NMR Verification of Diastereoselective Reduction of Substituted Cyclohexanones

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Abstract: This experiment provides an ideal opportunity to integrate NMR spectroscopy, stereochemical principles, and the use of enzymes in the sophomore-level organic chemistry laboratory. Students work in teams and reduce either 2-methylcyclohexanone or 4-*tert*-butylcyclohexanone using common baker's yeast (*Saccharomyces Cerevisiae*) and sodium borohydride. The stereochemistry and diastereomeric ratios of alcohols produced are determined using proton NMR. The students are asked to rationalize the contrast in stereoselectivity observed with the different reducing agents. This experiment has an advantage over other baker's yeast reduction experiments [1–4] in that the diastereoselectivity can be directly determined by NMR.

Introduction

During the last decade, enzymes and microorganisms have become widely used for stereoselective synthesis [5–8]. These biocatalytic systems present some advantages because they can produce reactions under mild conditions with high enantio- or diastereoselectivity. Obtaining purified enzymes is costly; however, microorganisms can be easily obtained and exploited to carry out enzymatic reactions. A whole-cell system has the advantage that it is inexpensive and contains all the entities necessary for the transformation (enzyme(s), cofactor(s), metal ion(s), etc.) within the cell. Common baker's yeast (*Saccharomyces cervisiae*) is an easily used, commercially available, inexpensive, and by far the most widely used microorganism for the asymmetric reduction of ketones [9]. A large number of different enzymes are present in yeast. Dehydrogenase enzymes catalyze oxidations and reductions in which nicotinamide cofactors serve as the immediate two-electron oxidants or reductants. Yeast contains multiple dehydrogenases, which are able to accept a wide variety of unnatural substrates.

Students reduce either 2-methyl- or 4-*tert*-butylcyclo-hexanone using common baker's yeast (*Saccharomyces Cerevisiae*) and sodium borohydride. Mixtures of cis- and trans-substituted cyclohexanols result from these reactions and the diastereomeric composition is determined by using ^{1}H NMR.

The Experiment

The experiment takes place over two laboratory periods. In the first period the yeast reductions are set up and the sodium borohydride reactions completed. The yeast reactions are carried out in Erlenmeyer flasks using one package of Fleischmann's dry active baker's yeast and 0.5 grams of substituted cyclohexanone suspended in warm water containing sucrose. The sodium borohydride reductions are carried out on a microscale (0.3 g cyclohexanone) in methanol and after workup are sufficiently pure for direct characterization via ^{1}H NMR. The students with the aid of the instructor obtain a ^{1}H NMR spectrum of the resulting mixture in $CDCl_3$. An expansion of the two low-field sets of peaks (3–4 ppm) is integrated and coupling constants determined. The students use this information to determine the ratio of cis to trans isomers formed. The relative amounts of the products can also be determined by gas chromatography.

During the second laboratory period the yeast reactions are centrifuged and the products isolated by extraction and purified by filtering the mixture through a short pad of silica gel. After removal of the solvent the resulting residue was characterized by ^{1}H NMR as described above.

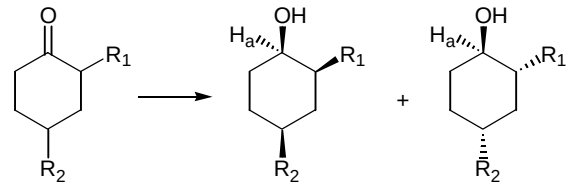
Experimental Method

General. All reagents can be purchased from Aldrich and used without further purification. The yeast, Fleischmann's active dry yeast was purchased from the grocery store. NMR spectra are obtained in $CDCl_3$ at 200 MHz (^{1}H), on a Varian Gemini 2000.

Yeast Reduction. To a 250-mL Erlenmeyer flask warm water (100 mL, 30 °C), sucrose (10 g), and one package of active dry granular baker's yeast (7 g) is added. The resulting suspension is stirred with a stirring bar for 10 min. Then, the 2-methylcyclohexanone (0.5 g) (or 4-tert-butycyclohexanone dissolved in ethanol (0.5 mL)) is added dropwise over a period of five min. The reaction flask is loosely plugged with a piece of cotton, and the mixture is stirred at room temperature for at least 24 h and up to one week. The fermentation broth is centrifuged and the supernatant extracted using methylene chloride (3×50 mL). The combined organic extracts are washed with saturated NaCl solution, dried over magnesium sulfate, filtered, and the solvent removed using a rotary evaporator. Finally, the substituted cyclohexanol is purified by passing the material through a small silica gel column eluted with a hexane/ethyl acetate (80/20) mixture. The yields obtained by students were (5–32%) and the product was characterized by IR and ^{1}H NMR.

Sodium Borohydride Reduction [10]. Dissolve the substituted cyclohexanone (0.3 g) in methanol (1.25 mL) in a small flask and cool the mixture in an ice bath. Then slowly add sodium borohydride (50 mg) to the mixture. After the vigorous reaction, remove the flask from the ice bath and stir the mixture for 15 min at room temperature. The borate ester is decomposed by the addition of sodium hydroxide (3 M, 1.2 mL) solution. The product is extracted with two 5-mL portions of methylene chloride, washed with saturated NaCl solution, and dried over $MgSO_4$. The methylene chloride solution is transferred to a tared flask and the solvent boiled off in the hood to yield a mixture of cis and trans alcohols. The yields obtained by students were (45–75%). The product was sufficiently pure for direct characterization by IR and ^{1}H NMR.

Table 1. Results of Reductions of 2-Methylcyclohexanone and 4-*tert*-Butylcyclohexanone



R_1	R_2	Yeast (cis/trans)[a]	$NaBH_4$ (cis/trans)
H	*t*-Bu	> 99/1	5/95
Me	H	62/39	41/59

[a]Cis/trans ratios were determined using the integrals of the ^{1}H NMR signals of H_a

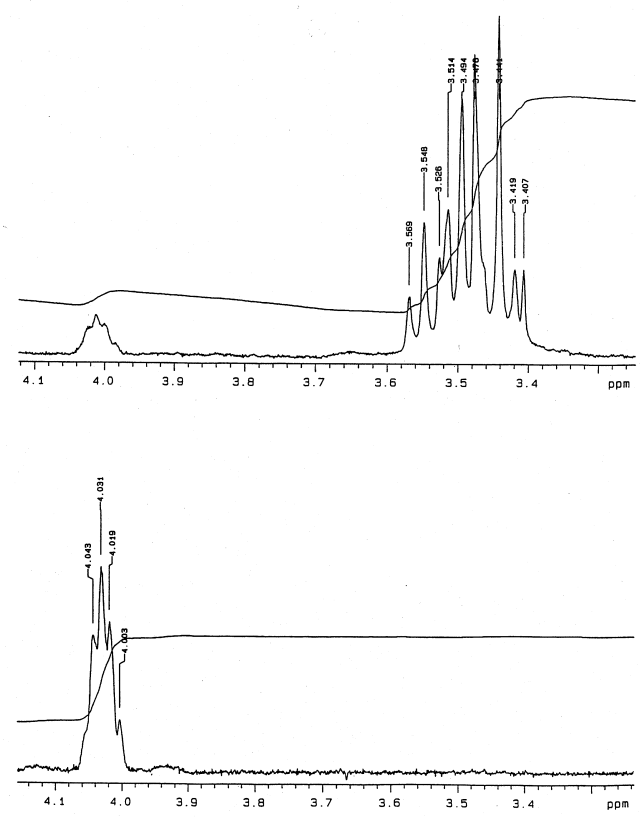


Figure 1. Expanded NMR spectrum from a typical student reduction of *tert*-butylcyclohexanone by sodium borohydride (top) and yeast (bottom).

2-methylcyclohexanol: bp 163–166 °C, IR (neat) $\tilde{\nu}_{max}$ (cm^{-1}): 3632, 2898, ^{1}H NMR ($CDCl_3$, δ): 3.74 (d of d of d, *J* = 2.7, 2.7, and 2.7 Hz, <1H), 3.08 (d of d of d, *J* = 9.8, 9.8, and 4.3 Hz, <1H), 1.2–2 (m, 8H), 0.97 and 0.91 (d, 3H).

4-*tert*-butylcyclohexanol: mp 62–70 °C: IR (KBr) $\tilde{\nu}_{max}$ (cm^{-1}): 3444, 2890: ^{1}H NMR ($CDCl_3$, δ): 4.031 (m, *J* = 2.8 Hz, <1H), 3.49 (t of t, *J* = 12.9 and 3.3 Hz, <1H) 1.2-2.2 (m, 8H), 0.84 and 0.82 (s, 9H).

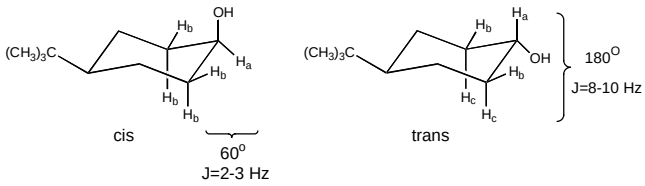
Safety Precautions and Disposal. The cyclohexanones are flammable liquids. Methylene chloride solutions need to be disposed of as halogenated waste and hexane and ethylacetate as nonhalogenated organic waste. The aqueous layer from the $NaBH_4$ reaction should be neutralized with acetic acid and flushed down the drain. The yeast and used silica gel can be placed in the nonhazardous solid waste container.

Results and Discussion

The results of yeast and sodium borohydride reductions of 2-methyl- and 4-*tert*-butylcyclohexanone are summarized in Table 1.

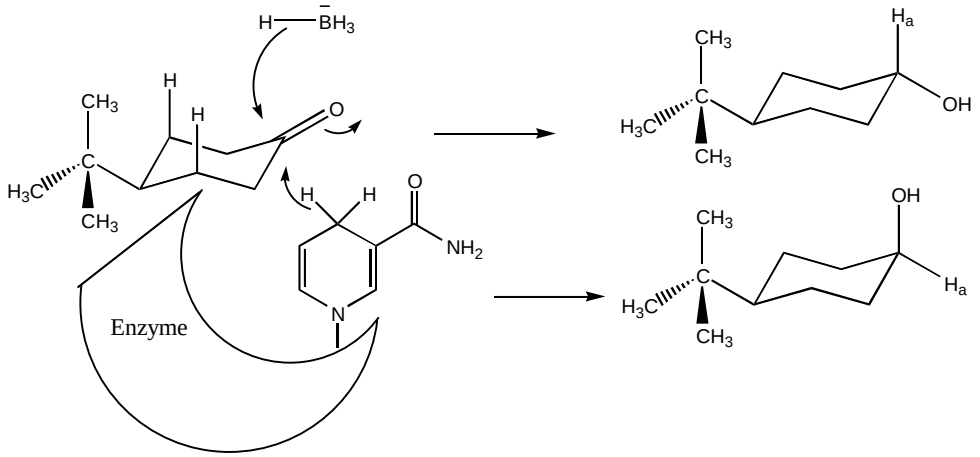
In the reduction of 4-*tert*-butylcylohexanone, both the cis and trans isomers of 4-*tert*-butylcyclohexanol are possible products. The ^{1}H-NMR can distinguish these diastereomers, and using integration, the diastereisomeric ratio can be determined. The NMR spectrum shown in Figure 1 illustrates the results obtained by a typical student. The two diastereomers gave clearly resolved peaks, the two signals at 4.03 and 3.48 ppm correspond to the proton on the carbon bearing the hydroxyl group. The peak at 3.48 ppm represents the trans alcohol, and the peak at 4.03 ppm represents the cis alcohol. Integration of these two peaks gives a 95:5 ratio of trans to cis for the sodium borohydride reduction; however, when yeast was used as the reducing agent, the cis isomer was formed almost exclusively in yields of 5–32%.

Structure determination was accomplished using coupling constants of these downfield signals. Coupling constants, *J*, can be predicted using the Karplus correlation, where the *J* values are related to the dihedral angle between protons on vicinal carbons atoms in conformationally restricted systems. When the dihedral angle between two coupled protons is 60º the coupling constant will be small (1–7 Hz, usually 2–3 Hz), but when the dihedral angle is 180º the coupling constant will be large (8–14 Hz usually 8–10 Hz) [11]. The quintet at 4.04 ppm implies that the proton is coupled with approximately equal coupling constants (*J* ~ 3 Hz) to four adjacent protons. The proton H_a must be equatorial because the dihedral angle between H_a and each of its four adjacent protons is approximately 60º. The triplet of triplets at 3.52 ppm implies that the proton is axial and is coupled to two adjacent (symmetry-equivalent) protons with a large coupling constant (*J* ~ 13 Hz) and two other adjacent protons with smaller coupling constant (*J* ~ 3 Hz) [12].



Yeast reduction of 2-methylcyclohexanone resulted in the cis isomer as the major product (62%), whereas sodium borohydride produced more of the trans isomer (59%). Coupling constants were also used in the assignment of configuration [10]. Yeast reduction of racemic 2-methylcyclohexanone should selectively produce one of four possible stereoisomers. Reduction of (*S*)-2-methylcyclohexanone can give the cis (1R, 2S) and trans (1S, 2S) diastereomeric pair of alcohols, whereas reduction of (*R*)-2-methylcyclohexanone can yield the cis (1S, 2R) and trans (1R, 2R) alcohols. The enantioselectivity of this reaction could be determined by NMR techniques using chiral shift reagents [13], but in light of the potential mixture of four stereoisomers, determination of the enantiomeric excess was not studied in this experiment.

The carbonyl group of substituted cyclohexanones has two distinct faces. The small borohydride anion attacks the



carbonyl group from the more sterically hindered axial position to give predominantly the thermodynamically more stable trans diequatorial isomer [14]. On the other hand, hydrogen transfer from nicotinamide adenine dinucleotide in yeast cells involves an equatorial approach. This leads to the formation of almost exclusively the cis isomer.

We have shown that a microorganism, such as yeast, can be used to reduce cyclic ketones to alcohols with enhanced diastereoselectivity. In addition, a contrast in diastereo-selectivity was observed between sodium borohydride and yeast. The trans isomer was the major diastereomer in the sodium borohydride reductions and the cis isomer for the yeast reductions. When yeast is used, the reduction is apparently controlled by steric influences that lead to the less stable cis isomer as the major product, while sodium borohydride forms the more stable trans diequatorial isomer. This experiment has the advantage over other baker's yeast reduction experiments in that NMR can directly determine diastereoselectivity.

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