Enzymatic Reduction of Methyl-Acetoacetate

p. 588 (576, 4th ed), Use Bakers Yeast to reduce carbonyl stereoselectively

Important Concepts

• Chirality - enantiomer, diastereomer
  • Stereoselective synthesis
  • Enantiomeric excess (e.e.)
• Assigning stereochemical configuration
  • Polarimetry, Chiral Shift Reagents
• Enzymes & Fermentation
  • Anaerobic conditions
Procedural Details - Week 1

- place starting materials in 250 ml Erlenmeyer flask and assemble apparatus.

- make sure the beaker with Ba(OH)$_2$ is lower than the Erlenmeyer flask to avoid back-pressure.

- you don’t need to put mineral oil in the beaker during the initial reaction.

- make sure that the rubber tubing is clean since the reaction foams a lot in the beginning, this tends to shoot up into the tubing.

- heat the reaction mixture ~35 °C for 1 hour and then transfer to shaker and place rubber tube into a community beaker of Ba(OH)$_2$. The reaction will continue until next week.

- monitor the initial reaction to make sure that CO$_2$ bubbles are forming in the beaker. If you do not see bubbles, then your rubber tube may be clogged.
Procedural Details - Week 2

- isolate product by filtering off the yeast biproducts.

- extract with dichloromethane, be careful not to form emulsions, you may have to add some brine to help separate layers.

- evaporate solvent, you will only have a small amount, so tare your RB flask beforehand.

- measure optical rotation of 3 sugar solutions.

- no ferric ion test.

**Required Data**

- yield, % yield

- TLC of product & starting material, you will probably need to make an iodine chamber to visualize the spots.

- IR and $^1$H NMR of product and starting material

- $^1$H NMR of racemate and product with chiral shift reagent, use to calculate enantiomeric excess (e.e.)

- optical rotation of three sugar solutions (+), (-), and racemate
Introduction to Chirality

Not Chiral

Chiral objects or molecules are NOT superimposable on their mirror image.

Enantiomers

Not Chiral

Chiral

Pasteur asked the question - why does tartaric acid, derived from wine yeast, rotate the plane of polarized light, but synthetic tartaric acid does not?

Pasteur noticed two types of crystals that were mirror images of each other in the synthetic tartaric acid. So he used a tweezers to separate them and found that they rotated the plane of polarized light in opposite directions!

The 50:50 mixture (i.e. racemate) rotates in both directions and averages to 0°.
Nature Exploits Chirality via Enzymatic Reactions

Enzyme: a biological catalyst, usually composed of amino acids (i.e. a protein)

Most enzymes create a chiral active site to yield a chiral product. In the case of Bakers Yeast the enzyme directs hydrogen addition from the top face.

The enantioselectivity of Bakers Yeast is poor when oxygen is present, therefore, we use anaerobic conditions (i.e without oxygen).

Enzymes work by lowering the activation energy for a reaction.
Why is Asymmetric Synthesis Important?

In the 1950’s doctors prescribed thalidomide as an effective treatment for morning sickness. In those days most synthetic drugs were synthesized as racemic mixtures (i.e. R:S ratio is 50:50). Unfortunately one enantiomer caused birth defects. Now the FDA requires all chiral drugs to be sold as pure enantiomers.

Today chemists try to devise new catalysts that are chiral and can synthesize new asymmetric stereocenters. The goal is to synthesize the product in high enantiomeric excess (e.e.).
What is an Isomer?

Isomer: compounds that have the same molecular formula, but different structure.

Constitutional isomer: isomers with a different order for the attachment of atoms.

Stereoisomers: isomers with the same order of attachment, but with different orientation of the atoms in space.

Enantiomer: compounds that are nonsuperimposable mirror images of each other.

Diastereomer: stereoisomers that are not mirror images of each other. Typically they have more than one stereocenter.

Conformational isomer: same molecule with different conformations in space.
Rules for Naming Chiral Compounds

1. Identify the stereocenter

2. Prioritize groups on the stereocenter
   (a) Atomic number of attached atom
   (b) If atoms are the same, go to next atom along chain, etc.
   (c) If there is a double bond, count it as two atoms

3. Arrange groups so that lowest priority is in the back

4. If clockwise = R, or if counterclockwise = S

5. If light is rotated to the left (levorotatory), then the stereocenter is (-) and if it is rotated to the right (dextrotdatory), then the stereocenter is (+).
How do we Detect Chirality?

1. Measure the optical rotation $\alpha$ of enantiomers by polarimetry.

$$\frac{\alpha_{sample}}{\alpha_{pure}} \times 100 = \text{e.e.}$$

In general, enantiomers have identical chemical properties (e.g. mp, $R_F$ value, reactivity)

2. Synthesize a diastereomer by adding another chiral group to the molecule.

3. $^1$H NMR can be used to integrate diastereomeric peaks.
Procedure for Polarimetry Measurements

You will measure $\alpha$ for three sugar solutions: (R), (S), and Racemate.

Set rotation to $0^\circ$

Rotate towards the dark side

Dark side left $\rightarrow$ (-) Dark side right $\rightarrow$ (+)

Record angle when both halves are equal in intensity. Take several measurements and average them.
Using an NMR Chiral Shift Reagent

The properties of enantiomers are identical, except for the rotation of light.

Add a chiral shift reagent to induce a diastereomeric relationship in the $^1H$ NMR spectrum.

\[
\text{Add chiral shift reagent} \quad \text{Mixture of R and S} \quad \text{Add chiral shift reagent} \quad \text{Enriched in (R)}
\]

Calculate e.e. by integrating the peaks equivalent to optical rotation $\alpha$.

\[
\frac{[95 - 5]}{[95 + 5]} \times 100 = 90\%
\]
Overall View of Fermentation of Glucose by Yeast

https://en.wikipedia.org/wiki/Fermentation
Fermentation is a metabolic process to convert energy under anaerobic conditions.

Sugars → Ethanol + CO₂ + ATP → Energy is released

Glucose → 2 eq. ethanol + 2 eq. CO₂

pyruvate → Ethanol + CO₂

NAD⁺ → NADH

NAD = Nicotinamide adenine dinucleotide

No net change in oxidation states of products and starting materials (i.e. when something is reduced, something else is oxidized)
Organic II: Unknown Lab (4/19 Tuesday to 4/21 Thursday)

(a) Sign up for unknown in each lab section with a partner – there are 10 unknown molecules. (during the 2nd week of “Enzymatic Reduction” – week of 4/12 Tuesday to 4/14 Thursday)

(b) You will have the molecular formula of the unknown and molecular weight. I will post NMR and Mass Spectra for the molecule online http://JulietHahn.com and D2L before the unknown week – print out your unknown NMR and Mass Spectra and take the spectra to the Unknown lab class.

(c) During the unknown lab, you will collect the IR of your unknown. You should take the Melting Point of the unknown (if it is a solid). You will also have your TA sign and date your lab report front cover. If you do not have your TA signature and date and turn in your lab report, you will lose 20% of your lab report grade. You may wish to consult your TA to help you determine the ID of your unknown.

(d) You will have 10 points for product points – which will be assigned after you turn in your Unknown Lab - Lab Report. The 10 points are for your final assignment of the identity of the unknown. The other 90% is for your write up and explanation.

(e) You lab report should have: (1) Introduction (2) degree of unsaturation calculation showing your work (3) your explanation of the IR explaining specific IR peaks and the functional group that your IR predicts (use charts page 251 to page 256 of your text) (4) your explanation of the NMR specifically mentioning chemical shift, integration and coupling information in detail (use charts pages 268 to page 271 of your text) (5) Your explanation of Mass Spectra. If you see the molecular ion peak, ID the peak. If you do not see the molecular ion peak, explain. (6) Discussion of how you decided on the identity of your unknown.

(f) There are more points for a good discussion of your process than in correctly identifying your unknown.