Experiment 20

Vanillin Reduction with Sodium Borohydride

![Chemical structure](structure.png)

Figure 20.1 Sodium borohydride reduction of \( p \)-vanillin.

**Introduction**

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a pleasant smelling aromatic compound formed through the enzymatic breakdown of a glucoside during the curing process of the vanilla bean. Widely used as a flavoring additive for cooking and fragrance preparation, the finest vanilla is obtained from natural vanilla, however synthetic vanillin costs much less. Vanillyl alcohol, the product formed by the reduction of vanillin, is a promising renewable starting material for the synthesis of biologically active molecules and flavoring ingredients. In this experiment, \( p \)-vanillin will be treated with a reducing agent to produce vanillyl alcohol. The reaction will be followed using TLC analysis, and the purity of the product will be determined using HPLC and melting point analysis. Reactants and products will be characterized using \( ^1H \)-NMR and IR spectroscopy.

**Reducing Agents**

In organic chemistry, the gain of hydrogen atoms, the loss of oxygen atoms, or both, is classified as a reduction. In this case, the carbonyl group of the aldehyde is reduced to an alcohol when its carbonyl group gains a hydride and a proton. The most common laboratory reagents for the reduction of the carbonyl group of an aldehyde or ketone to an alcohol are sodium borohydride (NaBH\(_4\)) and lithium aluminum hydride (LAH), shown in Figure 20.2. Both of these compounds behave as sources of a hydride ion, which is a very strong nucleophile (see McMurry text, pages 609 and 709).

![Reducing agents](reducing_agents.png)

Figure 20.2 Common reducing agents.

Lithium aluminum hydride is a very powerful reducing agent and reacts not only with aldehydes and ketones, but also with many other carbonyl containing compounds such as esters, carboxylic acids, and amides. One disadvantage of LAH is that it reacts *violently* with protic
solvents such as water and methanol, to produce metal hydroxides or alkoxides, and hydrogen gas, which could result in an explosion or fire.

Sodium borohydride is much milder reducing agent than lithium aluminum hydride. Unlike lithium aluminum hydride, sodium borohydride will not reduce carbonyl-containing compounds such as esters, carboxylic acids, or amides, therefore an aldehyde or ketone can be reduced with sodium borohydride in the presence of these other types of functional groups. These reductions can be performed in a wide variety of solvents, such as aqueous methanol or ethanol, with a good to excellent yield.

The key step in the metal hydride reduction of an aldehyde or ketone is transfer of a hydride ion from the boron atom of the reducing agent to the electropositive carbon of the carbonyl group to form a tetrahedral intermediate (Figure 20.3) called an alkoxide ion. The resulting alkoxide ion is protonated by the protic solvent to form a neutral alcohol. One mole of NaBH₄ is a source of four hydride ions, therefore can react with four carbonyl groups to produce four moles of the product. **Thus, one mole of the reducing agent reduces four moles of the carbonyl compound.**

![Figure 20.3 Mechanism for the sodium borohydride reduction of an aldehyde.](image)

**Reaction Conditions**

It is best to use a 50-100% excess of sodium borohydride to account for any that may react with a solvent or decompose during the course of the reaction. Using an excess of sodium borohydride will also increase the reaction rate, as the reaction is first order in both the sodium borohydride and the p-vanillin. Since the reagent is not stable at low pH or even in neutral aqueous solutions at room temperature, it is typically prepared in a dilute aqueous NaOH solution.

Sodium borohydride reacts slowly with alcohols, but ethanol is a suitable reaction solvent as long as there are no strongly acidic functional groups present, and the reaction time is no more than 30 minutes at room temperature. For this reason, the reaction will be performed at a temperature below room temperature. After the reaction is complete, the excess sodium borohydride must be decomposed by acidifying the reaction solution to pH 6 or below. This is accomplished in an ice water bath using aqueous hydrochloric acid. The acidification must be carried out slowly; as hydrogen gas is evolved during the process!

In order ensure that the reaction temperature does not exceed 25°C, an apparatus should be set up in such a way that the rate of addition of sodium borohydride can be controlled. This is accomplished using an apparatus such as the one shown in Figure 20.4. The sodium borohydride solution is added to a separatory funnel suspended above a stirring solution of p-vanillin in ethanol. The solution is slowly added drop wise at a controlled rate over a period of several minutes while the reaction solution stirs in an ice water bath.
**Chromatographic Analysis**

The reactant and product in this experiment contain aromatic rings which are chromophores easily visualized using a UV lamp. It is also optional to stain the TLC plate with certain compounds that not only aid in the visualization of compounds, but also provide a method for determining which functional groups are contained within a molecule. A solution of 2,4-Dinitrophenylhydrazine (2,4-DNP) is a stain mainly used to detect aldehydes and ketones (Figure 20.5). When these types of carbonyl compounds react with 2,4-DNP, they form the corresponding hydrazone, which is usually yellow or orange, thus resulting in the spot on the TLC plate to appear colored. Interestingly, 2, 4-DNP does not react with other carbonyl containing functional groups such as esters, carboxylic acids, and amides, therefore can be useful to distinguish between compounds containing more than one type of carbonyl functional group.

![Figure 20.4 Reaction apparatus.](image)

**IR Spectroscopy**

IR spectroscopy can certainly be used to determine whether the reducing agent successfully reduced the carbonyl substituent. This is based on the appearance or disappearance of certain types of absorptions characteristic of an aldehyde functional group. The phenolic O-H stretch typically appears as a strong, broad absorption in the range of 3200-3600 cm$^{-1}$. The
phenolic O-H, present in both the reactant and product, appears as a strong, broad absorption due to the intramolecular hydrogen bonding which occurs between the phenolic hydrogen and the neighboring methoxy oxygen in the ortho position. Also present is the C-O bond absorption, which typically appears within the range of 1000-1300 cm\(^{-1}\). Aldehydes usually exhibit two C-H stretches around 2700 and 2800 cm\(^{-1}\); however these are often difficult to distinguish when broad O-H absorptions are present. Finally, a strong C=O stretch absorption would appear between 1680-1740 cm\(^{-1}\).

The product of the reaction is the result of the conversion of a carbonyl group to a hydroxyl group. This should result in the presence of a new O-H signal in the product spectrum with a slightly different appearance. The new O-H signal of the alcohol does not participate in intramolecular hydrogen bonding, is considered a “free” O-H stretch, and appears as a strong, sharp absorption, typically in the range between 3500-3700 cm\(^{-1}\). The absence of a C=O absorption will also be a clear indication that the carbonyl group has been successfully reduced. The IR spectra of the reactant and product are shown in Figure 20.

NMR Spectroscopy

The \(^1\)H NMR spectra of the reactant and product are shown in Figure 20. The chemical shift of a hydroxyl proton varies depending on the purity of the sample, the temperature, and the sample solvent. The hydroxyl proton signal of an alcohol typically appears in the range of 0.5-6.0 ppm, depending on the experimental conditions. A phenolic proton signal, however, appears closer to the expected position for aromatic-ring protons, typically within the range of 5.0-10.0 ppm. The aldehyde proton signal is very distinctive, appearing as a singlet 10.0 ppm. Very few signals appear that far downfield in a \(^1\)H NMR spectrum. The aromatic region tends to be rather complicated, therefore note that some signals are already identified.

Objectives

In this experiment you will synthesize vanillyl alcohol by reducing \(p\)-vanillin with sodium borohydride in ethanol. The identity and purity of the product will be analyzed using TLC and HPLC analysis. Finally, reactants and products will be characterized using IR and \(^1\)H NMR spectroscopy.

Experimental

Synthesis: Reduction of \(p\)-vanillin

- Weigh between 2.5-2.6 g of \(p\)-vanillin into a 50 mL beaker. Record actual mass used in lab notebook.
- Clamp a 25 mL round bottom flask to the ring stand. Add a stir bar.
- Transfer the solid vanillin to the round bottom flask using a short stem powder funnel. Rinse the beaker and the funnel with ~ 5.0 mL ethanol.
- Lower the reaction flask into a shallow room temperature water bath. Stir the solution using a magnetic stirrer for several minutes until the vanillin is completely dissolved.

- **READ THOROUGHLY BEFORE STARTING THE NEXT STEP!!!**
  - Set up the apparatus as shown in Figure 20.4. Be sure to close the stopcock of the separatory funnel!
Add 5.0 mL of the NaBH₄ solution (3.42M prepared in 1.0M NaOH) to the separatory funnel. Cool the water bath by adding a few ice cubes.

**SLOWLY** open the stopcock of the separatory funnel to release 3-5 drops of the reducing agent, then close and allow the reaction to occur until the vigorous bubbling ceases.

Repeat this step until the entire reducing agent has been added. This controlled addition rate should take *several* minutes. Note any observations in your lab notebook.

- After the addition of NaBH₄ is complete, remove the ice bath. Stir the reaction solution at room temperature for 5 minutes to ensure the reaction has gone to completion.
- Return the ice bath to the apparatus. Add 6M HCl drop wise while stirring until H₂ gas is no longer evolved. Check the pH of the solution to verify that the solution is now acidic (pH 6 or below!)
- Continue to cool with stirring in the ice bath for 10 minutes. A precipitate will form during this time. Note any observations in your lab notebook.

**Product Isolation and Purification:**

- Set up a suction filtration apparatus (review Experiment 6 if necessary). Be sure to clamp the filter flask to a ring stand, and attach the filter flask to the vacuum line using a red rubber hose.
- Preweigh a small filter paper. Place the filter paper in the Buchner funnel, apply the vacuum, and then seat the filter paper with 1-2 mL ice cold water.
- Slowly pour the contents of the reaction flask into the center of the filter paper. Use as much ice cold water as necessary to completely transfer the entire solid from the flask.
- Rinse the solid product on the filter paper with additional 1-2 mL ice cold water, and then allow the solid to dry under vacuum for 2-3 minutes.
- Prepare a TLC sample of the solid by transferring a few crystals to a small test tube, then dissolving in 1 mL reagent acetone. Set this TLC sample aside for further analysis.
- Prepare an HPLC sample of the solid by transferring a few crystals to a small auto analyzer vial, then dissolving in 1 mL of HPLC solvent (2:3 ethyl acetate/hexane). Set this HPLC sample aside for further analysis.
- Transfer the remaining solid product to a preweighed large filter paper, label, and submit to instructor to dry until the next lab period.
- Proceed to **Product Analysis** with samples.
- During the next lab period, obtain mass of dry product and calculate yield.

**Product Analysis:**

**TLC Analysis**

- Perform TLC experiment on the sample along with the provided standards of *p*-vanillin and vanillyl alcohol. Develop this plate in 2:3 ethyl acetate/hexane and visualize under UV lamp. Sketch a diagram of this plate in your laboratory notebook.
- Using tweezers, dip this plate in 2, 4-DNP TLC stain. Blot excess stain on a paper towel, before transferring back to lab hood. Once completely dry, the 2, 4-DNP reagent with produce a dark orange spot with any compound containing an aldehyde functional group.
Sketch a diagram of this plate in your laboratory notebook, complete with color descriptions of the spots which appear. Complete Table 20.1 on the final lab report.

**IR Analysis**
- Using the provided spectra in Figure 20.6, identify all characteristic absorptions of reactants and products. Complete Table 20.2 on the final lab report.

**NMR Analysis**
- Using the provided spectra in Figure 20.7, identify and tabulate all characteristic resonances of reactants and products. Complete Table 20.3 on the final lab report.

**Melting Point Analysis**
- After obtaining mass of dry product, prepare a melting point capillary of your dry product.
- Obtain the experimental melting point of your product and compare to the literature value to determine purity. Complete Table 20.4 on the final lab report.

**Green Chemistry Analysis**
- Calculate the atom economy, experimental atom economy, \( E_{product} \), cost per synthesis, and cost per gram using the equations introduced in Experiment 13. Costs of all reagents and solvents are provided under the chemical equation on the final lab report.
- Be sure to show complete calculations in data section of lab notebook. Complete Table 20.5 with this data.

**HPLC Analysis**
- Submit the previously prepared HPLC sample for analysis.
- Once returned, compare HPLC results of the sample to the provided standard chromatogram. Identify any compounds present in the sample, and quantify compounds present. Complete Table 20.6 on the final lab report.

**WASTE MANAGEMENT**
Place all solid waste into the container labeled “SOLID WASTE” located in the waste hood. Place all liquid waste into the container labeled “LIQUID WASTE”. Place any used melting point capillaries and TLC capillaries in the “BROKEN GLASS” container. TLC plates and filter papers can be discarded in the yellow trashcan.

**References**


\(^1\)H NMR spectra on page 176 are from [http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng](http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng)
Figure 20.6 IR spectra of reactant and product.
Figure 20.7 $^1$H NMR spectra of reactant and product.
Exp. 20*: Vanillin Reduction with Sodium Borohydride

EXPERIMENTAL RESULTS
(Tables in INK only!)

Table 20.1 TLC Analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Standard R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>Sample R&lt;sub&gt;f&lt;/sub&gt;</th>
<th>TLC Diagram</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-vanillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanillyl Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 20.2 IR Analysis

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Base Values</th>
<th>p-vanillin</th>
<th>Vanillyl alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH stretch</td>
<td>3200-3600</td>
<td>Frequency (cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Frequency (cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>C-O stretch</td>
<td>1000-1300</td>
<td>Frequency (cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Frequency (cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>C=O stretch</td>
<td>1680-1740</td>
<td>Frequency (cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>Frequency (cm&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

Table 20.3 <sup>1</sup>H NMR Analysis
Table 20.4 Experimental Results

<table>
<thead>
<tr>
<th>Theoretical yield (g)</th>
<th>Actual yield (g)</th>
<th>Percent yield</th>
<th>Product Appearance</th>
<th>Experimental Melting Point (°C)</th>
</tr>
</thead>
</table>

Table 20.5 Green Chemistry Results

<table>
<thead>
<tr>
<th>Atom Economy (%)</th>
<th>Experimental Atom Economy (%)</th>
<th>“E_{product}”</th>
<th>Cost per synthesis ($)</th>
<th>Cost per gram ($/g)</th>
</tr>
</thead>
</table>

Table 20.6 HPLC Analysis

<table>
<thead>
<tr>
<th>Compound</th>
<th>HPLC Retention Times (min)</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-vanillin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanillyl alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION/CONCLUSIONS

(In the space provided, briefly answer the following questions. Use numerical values to support conclusions where applicable.)

1. Based on TLC and HPLC analyses, was the p-vanillin completely reduced to vanillyl alcohol? Identify any compounds present in your sample. Give HPLC retention times, area percent values, and TLC R_{f} values for any compounds present in your sample.

2. What is one type of IR absorption band that could be used to indicate that the conversion from the aldehyde to the alcohol took place? What is the typical frequency for this type of absorption? What is the actual frequency of the absorption in the provided spectrum?

3. What is one signal in the ¹H NMR spectrum that indicates that the reduction took place? What are the typical chemical shifts that these types of protons may appear? What is the actual chemical shift of this proton signal?
4. Draw the product, and a *complete mechanism* for its formation, for the following reaction. In your mechanism, use only the hydride ion as your nucleophile.

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1. NaBH₄
2. H₂O⁻ workup

**Attach sample chromatogram!**
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