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The Separation and Identification of Two Unknown Solid Organic Compounds

An Experiment for the Sophomore Organic Chemistry Laboratory

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The separation and identification of organic compounds are frequent tasks of researchers in organic chemistry. Fueled by a desire to reflect modern organic laboratory separation practices, we developed a new experiment for our sophomore-level organic chemistry students. Entitled "Separation and Identification of Two Unknown Compounds", this experiment employs microscale flash chromatography to separate a mixture of two singly-functionalized solid organic compounds, and employs spectroscopy and melting point determination to identify them by matching observed data with that of known compounds. This is a technique and puzzle-solving experiment and is more challenging and more interesting than cookbook-style experiments. The experiment reinforces good laboratory technique: the student who is sloppy and does not pay attention to details is not able to isolate compounds pure enough for spectroscopic and melting point identification.

The separation scheme outlined in this experiment emphasizes chromatographic procedures. Modern organic research laboratories utilize a chromatography step in almost every purification scheme, either high-performance liquid chromatography (HPLC), flash chromatography, or microscale flash chromatography. Of these, microscale flash chromatography is well-suited to teaching labs because it does not require expensive, specialized glassware nor does it require compressed air or nitrogen. Although our teaching curriculum has included for several years an experiment that incorporates a microscale flash chromatography step, we find that students do not become competent in this technique by doing it only once, nor do they understand the rationale behind elution-solvent choice when given an explicit chromatographic procedure. In the current experiment, students are not told which solvent system to use to effect the separation of the two compounds. Instead, each student must run a series of thin-layer chromatography (TLC) plates in different polarity solvents to determine the best system, just as they would in a research laboratory. The benefits of this practice are twofold: students hone their TLC technique, and they see firsthand the relationship between solvent polarity, compound polarity, and R_f value.

The students identify their separated unknowns by comparing the melting points and spectra with the values for known compounds; we limit the students' searches by giving them a list of possible unknowns. Institutions that do not have an FT-IR instrument can have students use melting point data alone to match their unknowns with the possible compounds. Although melting points are not commonly used to determine structure in modern research labs, they are still a good indication of purity and can be helpful in compound identification. Furthermore, most organic chemistry teaching laboratories have and use melting point devices because they are relatively inexpensive. If FT-IR instruments are available, students should run an IR of each purified unknown as a thin-solid film *(1),* a method of preparing solid samples for IR that yields good spectra in a short time. The observed spectra are compared to printed spectra of possible compounds or matched in a computer database.

NMR spectra of the unknowns aid students in their assignment of structures. Ideally, each student would run the ^{1}H NMR spectrum of each unknown. At our university, students in our nonmajors courses do not have routine access to the departmental NMR instruments. Therefore, we give these students an NMR spectrum of each of their unknowns as if they had run it themselves. In our course for chemistry majors, each student submits a sample of each unknown for ^{1}H NMR analysis. In either case, the students assign the ^{1}H NMR spectrum for each unknown as part of the write-up for the lab. All of the unknowns that we currently use have relatively complex aromatic ^{1}H NMR patterns; we do not hold students responsible for assigning each aromatic shift. Since students are given a list of possible unknowns, this part of the exercise is a "matching" effort rather than a true NMR interpretation.

Experimental Overview

We schedule two three-hour laboratory periods for this experiment. Each student is given a vial and told that it contains both a solid alcohol and a solid ketone. During the first laboratory period, the students run the mixture on a series of thin layer chromatography plates in solvent systems of different polarity (we use mixtures of hexanes–ethyl acetate in various concentrations). The students determine the solvent system that gives good separation of the two components and that moves the faster-moving compound to an R_f of about .35, the value given as ideal for flash chromatography *(2).*

Students run the microscale flash chromatography column during the second laboratory period according to the method in *Organic Laboratory Techniques (3).* Although several useful methods for flash chromatography have been pre-

sented in this *Journal* in recent years *(4–6),* we prefer this method because it does not require equipment other than a Pasteur pipet and a bulb. Note that this method is faster than microscale gravity chromatography. A cotton-plugged Pasteur pipet is filled with silica gel (230–400 mesh) and the column is pre-eluted with hexanes, using a pipet bulb to force solvent through the column. A small quantity of silica is preloaded with the sample and the dry silica–unknown mixture is placed on top of the column. The column is eluted with the solvent system determined during the previous lab session; fractions are collected and analyzed by TLC. When the first compound is off the column, a more polar solvent is applied to the column until the second compound has eluted.

Fractions that contain the same pure unknown compound, as assessed by TLC, are combined and the solvent is removed. Students determine the melting point and run a thin-solid film IR spectrum (and, if possible, the ^{1}H NMR spectrum) of each compound. Students consider the melting point, IR, and NMR data and identify each unknown. They then write an argumentative paper supporting their identifications.

Hazards

Hexanes, ethyl acetate, and acetone are used in this experiment. Hexanes and ethyl acetate are flammable and considered mild health hazards. Students should wear gloves, eye protection, and appropriate clothing while handling these solvents. The unknowns chosen for this experiment are neither carcinogenic nor toxic; however, students must be aware that they may be moderate health hazards and that they should be handled using appropriate personal protection. If possible, students should handle all of these chemicals in a fume hood.

Discussion

About 95% of our students successfully identify both of their unknown compounds. Our students consider this a long and difficult experiment, but they are pleased when they finally run the IR of a purified compound and find an exact match with a compound in the database. We like the experiment because it is inexpensive, microscale, and uses relatively nonhazardous chemicals. In addition, the experiment gives practice in chromatographic and spectroscopic techniques and concepts, shows us which students have good lab technique and are able to work on their own, and reinforces critical thinking.

[W]Supplemental Material

A list of suitable unknown compounds, preparation and procedure notes for the instructor, and a student handout are available in this issue of *JCE Online*. Photographs of the microscale flash chromatography procedure are online at *http://orgchem.colorado.edu/experiments/idunk/idunklab.html* (accessed Sep 2003).

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