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

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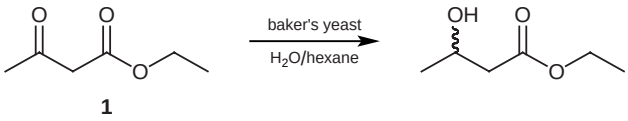
Important and interesting additions to the undergraduate organic laboratory are biological reaction systems that can enhance stereochemical outcomes of common organic reactions. Stereochemistry is a vital part of the organic chemistry curriculum and is often one of the most challenging for the organic 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 acetoacetate 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 ^{1}H 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 ^{1}H NMR analysis of diastereotopic proton systems.

The reduction of ethyl acetoacetate 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 ^{1}H 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 ^{1}H 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.



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Scheme I. The reduction of ethyl acetoacetate using baker's yeast.

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 ^{1}H NMR spectra of the esters revealed very interesting AMX coupling patterns that could be used for ^{1}H NMR spectra simulation.

Our Experiment

In our method of reducing ethyl acetoacetate 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 mixture was coupled with (*S*)-(+)-α-methoxyphenylacetic acid using dicyclohexylcarbodiimide (DCC) to form a diastereomeric ester pair (Scheme II) that was analyzed by ^{1}H NMR to determine relative quantities of SS and SR diastereomers.

The ^{1}H NMR spectra for the individual isomers are shown in Figures 1 and 2. The critical region for the ^{1}H 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*)

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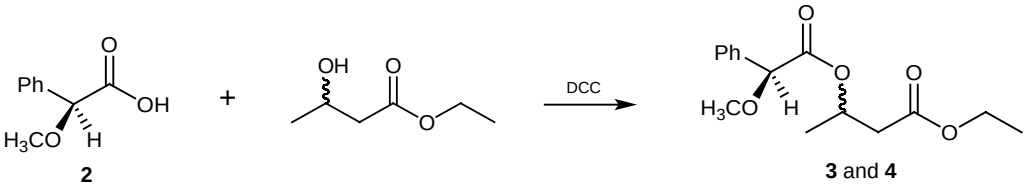
Scheme II. Esterification of alcohol product mixture with chiral acid.



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

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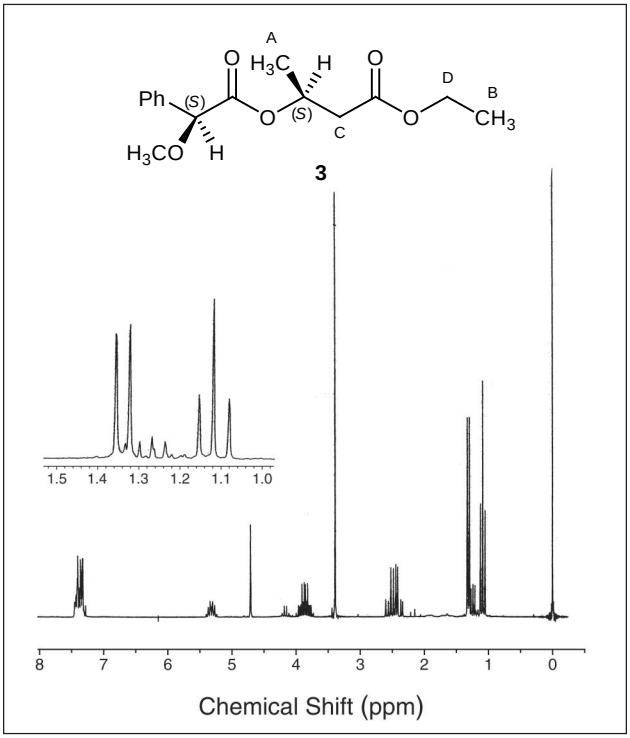

Figure 1. ^{1}H NMR spectrum (200 MHz) of compound **3**, (*S,S*) isomer. Inset shows the peaks from the methyl groups labeled A and B.

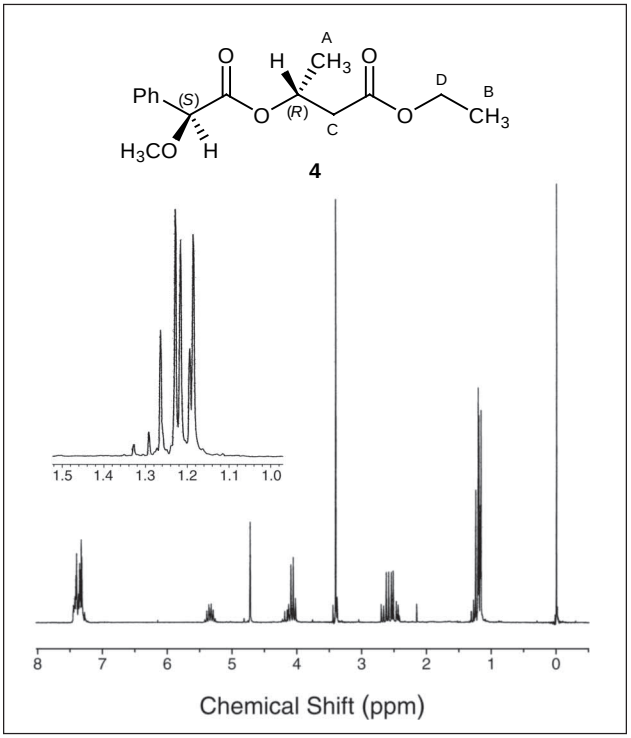

Figure 2. ^{1}H NMR spectrum (200 MHz) of compound **4**, (*S,R*) isomer. Inset shows the peaks from the methyl groups labeled A and B.