Hydroboration–Oxidation of (1R)-(+)−α-Pinene to Isopinocamphene

A Microscale Experiment that Displays Regio- and Stereochemistry Using NMR Spectroscopy and Molecular Mechanics Calculations

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The hydroboration–oxidation of alkenes is an important route to alcohols and therefore receives considerable treatment in standard organic textbooks. Although this synthetic method exhibits both regiochemistry (i.e., anti-Markovnikov addition) and stereochemistry (i.e., syn-addition) if a suitable alkene is chosen, we are not aware of an example that displays both of these features that has been adapted for use in the undergraduate laboratory. Experiments demonstrating only the regiochemistry of this sequence of reactions have appeared in this Journal in the macroscale setting (1) and in laboratory textbooks in the microscale setting (2, 3). We searched, then, for an alkene that would undergo the hydroboration–oxidation process in a manner that would demonstrate both the regio- and stereochromical features of the reaction.

Our choice of (1R)-(+)−α-pinene (1), hereafter referred to as α-pinene, as the substrate not only meets these requirements but has other advantages as well (see Fig. 1). Hydroboration–oxidation of 1 produces isopinocamphene (2) in high yield (4). Alcohol 2 is a low-melting solid that can be easily purified by sublimation in milligram quantities. This important purification technique is utilized rather infrequently in many undergraduate laboratories and needs more exposure. Another advantage in using 1 as the substrate is that it is commercially available in high optical purity. This provides the option of monitoring the reaction with a polarimeter. Finally, it should be noted that proton NMR coupling constants for the rigid, bicyclic alcohol 2 can be calculated accurately using molecular mechanics methods (5–9). In this way, the observed coupling constants in the NMR spectrum of the product can be compared with those calculated by the molecular mechanics program and used to help establish the stereochemistry of the product. Considerable interest in integrating computational methods into the undergraduate chemistry curriculum has been expressed in this Journal (9–13), and we believe this experiment utilizes these technologies in a meaningful way in the laboratory.

Experimental

Materials and Reagents Required

Standard microscale kit including a dry 1-mL syringe.
Sublimation apparatus (125-mL filtering flask fitted with a one-hole rubber stopper containing a 16-mL conical centrifuge tube). (1R)-(+)−α-pinene, optical purity 98.4%.
Borane–THF complex, 1.0 M (caution: avoid contact with water).
30% H₂O₂ (caution: strong oxidant, avoid skin contact).

Procedure

Hydroboration. Add 150 mg (1.10 mmol) of (1R)-(+)−α-pinene to a dry 5-mL conical vial. Equip the vial with a magnetic stirrer, a Claisen head fitted with a CaCl₂ drying tube, and a rubber septum. Using a dry syringe, add 600 μL (0.600 mmol) of 1.0 M BH₃−THF (caution: hydrogen gas is produced upon contact with water) to the vial through the rubber septum.¹ While stirring, heat the reaction mixture to ~50 °C for 5–10 min. Cool the reaction mixture in an ice bath, remove the rubber septum, and, while stirring, add a single drop of H₂O₂. Considerable frothing may occur due to the production of hydrogen gas.² After bubbling diminishes, add two more drops of water.

Oxidation. Attach a condenser to the 5-mL vial containing the reaction mixture. While stirring, use a syringe to add 375 μL of 3.0 M NaOH through the top of the condenser all at once, followed by 375 μL of 30% H₂O₂ (caution: this reagent causes instant burns to skin). The oxidation of the dialkylborane with hydrogen peroxide is mildly exothermic. Heat the reaction mixture to a gentle reflux for 15–20 min.

Isolation of Product. Cool the reaction mixture in an ice bath, and transfer the aqueous layer to a 3-mL vial with a Pasteur pipet, saving the organic layer in the reaction vial. Extract the aqueous

¹ Due to steric factors a dialkylborane rather than a trialkylborane is formed in the reaction, thereby requiring a 2:1 mole ratio of alkene to borane.
² Any hydride that may be present will react with water producing hydrogen gas.
layer with 1.5 mL of petroleum ether. Discard the aqueous layer, and
transfer the petroleum ether extract to the first vial containing the
organic layer. Extract this solution with 2.0 mL of 0.20 M HCl
followed by water (2 × 1.0 mL), saving the organic layer in each case.
Dry the organic layer over anhydrous Na2SO4. Transfer the organic
layer to a dry 125-mL Buchner filtering flask, and remove the sol-
vent by evaporation in a hood under a slow stream of air, leaving a
viscous liquid that partially solidifies upon cooling.

**Filtration of the Product by Sublimation.** Drill a hole large
enough to accommodate a 15-mL glass centrifuge tube in a #5
rubber stopper. Place the centrifuge tube into the rubber stopper to
a depth such that when the stopper is placed into the 125-mL
Buchner flask containing the crude product, the bottom of the cen-
trifuge tube is about 1 in. from the bottom of the Buchner flask.
Fill the centrifuge tube with small chips of ice. Connect the side arm
of the Buchner flask to a water aspirator, and immerse the Buchner
flask in a water bath (a half-full 400-mL beaker works well) main-
tained at about 80 °C on the hot plate. The product sublimes onto
the cold surface of the centrifuge tube.3

**Results and Discussion**

The alcohol isolated from the hydroboration–oxidation of
α-pinene using the above procedure has a melting point of
51–53 °C and can be obtained in a yield of 50–60%. The proton and
C-13 NMR spectra of this product (Fig. 2) show that a single substanece is formed demonstrating that the
reaction is highly regio- and stereospecific.

The fact that a single alcohol is formed in high yield in this
reaction suggests that the addition of H2O occurs exclusively from
the less sterically hindered face of the double bond in
α-pinene. Thus, only alcohols 2–5 are considered as possible
products. The alcohols 2 and 3 are the result of anti-Markov-
nikov addition in a syn and anti fashion respectively, whereas
alcohols 4 and 5 are the result of Markovnikov addition.

It is clear that the product of the reaction is neither cis-pinanol (4) nor trans-pinanol (5). Not only are their melting
points higher than that of the product, but the proton NMR spectrum shows a signal (a doublet of 1:2:1 triplets
with J = 8.5 and 5.0 Hz, respectively) at approximately 4
ppm for one hydrogen. The chemical shift of this hydrogen is in
the range expected for a secondary alcohol proton (CHOH).
Such a signal should be present in the PMR spectrum of either 2 or 3 but absent in the spectra of both 4 and 5.
Furthermore, the PMR of the product displays a doublet at 1.05 ppm, which integrates for three hydrogens. This signal
is attributed to a methyl group attached to a tertiary carbon
(CH3CH) and would be expected in the PMR spectrum of
either 2 or 3 but not in the spectrum of either 4 or 5. On this
basis we may conclude that the reaction involves anti-Markov-
nikov addition of water across the double bond.

With the regiochemistry of the hydroboration–oxidation
of α-pinene now established, we are left with the question of
stereochemistry. Since the melting points and specific rota-
tions of isopinocamphor (2), the syn-addition product, and
neoisopinocamphor (3), the anti-addition product, are
quite similar, we decided to try to use proton NMR spectra
and 13C NMR spectra correlated with molecular mechanics calculations to distinguish between these two possible products.

A small amount of isopinocamphor (2), was converted
into the corresponding ketone (pyridinium chlorochromate oxidation). Reduction of this ketone (LiAlH4) produced a
20:80 mixture of isopinocamphor (2), and its epimer, neoi-
soinocamphor (3), respectively. The PMR spectra of both
2 and 3 contain a signal in the 4-ppm region attributable to a
secondary alcohol proton (CHOH). However, the proton in 2
is coupled to three vicinal protons with coupling constants of
8.5, 5.0, and 5.0 Hz and occurs as a doublet of triplets cen-
tered at 4.05 ppm. The corresponding proton in 3 also is
coupled to three vicinal protons but with coupling constants of
9.7, 9.7, and 5.1 Hz and appears as a triplet of doublets centered at 4.4 ppm.

Using the MM2 force field calculations, the geometry of
the alcohol 2 was energy-optimized, and the coupling con-
stants for the secondary alcohol (CHOH) proton were
obtained. The calculation suggests that this proton in 2 should
be coupled to three vicinal protons with coupling constants of
9.7, 4.3, and 3.9 Hz. This is in very good agreement with the
experimental spectrum we obtained for the product of the
hydroboration–oxidation reaction. Similarly, the geometry of
the alcohol 3 was energy-minimized and the coupling constant for the secondary alcohol (CHOH) proton
obtained. The calculation suggests coupling to three neighbor-
ing protons with coupling constants of 9.7, 8.4, and 3.9 Hz.
Once again this agrees well with the experimental result
obtained for the alcohol 3. The calculated and observed
coupling constants for this proton in 2 and 3 are summarized
in the table. These results demonstrate that molecular
mechanics calculations can have considerable utility in making
stereochemical assignments. On the basis of these results we
may conclude that hydroboration–oxidation of (1R)-(+)–α-
pinene involves syn-addition of water across the double bond.

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3 Initially, the product condenses on the cold tube as a liquid that
freezes. Then deposition occurs resulting in the formation of long,
white needles. The melting points of these two sources of sublimed
product are nearly identical.

4 This software, PC Model, is available at Serena Software (Box
3076, Bloomington, IN 47402-3076) and can be used on IBM or
Macintosh personal computers.
Coupling Constants (Hz) for H₂ in Alcohols 2 and 3

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Protons</th>
<th>Dihedral Angle (°) (calc)</th>
<th>(J_{\text{calc}})</th>
<th>(J_{\text{obs}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>H₃-H₂</td>
<td>131</td>
<td>4.4</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>H₃-H₁', H₃-H₂'</td>
<td>6</td>
<td>9.7</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>H₃-H₄</td>
<td>124</td>
<td>3.9</td>
<td>5.0</td>
</tr>
<tr>
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<td>H₃-H₃</td>
<td>1</td>
<td>8.4</td>
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<td>H₃-H₁', H₃-H₂'</td>
<td>124</td>
<td>3.9</td>
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<tr>
<td></td>
<td>H₃-H₄</td>
<td>8</td>
<td>9.7</td>
<td>9.7</td>
</tr>
</tbody>
</table>

* MM2 force field calculation using optimized geometries.

At Calvin College this experiment is done early in the second semester of organic chemistry soon after students are introduced to NMR spectroscopy. Before students do the experiment, they are told that alcohols 2–5 are possible products of the hydroboration–oxidation of α-pinene. Based upon the PMR spectrum of the product and the coupling constants in the table, students are required to deduce the structure of the alcohol. In the near future, we hope to have a computational chemical laboratory that will allow students to determine independently the MM2 force field calculated coupling constants for the secondary alcohol (CHOH) proton.

**Acknowledgment**

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**Literature Cited**


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**Testing for Chemical Toxicity Using Bacteria**

An Undergraduate Laboratory Experiment

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The impact of chemicals on people, animals, and the environment in general has become a major concern of scientists in recent years. Many new analytical methods have been developed to detect trace levels of hazardous chemicals, and a wide variety of biological screening tests are being employed in testing chemicals and environmental samples. Yet, most laboratory courses tend to omit toxicity studies since many of the tests are expensive and time-consuming. In this laboratory activity a relatively simple and inexpensive screening test using a bacterial culture is described for determining chemical toxicity.

Chemicals may be harmful to an organism in a variety of ways. *Acute toxicity* refers to the short-term effect of a toxic material on an organism and is usually measured by the symbol LD₅₀ or EC₅₀. This is the lethal dose (LD) causing death in 50% of the test organisms or the effective concentration (EC) causing a 50% decrease in a specific effect or response. The former is often measured in milligrams of the chemical per kilogram of body weight, whereas the latter may be measured in milligrams per liter (ppm) of solution (1). Acute toxicity is determined from the mortality rate when the test organism is exposed to the chemical or environmental sample for a prescribed period of time. *Chronic toxicity*, on the other hand, involves exposing the test organism to low levels of the chemical for a longer period of time, sometimes for several generations, to determine if the chemical has any adverse effects.

Since living organisms may respond differently toward chemicals, a battery of screening tests involving different organisms is normally performed to evaluate the toxicity of a sample. Due to the high costs associated with common laboratory animals, microbes are often employed in screening tests since their costs are low, they have a short life cycle, and a large number of individuals will be exposed to the test chemical. Two tests in particular have received wide application. The Ames test detects the presence of reverse mutations in a special bacterial culture upon exposure to a test chemical. The Microtox test measures the decrease in light emission from photobacteria when exposed to the sample. Numerous other methods have been developed for measuring the inhibition of microbial activity (2,3).

The procedure described in this paper is a modification of a screening test developed by D. Liu and co-workers (4-8). The test employs an electron acceptor dye, resazurin, which changes color in the presence of dehydrogenase enzyme activity resulting from microorganisms actively growing in a culture medium. In the presence of an active bacterial culture, resazurin changes color from blue to pink, as follows:

\[
\text{HO} + \text{NADH} + \text{H}^+ \xrightarrow{\text{ENZYME}} \text{RESAZURIN} \quad \text{(BLUE 610 nm)}
\]

\[
\text{HO} + \text{NAD}^+ + \text{H}_2\text{O} \xrightarrow{\text{ENZYME}} \text{RESORUFIN} \quad \text{(PINK 580 nm)}
\]

The visible spectra of resazurin (blue) and its reduced product, resorufin (pink), are shown in Figure 1. If bacterial growth is inhibited, no reduction of the resazurin occurs, and such a sample would remain blue. If the test chemical has no