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Enzymatic Resolution of 1-Phenylethanol and Formation of a Diastereomer: An Undergraduate ¹H NMR Experiment To Introduce Chiral Chemistry

David H. Smith[†], Mark Wilson, Kyla Ronhovde, Erin Wilson, David Clevette, Kerry Lucas^{††,*}, and Andrea Holmes^{**}

Department of Chemistry, Doane College, Crete, Nebraska 68333

Abstract

This organic laboratory experiment introduces students to stereoselective enzyme reactions, resolution of enantiomers, and NMR analysis of diastereomers. The reaction between racemic 1-phenylethanol and vinyl acetate in hexane to form an ester is catalyzed by acylase I. The unreacted alcohol is then treated with a chiral acid and the resulting ester diastereomer is analyzed by NMR. This experiment is suitable for group work in the laboratory as several diastereomers are synthesized and compared to determine which enantiomer of 1-phenylethanol reacts with the enzyme.

Keywords

Laboratory instruction; second-year undergraduates; organic chemistry; collaborative learning; chirality/optical activity; diastereomers; stereochemistry; enzymes; NMR spectroscopy; hands-on learning/manipulatives

Stereochemistry is usually introduced in the first semester of undergraduate organic chemistry and is a crucial concept for organic chemistry (racemization, S_N2 reactions), biochemistry (peptides, proteins, helicity of B- and Z-DNA), and inorganic chemistry (chiral catalysis). Unfortunately, students often struggle with the abstract concepts of stereochemistry. There are many experiments described in this *Journal* and others that attempt to enhance student understanding of stereochemistry (1–22). An organic laboratory experiment is presented that focuses on stereochemistry by introducing students to stereoselective enzyme reactions, resolution of enantiomers, and NMR analysis of diastereomers.

This experiment uses an enzyme for the preparation of an enantiopure product from a racemic precursor. Acylase I from *Aspergillus melleus* (27), which catalyzes the transformation of a range of aromatic alcohols to esters (24) by transferring an acyl group, is used. A racemic mixture of 1-phenylethanol is reacted with vinyl acetate. The reaction is shown in Scheme 1 and reflects the stereoselectivity of the enzyme. The reaction follows a greener reaction route (23) and is ~80% less expensive than the formation of Mosher

*lucask@kenyon.edu

**andrea.holmes@doane.edu

[†]Dr. David Smith (1942–2008), Professor Emeritus of Chemistry, Doane College, Crete, Nebraska.

^{††}Current address: Department of Chemistry, Kenyon College, Gambier, Ohio 43022.

diastereomers (5). It is also carried out in hexane, which is more suitable for many organic molecules than the aqueous environment most enzymes require (24). Previously described enzymatic methods include the stereospecific, catalyzed reactions of penicillin acylase derivatives (25) and their use in the chiral resolution of isomers (26). Students benefit from the hands-on experience and observe firsthand how enzymes can resolve racemic compounds. To determine which enantiomer the enzyme preferred, the students derivatize the unconverted alcohol(s) to a set of diastereomeric esters using a chiral carboxylic acid and a coupling reagent.

The experiment is divided into two parts and conducted over three, two-hour laboratory periods one week apart. Part 1 is the stereoselective formation of an ester from a racemic alcohol by acylase I, followed by the separation of the product ester and the unreacted alcohol and part 2 is the formation of a diastereomeric compound from the unreacted alcohol and use of ^1H NMR spectroscopy to determine the chirality of the unreacted alcohol.

Experimental Details

Synthesis

In part 1, a racemic mixture of 1-phenylethanol was reacted with vinyl acetate, catalyzed by acylase I, in hexane for one week at room temperature. No stirring was required and the reaction occurred by simply storing the mixture for one week at the student's lab stations. If the reaction continued longer than seven days, increasing quantities of the (*S*)-enantiomer were consumed, complicating the analysis. After the reaction was complete, the alcohol and ester products were separated by silica gel column chromatography (monitored by TLC).

In part 2, students carried out three reactions to investigate the stereochemistry of the reaction in part 1. The unreacted 1-phenylethanol was derivatized with (*R*)-(-)-acetoxypheylacetic acid ((*R*)-(-)-O-acetoxy-2-phenyl-2-ethanoic acid) in the presence of a coupling reagent, ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and a catalyst, 4-dimethylaminopyridine (4-DMAP), to prepare the ester (Scheme 2). Students then reacted the racemic starting alcohol with (*R*)-(-)-acetoxypheylacetic acid to make the pair of diastereomeric esters. Finally they reacted the enantiopure (*R*)- or (*S*)-alcohol with (*R*)-(-)-acetoxypheylacetic acid to make the diastereomeric ester. Proton NMR analysis was used to assess the stereochemistry of the products.

The details of the experimental setup are provided in the supporting information.

NMR Analysis

The ^1H NMR (300 MHz) spectrum from the reaction of the racemic starting alcohol with (*R*)-(-)-acetoxypheylacetic acid is shown in Figure 1. The two diastereomers can be distinguished by NMR as the methyl protons in the 1-phenylethyl group of the diastereomers appear as two doublets centered at 1.5 ppm. An expansion of the 1–3 ppm region of the ^1H NMR spectrum of a 50:50 mixture of diastereomers (Figure 2A), shows the benzylic methyl group from the two diastereomers have slightly different chemical shifts. The same region from a spectrum of the diastereomer derived from commercially available (*R*)-phenylethanol (Figure 2B) shows the doublet at ~1.5–1.6 ppm is attributable to the (*R,R*) diastereomer whereas the doublet at ~1.4–1.5 ppm comes from the (*S,R*) diastereomer.

The ^1H NMR (90 MHz) spectrum of the ester (Figure 3) from part 1 (Scheme 1) indicates that the majority (approximately 3:1) of the unreacted alcohol was the (*S*)-enantiomer leading to the (*S,R*)-diastereomer, indicating the enzyme preferred (*R*)-(+)-1-phenylethanol. The student data showed 76% (*S,R*) isomer and 24% (*R,R*) isomer leading to an enantiomeric excess of 52% by ^1H NMR.

Optical Purity

Students determined the optical purity of their unreacted alcohol by measuring its specific rotation and comparing to a purchased standard, (*R*)-1-phenylethanol (99.9% from Aldrich). The specific rotation of the standard was +43.03 and the resolved alcohol -25.02 (both in methanol), giving an optical purity of 58% (*S*)-enantiomer by polarimetry.

Hazards

1-Phenylethanol, vinyl acetate, dichloromethane, and deuteriochloroform are cancer suspect agents. 4-Dimethylaminopyridine (DMAP) is highly toxic. EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride is an irritant. These materials must be used in small quantities in fume hoods. Student should use chemically resistant gloves and protective eye wear.

Organization of the Group Work

The students each performs the enzyme-catalyzed transesterification with the racemic alcohol (part 1). Each student in a group of 3–4 then either reacts the (*R*)-enantiomer of the alcohol, the experimentally unreacted (*S*)-enantiomer (collected from all group members), or the racemic mixture with the acetoxypheylacetic acid so that each group had a complete set of complexes to analyze (part 2). This allows students to gain enough information to determine which enantiomer reacted preferentially with the enzyme in part 1. The weekly experimental schedule for the laboratory exercise is

Week 1: Each student reacts racemic 1-phenylethanol with vinyl acetate using acylase I from *A. melleus* as a catalyst. This reaction takes 10–15 min to prepare and can be performed at the end of another lab experiment session. If possible, students should come in during the course of the week to test the reaction mixture via TLC and observe the disappearance of the starting alcohol peak and the increase in the product ester peak.

Week 2: Each student performs TLC analysis of their reaction mixture and then separates the alcohol and ester by microscale column chromatography. Each fraction is analyzed by TLC, and pure fractions are combined. The students in each group are then assigned an alcohol:

Student 1: Reacts the racemic alcohol with (*R*)-(-)-acetoxypheylacetic acid to make the pair of diastereomeric esters.

Student 2: Reacts the unreacted alcohol with (*R*)-(-)-acetoxypheylacetic acid to make the diastereomeric ester.

Student 3: Reacts the enantiopure (*R*)- or (*S*)-alcohol with (*R*)-(-)-acetoxypheylacetic acid to make the diastereomeric ester.

For larger groups: Additional students can replicate another student.

Week 3: Students collect and share ^1H NMR spectra and analyze their results to determine which enantiomer of 1-phenylethanol was the preferred substrate in the enzyme reaction. Student 1 obtains a ^1H NMR spectrum showing the doublets from both diastereomeric esters (Figure 1). The ^1H NMR spectrum from student 2 shows the doublet from the ester of the unreacted alcohol (Figure 3). Student 3 has an ^1H NMR of the ester from the pure, known (*R*)- or (*S*)-alcohol (Figure 2B). From this data, the group can deduce which enantiomer of the starting alcohol was consumed by the enzyme and calculate by how much it was preferred.

Costs

An attractive aspect of this enzyme-facilitated enantioresolution is its low cost. Based on catalog prices from commercial suppliers, the average cost per student is about \$3.50, exclusive of solvents, TLC plates, and other common reagents. The majority of the cost is (*R*)-(-)-acetoxypheylacetic acid, EDC, and DMAP at about \$2.75 per student. In comparison, on a similar scale using an acid chloride, the experiment costs approximately \$43 per student (Sigma-Aldrich Handbook of Fine Chemicals 2007–2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. ¹H NMR (300 MHz) spectrum of (*S*)-1-phenylethyl (*R*)-acetoxypheylacetate and (*R*)-1-phenylethyl (*R*)-acetoxypheylacetate.

Figure 2.

Expanded ^1H NMR spectra of Figure 1: (A) 50:50 mixture of the two diastereomers (*S*)- and (*R*)-1-phenylethyl (*R*)-acetoxyphenylacetate and (B) the (*R,R*)-diastereomer derived from commercially available (*R*)-phenylethanol. The trace amount of the (*S,R*)-diastereomer indicates that the commercial alcohol was not 100% pure.

Figure 3.

¹H NMR (90 MHz) of 1-phenylethyl acetoxyphenylacetate, after the unreacted 1-phenylethanol was reacted with (*R*)-(-)-acetoxyphenylacetic acid leading to the diastereomers (*R,R*) and (*S,R*). The 3:1 ratio of (*S,R*) to (*R,R*) indicates a preference of acylase I for the (*R*) enantiomer of 1-phenylethanol.

Scheme 1.

The Enzymatic Acylation of (R,S)-(+)-1-Phenylethanol

Scheme 2.

The Formation of (*S*)-1-Phenylethyl (*R*)-acetoxypheylacetate from the Unreacted Starting Alcohol