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Laboratory Experiment

Reduction of Vanillin Isobutyrate: An Infrared Spectroscopy Structure Determination Experiment

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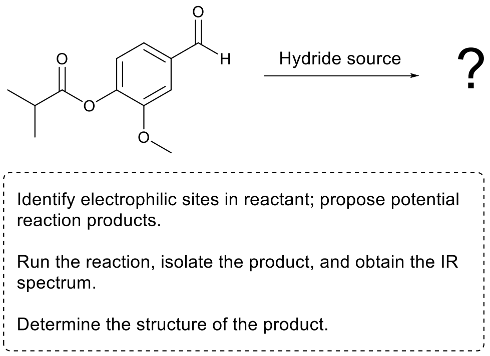
ABSTRACT: In this laboratory experiment, first-semester organic chemistry students use mechanistic reasoning to propose possible outcomes for the reaction of vanillin isobutyrate with a hydride source. The students subsequently run the reaction, isolate the product, and acquire its infrared spectrum. The experiment provides students with the opportunity to hone their skills at proposing mechanisms and interpreting IR spectra. It also serves as an introduction to the chemistry of carbonyl-containing functional groups.



KEYWORDS: *Second-Year Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Inquiry-Based/Discovery Learning, IR Spectroscopy, Mechanisms of Reactions*

Structure determination experiments are a staple of the organic chemistry instructional laboratory. Such experiments lend themselves well to the discovery-based pedagogical approach, and a wide variety of such experiments appear in the chemical education literature. Given the wealth of structural information that can be derived from even simple NMR experiments, most structure determination laboratory exercises make use of NMR spectroscopy, either as the sole spectroscopic technique or in combination with infrared spectroscopy and/or mass spectrometry.[1] Many fewer laboratory experiments rely solely on infrared spectroscopy for structure determination.[2,3]

In our year-long organic chemistry sequence, infrared spectroscopy is taught in the first semester while mass spectrometry and NMR spectroscopy are introduced in the second semester. We usually include an IR-based structure determination experiment in the first semester laboratory. One challenge from the instructor's point of view is the inevitable "leakage" of information from one class of students to the next. Thus, it is valuable to have a sizable set of such experiments to use in rotation.

Recently, we set out to develop a new exercise to add to our menu of structure determination experiments. We envisioned using a multifunctional compound in a chemoselective reaction where, at least in theory, multiple products would be possible. Because sodium borohydride mildly and selectively reduces ketones and aldehydes in the presence of carboxylic acid-derived functional groups, this reagent seemed like a logical choice.[4] Furthermore, the first-semester organic students would not yet be familiar with hydride reductions of carbonyl-containing functional groups. Finally, we could represent sodium borohydride as a generic "hydride source" to make an online search for the "answer" less user-friendly.

Thus, we focused our attention on the borohydride reduction of keto- and formyl-esters. Experiments which examine chemoselective reductions of such compounds by sodium borohydride appear in the chemical education literature.[5] Notably, Mohan describes a discovery-based experiment involving the sodium borohydride-mediated reductions of vanillin acetate and 4-formylbenzoate, both of which were investigated as potential substrates for the experiment described herein. In Mohan's experiment, the structures of the reaction products are determined using ^{1}H and ^{13}C NMR spectroscopy along with traditional functional group chemical tests.[6] In contrast, we designed an experiment that asks students to

- Use mechanistic reasoning to propose possible products from a reaction involving unfamiliar reagents.

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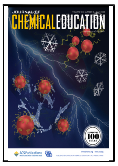

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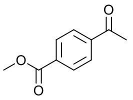
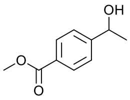
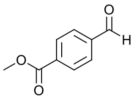
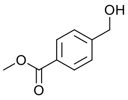
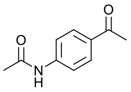
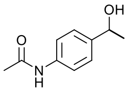
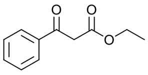
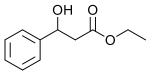
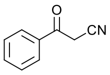
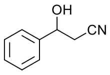
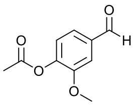
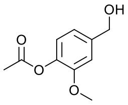
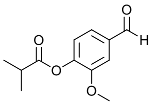
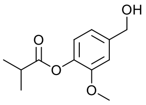
Table 1. Substrate Candidates[a]

Entry	Substrate	Substrate $\bar{\nu}_{CO}$ (cm^{-1})	Product	Product $\bar{\nu}_{CO}$ (cm^{-1})	Yield (%)
1	 **1a**	1719, 1676	 **1b**	1718	92
2	 **2a**	1720, 1681	 **2b**	1719	92
3	 **3a**	1671, 1588	 **3b**	1663	28
4[b]	 **4a**	1736, 1684	 **4b**	1716	78
5	 **5a**	2254[c], 1686	 **5b**	2254[c]	82
6	 **6a**	1747, 1677	 **6b**	1759	77[d]
7	 **7a**	1760, 1698	 **7b**	1756	86

[a] Reaction conditions: $NaBH_4$ (1.2 equiv), methanol solvent, 30 min, quenched with saturated NH_4Cl (aq). [b] Reaction run in ethanol solvent. [c] C≡N stretching vibration. [d] Obtained as a 4.5:1 mixture with **8**.

- Use IR spectroscopy to identify the functional groups present in the unknown reaction product and eliminate all but one of the candidate structures.

SUBSTRATE CHOICE

The optimal substrate for this experiment contains a ketone or aldehyde along with a carboxylic acid derivative, such as an ester, amide, or nitrile. The reaction with sodium borohydride should go to completion in less than an hour, to allow for prelab discussion, reaction setup, and workup to be completed within a single lab period, and cleanly yield a single product. Finally, interpretation of the IR spectra of the reactant and the product should provide an appropriate level of challenge, such as requiring students to differentiate between carbonyl-containing functional groups and to consider the effect of conjugation on the frequency of the carbonyl stretching vibration.

Seven commercially available aldehydes and ketones, **1a**–**7a**, were evaluated (Table 1). The methyl benzoate-derived ketone, **1a**, and aldehyde, **2a**, cleanly produce the corresponding alcohols in high yield (Entries 1 and 2). However, in addition to the ester C═O stretching vibration at 1718 cm^{-1}, **1b** exhibits a signal at 1702 cm^{-1} as a result of Fermi resonance.[7] Students new to IR spectroscopy would likely misinterpret this absorbance as a second carbonyl signal; thus, ketone **1a** was ruled out. Although alcohol **2b** exhibits a single band in the carbonyl range, it was thought that compounds where just one of the C═O stretching vibrations were shifted due to conjugation (Entries 3–7) might better demonstrate the effect of resonance on IR frequencies. While ketoamide **3a** is cleanly converted to a single product, as judged by TLC, significant amounts of the polar hydroxy amide, **3b**, are presumably lost to the aqueous layer during the standard extractive workup, thus the low yield. Ketone **4a** was ruled out as the C═O stretching absorption of the *β*-hydroxy ester product, **4b**, is shifted well below the typical frequency for an unconjugated ester as a result of the strong intramolecular hydrogen bond. Ketone **5a** is a suitable substrate for the experiment, cleanly producing hydroxy nitrile, **5b**, in good yield. However, the distinctive location of the nitrile CN stretch makes the determination of the product structure straightforward; we were hoping to find a substrate that would present a more challenging IR interpretation problem for the students.

Finally, we examined vanillin acetate, **6a**, and vanillin isobutyrate, **7a**. While both reacted smoothly with sodium borohydride, the acetate, **6a**, also yielded **8** (Figure 1), due to concurrent reduction and transesterification of the acetate with the methanol solvent. The analogous transesterification was not observed for the more hindered isobutyrate ester. Not only does the isobutyrate ester provide for a cleaner reaction, but also it is less expensive than the acetate. Thus, we settled on vanillin isobutyrate, **7a**, as the substrate for the student version of the experiment.

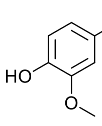

8

Figure 1. Diol product obtained as a result of the transesterification reaction in reduction of vanillin acetate.

■ EXPERIMENTAL OVERVIEW

The students completed the experiment over two laboratory periods. The first week of the experiment, including the classroom discussion described below, required three hours. The second week of the experiment required less than an hour for each student to complete and was paired with other lab activities. Students completed online prelab assignments ahead of each laboratory meeting and submitted a postlab writeup.

Experimental Procedure

A 25 mL round-bottom flask was charged with vanillin isobutyrate (0.250 g, 1.12 mmol), methanol (5.00 mL), and a stir bar. The stirred solution was cooled in an ice–water bath, and sodium borohydride (0.050 g, 1.32 mmol) was added in three portions over a period of 5 min. After 20 min, an aliquot (1 to 2 drops) of the reaction mixture was added to a test tube, quenched with saturated, aqueous ammonium chloride (1 mL), and extracted with diethyl ether (1 mL). The ether solution was spotted on a TLC plate along with a sample of vanillin isobutyrate in diethyl ether. The TLC plate was developed with 70:30 hexane/ethyl acetate and visualized using a UV lamp. In the event that vanillin isobutyrate was still present in the reaction mixture, the reaction progress was checked by TLC at 10 min intervals.

When all of the vanillin isobutyrate had been consumed, the reaction, still at 0 °C, was quenched with saturated, aqueous ammonium chloride (5 mL). After the fizzing subsided, the mixture was added to water (30 mL) and extracted with diethyl ether (3 × 10 mL). The combined ether extracts were washed with brine (1 × 10 mL) and dried over sodium sulfate in a sealed container.

At the subsequent laboratory meeting, the solution was gravity filtered into an appropriately sized, preweighed round-bottom flask. The diethyl ether solvent was removed by rotary evaporation, and the last traces of solvent were removed under high vacuum. The mass of the product was determined, and an IR spectrum was obtained.

■ HAZARDS

All compounds should be treated as if they are potentially hazardous. To minimize risk due to exposure, all manipulations should be performed in a fume hood and while wearing eye protection, gloves, and clothing that completely covers the body. Sodium borohydride reacts with water, releasing hydrogen gas, which may spontaneously ignite: keep the reagent bottle tightly capped and away from water sources. Sodium borohydride can cause skin burns and eye damage and should be handled with care. Inhalation of the dust can irritate or damage sensitive mucous membranes. Methanol may damage the kidneys, liver, or eyes. Ammonium chloride and ethyl acetate may cause eye irritation. Methanol, diethyl ether, hexane, ethyl acetate, vanillin isobutyrate, and 4-(hydroxymethyl)-2-methoxyphenyl 2-methylpropanoate are flammable.

■ RESULTS AND DISCUSSION

This experiment has been completed by 64 students over two semesters of Organic Chemistry I Laboratory. The experiment is timed to run concurrently with the unit on IR spectroscopy in the corequisite Organic Chemistry I Lecture. At this point in the semester, the students have completed units on substitution and elimination chemistry and have had ample practice with writing reaction mechanisms and identifying electrophiles and nucleophiles. The students have not yet encountered hydride reagents or nucleophilic addition to carbonyls in their coursework.

Week 1: Identifying Possible Products and Running the Reaction

The students completed an online (Moodle) prelab assignment ahead of each laboratory meeting. As part of the first prelab assignment, students were asked to identify whether each of six indicated atoms in vanillin isobutyrate possessed a partial positive charge. The majority of the students were able to correctly identify electrophilic sites (Figure 2).

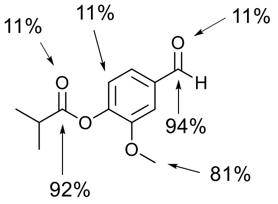


Figure 2. Percentage of students identifying each of the six indicated atoms as electrophilic.

The lab meeting began with a review of the results of the prelab assignment. The students were then asked to predict the structure of the product that would result from the attack of a hydride ion at each of the three electrophilic sites. As a prompt, the instructor presented three separate structures of vanillin isobutyrate, each with a single arrow showing the attack of hydride at one of the electrophilic carbon atoms. The students worked in small groups to complete each mechanism and arrive at structures for the theoretical products (Figure 3). Alcohols **7b**, **9**, and **10** were ultimately proposed as the result of nucleophilic addition, nucleophilic acyl substitution, and S_N2 mechanisms, respectively. When writing the mechanism for the attack of hydride at the ester functional group, the students initially proposed the hemiacetal intermediate as the

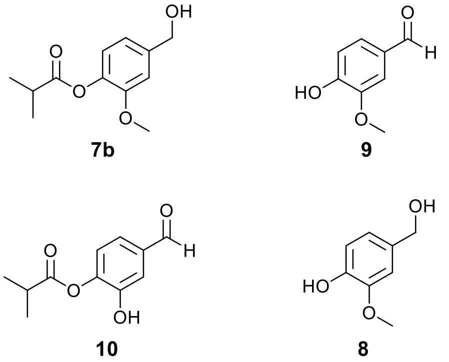


Figure 3. Reaction products proposed by students.

final product. With some gentle coaching from the instructor, they eventually arrived at the structure for alcohol **9**. In some laboratory sections, students also suggested that reduction could occur at both the ester and aldehyde to yield diol **8**.

The remainder of the laboratory period was dedicated to experimental work. Students were cautioned to handle the hydride reagent carefully and to add it in small portions to the reaction mixture over a period of 5 min. The vanillin isobutyrate was completely consumed and cleanly converted to a single product, as judged by TLC, after 20−30 min of reaction time. Quenching of the reaction and subsequent extraction proceeded smoothly. The ether extracts were stored over sodium sulfate, at room temperature, until the next laboratory period.

Week 2: Product Isolation

The prelab assignment for the second laboratory meeting asked students to predict at which site the hydride was most likely to add and to provide a rationale for the choice. The class was nearly evenly split with 40% predicting that hydride would attack at the ester, 30% choosing the ether, and 30% choosing the aldehyde. Those who predicted that the reaction would occur at the ester argued that the largest build-up of positive charge would be at the ester carbon, as a result of induction by the two attached oxygen atoms. While students noted that the ether carbon atom was not as electrophilic as the ester or aldehyde, those who chose it as the most likely site of attack did so based on a steric argument. Those who predicted reaction at the aldehyde generally cited a combination of two effects: a significant partial positive charge on the carbonyl carbon and a less sterically hindered environment than at the ester.

Students were also tasked with assigning four stretching vibrations from the IR spectrum of vanillin isobutyrate: the aldehyde C−H at 2839 and 2735, the ester C═O at 1760, the aldehyde C═O at 1698, and the benzene C═C at 1599 cm^{-1} (Figure 4). Approximately 90% of the students assigned the aldehyde C−H and benzene C═C stretches correctly. Most (82%) assigned the ester C═O stretching vibration correctly. Those who did not had attributed the signal to the aldehyde C═O. The signal at 1698 cm^{-1}, not surprisingly, had the lowest rate of correct assignment (70%). Of those who missed this assignment, half attributed it to the C═O stretch of the ester. The remainder of the attributions (15%) were distributed among the other choices.

In lab, the students completed the isolation of the product and obtained the IR spectrum. Yields of the product ranged from 16% to 89%, with an average of 57%. Lower yields were attributed to spills or insufficient rinsing of glassware during transfers or of the sodium sulfate during the gravity filtration. Even so, all of the students had sufficient product for acquisition of an IR spectrum (Figure 5). The IR spectra of the products were remarkably consistent from student to student, with no apparent spurious signals.

Post Lab: Structure Identification and Reasoning

The postlab assignment asked students to identify the product, cite evidence from the IR spectrum to support their conclusion, assign the spectrum, and answer questions related to structure determination. Of the 64 students who completed the experiment, 58 (91%) arrived at the correct structure for the product, **7b**. Of the six who did not arrive at the correct structure, five indicated that reduction had occurred at the ester functional group to yield **9** and one indicated that the diol

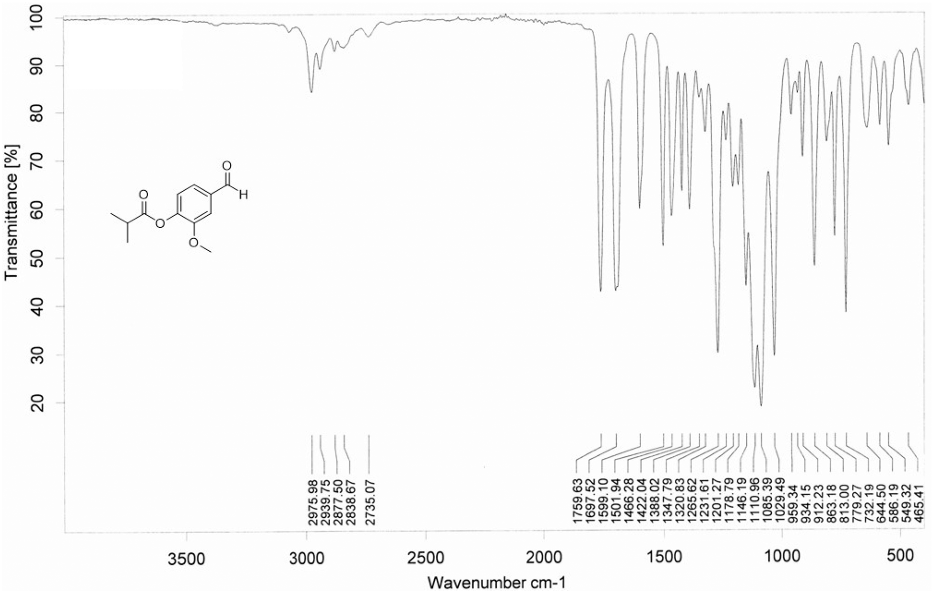


Figure 4. IR spectrum of the vanillin isobutyrate, **7a**.

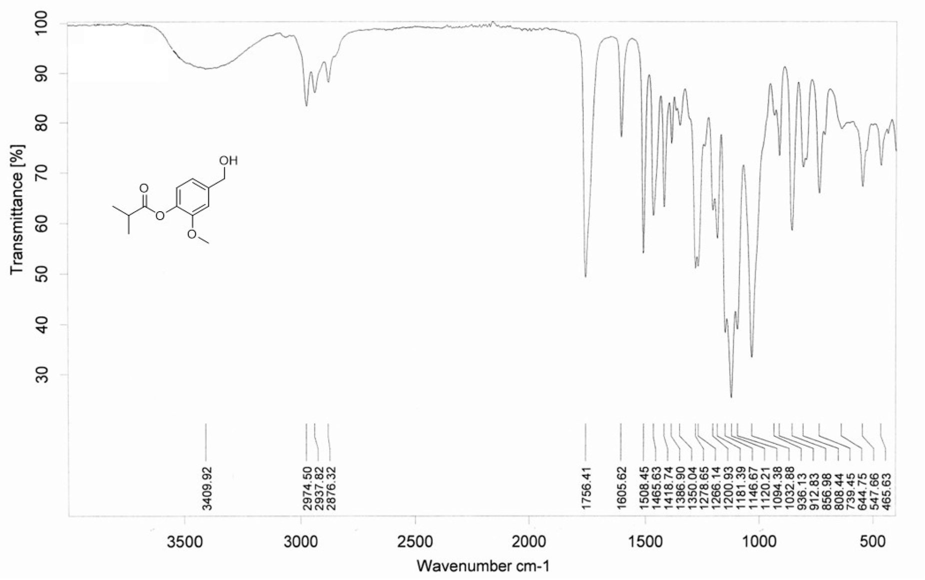


Figure 5. IR spectrum of the vanillin isobutyrate reduction product, **7b**.

8 was the product of the reduction. The most commonly cited evidence in support of the **7b** as the structure included the presence of the ester carbonyl stretch at 1756 cm^{-1}, the alcohol OH stretch at 3409 cm^{-1}, and the absence of the aldehyde signals at 2839, 2735, and 1698 cm^{-1}.

Questions inspired by the student responses on the prelab assignments were included with the intent of correcting any misconceptions. For example, nearly a third of the students had mis-assigned the aldehyde C═O stretching vibration in the IR spectrum of vanillin isobutyrate, presumably because it appears at 1697 cm^{-1}, well below the typical range for an *unconjugated* aldehyde. We therefore asked the students to draw resonance structures to illustrate how the bond order of one of the carbonyls is reduced as a result of conjugation with the benzene ring. The vast majority of students correctly attributed this signal to the aldehyde, and most were able to draw correct or nearly correct resonance structures.

On the week 2 prelab assignment, the students routinely cited inductive and/or steric effects to support their predictions regarding the likely site of hydride addition to vanillin isobutyrate. Little attention was paid to the impact of resonance on the relative nucleophilicity of the two carbonyl functional groups. Given that the students had not yet studied the chemistry of carbonyl-containing functional groups, this was not entirely surprising. However, we opted to include a question in the postlab report that was intended to encourage the students to consider the effect of resonance. The students were asked to suggest a reason a particular hydride reagent, sodium borohydride, reacts with ketones but not esters.[8] Most (77%) identified resonance donation of electron density from the sigma-bound ester oxygen to the carbonyl as the reason for the reduced electrophilicity of the ester carbonyl carbon atom.

CONCLUSION

In this experiment, students used mechanistic reasoning to predict several compounds that could result from the reaction of a highly functionalized compound, vanillin isobutyrate, with a hydride source. The students successfully ran the reaction, characterized the product by its IR spectrum, and identified the product. Through this experiment, students honed their IR spectroscopy interpretation skills and were introduced to the chemistry of carbonyl-containing functional groups.

ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.3c00009.

Notes for instructors, student handout and report form, and copies of spectra (PDF; DOCX)

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Notes

The authors declare no competing financial interest.

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