aforementioned methods. Addition of a small quantity of organic solvent to the aqueous reaction system dramatically increased the yields and the reproducibility of the experiment. Furthermore, preparation of the Mosher’s esters for analysis of the stereoselectivity of the reduction was accomplished in a short time period and did not require a complex chromatographic purification of the products. (Purification was accomplished by a simple filtration through a bed of silica gel and sodium sulfate). The outcome of this new lab was moderate yields of the final products, minimal quantity of organic solvent used, ease of workup, and an interesting and easy ascertainment of the enantiomeric excess of the reduction product. In addition, the 1H NMR spectra of the esters revealed very interesting AMX coupling patterns that could be used for 1H NMR spectra simulation.

The 1H NMR spectra for the individual isomers are shown in Figures 1 and 2. The critical region for the 1H NMR analysis is between 1.1 and 1.3 ppm (see insets in Figures 1 and 2). In this region the methyl group protons labeled A and B on structures 3 and 4, respectively, are clearly resolved when comparing the spectra of the pure (S,S) and (S,R) isomers. By integration of the student’s ester product mixture we can ascertain the relative quantities of (S,S) and (S,R)
diastereomers. Of additional interest to advanced students of $^1$H NMR spectroscopy is the AMX splitting pattern at 2.6 ppm of the CH$_2$ group (labeled C) and the ABX pattern for the CH$_2$ group at 3.8 ppm (labeled D) in compound 3 in Figure 1.

This lab is now routinely carried out by sophomore level students at the University of Washington and is completed in roughly two, three-hour lab periods. This yeast reduction will produce a 95% enantiomeric excess of the (S) isomer. However, students are not told which stereoisomer will be produced by the yeast but have to come to their own conclusions using their $^1$H NMR data.

Hazards

Dicyclohexylcarbodiimide (DCC) used in the esterification step is a contact allergen and is considered highly toxic. It is advised that the instructor preweigh the sample for each student to minimize contact. 4-Dimethylaminopyridine is used in a small catalytic quantity but is highly toxic and is readily absorbed through the skin.

Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of JCE Online.

Literature Cited

Lab Documentation

This lab is completed over 3 separate sessions. On day 1, only 30 minutes of lab time is required and this part is run concurrently with another experiment. Days 2 and 3 require 2-3 hours each to complete.

Procedure for day 1-Yeast reduction of a Ketone: To a 500 mL Erlenmeyer flask, add 150 mL water and warm to 35 °C using a hot plate set on low. Once the temperature is stabilized at 35 °C, add 7 g of sucrose and 7 g Baker’s yeast. Let this solution sit for 15 minutes at 35 °C. Dissolve 3 g of ethyl acetoacetate in 8 mL of hexane. Add this solution to your yeast mixture along with a stirbar and begin stirring for 3 hours while maintaining the temperature at 35 °C. Before the end of lab, take an IR spectrum of ethyl acetoacetate. At the end of the period you will store your reaction mixture at room temperature until the next lab meeting.

Procedure for day 2-Isolation of the alcohol product: To the yeast solution add 5 g of celite and stir for 1 min. Let the solid settle as much as possible (wait about 5 minutes). While the solution is settling, set up a vacuum filtration apparatus with a trap using the large Buchner funnel. Add one sheet of filter paper and wet. Obtain a square of cheese cloth and fold it 2 times to make a 3x3 square. Wet it and place it on top of the filter paper. You are now ready to filter your solution. First, decant and filter as much of the clear supernatant liquid as possible before adding the celite slurry. Wash the celite residue with 20 mL of water. Finally, filter the solution one more time using the plastic steri-cup filtration apparatus.

To the filtered solution add 20 g of sodium chloride and swirl the solution until it dissolves. Extract the aqueous solution 2 times with 30 mL of diethyl ether using a 250 mL separatory funnel. Occasionally, an emulsion forms. If that is the case, drain off the lower aqueous layer up to the emulsion. By gently stirring the emulsion with a stirring rod you may help break it up. If necessary, you may also transfer the emulsified portions to your glass centrifuge tubes and centrifuge the mixture in order to separate it.

Dry the combined ether extracts in an Erlenmeyer flask over 1 g of magnesium sulfate for 5 min. Obtain 1 mL of this solution for analysis by gas chromatography. Decant the liquid to a tared beaker and
evaporate using an air stream until the volume of liquid remains constant (approx. 1-2 mL). This is your final product, ethyl 3-hydroxybutanoate (an alcohol). Determine and record the weight of the product.

**IR spectroscopy**: Obtain an IR spectrum of your isolated product. Look for presence of an alcohol group (~3300 cm⁻¹) and compare with the spectrum of the starting ketone.

**Procedure for day 3-Esterification of the alcohol products with a chiral acid**: In a 5 mL conical vial, equipped with an air condenser and drying tube packed with drierite, prepare a solution containing 2 mL of methylene chloride, 50 mg of (S)-(+)α-methoxyphenylacetic acid (0.3 mmol), 3 drops of your alcohol (50 mg, 0.4 mmol, d 1.017 g/mL). Cool this solution to 0 °C and then add 65 mg of 1,3-dicyclohexylcarbodiimide (DCC, MW 206, 0.34 mmol) and ~2 mg 4-dimethylaminopyridine (~0.02 mmol). **Caution**: DCC and dimethylaminopyridine are highly toxic, wear gloves--avoid skin contact! Allow the mixture to stir at 0 °C for 30 min. During this time dicyclohexylurea (DCU) will precipitate. At the end of the reaction time, filter off the DCU precipitate using a Hirsh funnel. Add 2 more mL of methylene chloride to the solution once it has been filtered. Using a centrifuge tube with a cap, extract the methylene chloride solution twice with 2 mL of 5% acetic acid solution and then twice with 2 mL of 5% sodium bicarbonate solution. (Methylene chloride will be the bottom layer in these extractions).

Prepare a microcolumn for filtration by placing a small wad of cotton at the constriction of a pipet and then filling the pipet with 0.5 g of sodium sulfate followed by 0.2 g of silica gel. Clamp the microcolumn upright and carefully add 1 mL of methylene chloride onto the column (do not disturb the surface) and let it drain to where the solvent level just approaches the surface of the silica gel. Just before the solvent reaches the surface of the silica gel add the methylene chloride solution containing your product and collect the eluent in a tared test tube. Once the solution has drained to the top of the silica add another 2 mL of methylene chloride to rinse off any product that remains on the column. Evaporate the methylene chloride solvent with an air stream. Weight the test tube after evaporation and record the weight of the recovered product.

**NMR** Prepare an NMR sample of your product by dissolving all of your product in 0.75 mL of CDCl₃ NMR solvent.
NMR analysis: Since the S,R and S,S isomers have a diasteromeric relationship, their chemical and physical properties, such as NMR spectra, will be different. A difference in the NMR spectra of the SS and SR isomers can be seen by comparing each diastereomer’s two methyl groups (\(-\text{CH}_3\)) NMR signals. The two methyl groups of interest are labeled (A) and (B) in figure 2. The NMR spectra of the pure compounds 3 and 4 are shown in figures 3 and 4 respectively. The methyl groups give rise to a doublet and triplet in the 1.1 to 1.3 ppm region (see figure 5). The (A) and (B) methyl groups of the SS and SR diastereomers have slightly different chemical shifts and can be discerned from each other. Because of these differences we can ultimately, by integration, determine the relative amount of SS and SR in a mixture of the two.

The full interpretation of the SS isomer (figure 3): \(\delta 7.4 \text{ (m, 5H)}, \delta 5.4 \text{ (m, 1H)}, \delta 4.8 \text{ (s, 1H), } \delta 3.4 \text{ (s, 3H)}, \delta 2.5 \text{ (m, 2H), } \delta 1.3 \text{ (d, 3H), } \delta 1.1 \text{ (t, 3H).}

List of substances used:

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Instructors notes

Yeast and sugar were bought from a local grocery store. Red star and Fleishman’s are the two brands of yeast used and we found no preference for either. Sugar is C & H brand. All reagents except for the yeast and sugar were purchased by the Aldrich chemical company (ethyl acetoacetate, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, (S)-(+) - α−methoxyphenylacetic acid). The Steri-cup filtration apparatus was purchased from Nalgene. The product number is MF75 filter unit NNI #166-0020.

The yeast solutions were heated and stirred at ~ 35-40 °C for 2 hours. After this time the solutions were collected and stored at room temperature. It is recommended that for storage past 3 days the solutions should be refrigerated to prevent epimerization of the alcohol product.

The alcohol from the initial reduction was analyzed by GC-MS. The GC-MS system used was an Agilent 6890. Column was purchased from Agilent; catalogue number 19091S-433 HP 685. The GC temperature ramp: 50 °C for 1 minutes; 15 °C ramp until 250 °C. The retention time for the alcohol product was 4.5 min and the starting ketone 4.6 min.

We also attempted the yeast reduction of acetophenone but with no success. Students only isolated starting material after following the standard procedure outlined above.