Identification of an Unknown Compound by Combined Use of IR, ^{1}H NMR, ^{13}C NMR, and Mass Spectrometry: A Real-Life Experience in Structure Determination

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Mass spectrometry, IR, ^{1}H NMR, and ^{13}C NMR are four of the most commonly studied spectroscopic techniques in the introductory organic chemistry curriculum *(1)*. These techniques are powerful tools often used for qualitative analysis of unknown compounds and thus the instrumentation is widely available in organic laboratory facilities. Students enrolled in the first year of organic chemistry should be provided with an opportunity to have hands-on experience operating the instrumentation and to have a real-life experience in determining the structure of an unknown compound using all four spectroscopic techniques simultaneously. Such an opportunity will help to reinforce the spectrometric concepts covered in the lecture and to learn the usefulness and limitations of each technique. For these reasons, we present a laboratory experiment in which students have to determine the structure of an unknown organic compound. In order to do so, they have to learn how to operate a mass spectrometer, an IR spectrometer, and a NMR spectrometer to obtain the spectra and then they must analyze the spectra to determine the compound's structure. The students can either work in teams or alone depending on the number of instruments available and the time constraints.

This laboratory exercise is unique to other published work in that it combines GC–MS with IR and NMR to elucidate the structure of unknown substance as well as providing the students with hands-on experience operating these three instruments *(2)*. Unlike other experiments utilizing these spectroscopic tools, this one requires that the students perform a structure determination of a complete unknown instead of simply characterizing the structure of an expected product after an organic synthesis experiment *(3)*. Furthermore, the students are not provided with a list of possible unknowns to narrow their search or allowed to search a database to find a match to their spectra *(4)*.

The compounds chosen as unknowns for this experiment have structures that can be elucidated only if the four different spectroscopic techniques are utilized. For each unknown, the ambiguities in its mass spectra are minimal since the molecular ion peak is present and the isotope effect is illustrated because either chlorine or bromine is present. Furthermore, the analysis of ^{1}H NMR for the chosen unknowns is relatively simple, mostly first-order splitting is observed with no signals overlapping, so that first-year organic students should be able to interpret the spectrum.

This laboratory exercise is used to introduce the students to organic spectroscopic techniques. Later experiments reinforce the skills learned here but the emphasis is on verifying the presence of a desired product after organic synthesis rather than on identifying a complete unknown. Students overwhelmingly feel that this experiment is the most effective learning experience of all the experiments done during first-semester organic chemistry. When completing the laboratory course evaluations, in response to the open-ended question, "Which experiment was most effective in enhancing your understanding of the course material?", 67% of the students over the past two years selected this experiment. The next most selected experiment was chosen by only 7.5% of the students.

Procedure

Preparation and Prelab Lecture

The first week all of the students attend a one-hour prelab lecture that covers the basics of preparing samples for and running the different instruments. During the remaining three hours of the lab period, the students have 30 minutes available on each instrument to collect their data. To ensure an orderly and efficient use of instrument time, the students are required to sign up for their 30-minute time slots a week in advance. For a 4-hour lab period, up to 6 student teams can obtain the required spectra during the first week and up to 8 more teams can obtain the required spectra on the second week since there is no need for prelab lecture. If this is not sufficient to accommodate all the teams, other time slots are made available outside the scheduled lab period.[1] The theory behind and interpretation of the spectroscopic techniques is reserved for the lecture component of the course and to one-on-one instruction after students have systematically worked through the interpretation described in the laboratory manual.

Each student team is given either 0.2 mL (if liquid) or 0.2 g (if solid) of an organic unknown sample in a small test tube labeled with a three-digit number. The last two numbers of the three-digit number identifies the unknown but only the instructor has access to the code. Adding a random third digit to the front allows the same unknown to be assigned to more than one team without the students being aware of the duplicity. There are 17 different organic compounds currently used for this exercise (List 1) and others can be added based on availability and budget.

List 1. Organic Unknowns

4′-Bromoacetophenone	4-Bromo-α-methylbenzyl alcohol
(2-Bromoethyl)benzene	1-(4-Chlorophenyl)ethanol
4-Bromoethylbenzene	2,4′-Dibromoacetophenone
4-Chlorophenethyl alcohol	(1,2-dibromoethyl)benzene
2-Bromophenethyl alcohol	2,4′-Dichloroacetophenone
4-Bromophenethyl alcohol	2,4-Dichloro-α-methylbenzyl alcohol*
4′-Chloroacetophenone	2,4-Dichlorophenethyl alcohol*
(2-Chloroethyl)benzene	2-Chlorophenethyl alcohol
α-Chloromethyl-2,4-dichlorobenzyl alcohol*	

*Specific benzylic substitution pattern will be ambiguous.

Data Collection

The operation of each instrument is overseen by either the laboratory professor or an upper-class student working as a teaching assistant.[2] As each team of students runs their sample on a particular instrument, the person overseeing that instrument points out the main parts of the instrument and their function. The students operate the instruments and obtain a spectrum while the overseer stands by to answer questions and to provide useful theory and instrument operation information about the different techniques when relevant.

Hazards

All chemicals used in this experiment should be considered toxic. All the unknowns are flammable and can cause eye, skin, and respiratory track irritation. Protective eyewear and gloves must be worn at all times, and the chemicals should be handled under a hood to avoid skin contact and inhalation. Chloroform is a possible carcinogen and an irritant. Dichloromethane is also an irritant. A syringe is needed to inject the unknown sample into the GC–MS so the lab instructor should closely monitor this step.

Results

Students are expected to record the signals obtained from each spectrum and tabulate their results. They are instructed to simultaneously consider all the pieces of data to elucidate the structure of the unknown. This task at first seems overwhelming but the laboratory manual provides the students with a sequential approach to performing this assignment, which makes the task very doable. As a result of tackling and completing what at first seems to be an overwhelming task students' confidence in their abilities is greatly increased.

Each student writes a lab report that includes copies of all the spectra, a completed report worksheet with tables that list the peaks and their assignments, and a step-by-step detailed explanation of the reasoning used to determine the final structure of the unknown. If there are any ambiguities in the structure, these are also included in the lab report. For example, the specific benzylic substitution pattern of the chlorines in the three alcohols, marked with an asterisk in List 1, will be challenging for first-year organic students. For these unknowns, the students should list all the possible substitution patterns. The report reflects the student's understanding of the material covered in lecture and reinforces the advantages and limitations of each type of analysis.

Conclusions

This experiment provides a comprehensive review of the concepts covered in spectroscopy and the students get hands-on experience at collecting and analyzing spectral data in a way that is similar to how they would in a research laboratory situation. Most students (>90%) identify their unknown compounds correctly; however, many need work in developing their ability to explain their reasoning in writing. In a larger institution where it would not be practical to allow large number of students access to the instrumentation, all or parts of the experiment could be adapted to be an exercise in the analysis of spectra to elucidate the structure of an unknown compound.

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Notes

1. We actually run this experiment over multiple weeks. Simultaneously we run traditional UV–vis and gas chromatography experiments so that students are busy during the weeks they are not running this experiment.
2. At Stonehill College the proximity of the instruments allows for one lab instructor to oversee both the operation of the NMR and the GC–MS, while a TA supervises the IR.

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