Experiment 15

Identification of Unknowns: Isolation of an Alcohol and a Ketone

Reading: Handbook for Organic Chemistry Lab, sections on TLC (Chapter 12) and Column Chromatography (Chapter 13).

Unknown compounds are frequently encountered by the organic chemist. Often they are reaction byproducts, but may also be unidentified substances isolated from natural sources. Years ago, the structural determination of organic molecules rested solely on chemical reactions of unknown substances (i.e., chemical transformations of unknown compounds to known derivatives through unambiguous chemistry), and on physical properties such as melting points, boiling points and solubility. The advent of spectroscopic techniques (UV, IR, NMR, mass spectroscopy, and X-ray diffractometry) has since revolutionized the manner in which structural determinations are performed. Today, a combination of chemical methods, measurement of physical properties, and spectroscopic techniques are often utilized for rapid and complete structural determination.

Your goal for this lab is to identify two unknown organic compounds—an alcohol and a ketone. You will be given the compounds as a mixture; therefore, you must separate them before you can identify them. Take a moment to recall the organic chemistry separation techniques you have learned:

- Distillation
- Chemically active extraction
- TLC
- Column chromatography

Both of the compounds you will be given are solids at room temperature, so you will not be able to separate them by distillation. One is an alcohol and the other a ketone; neither of these functional groups has ionic character in acid or in base, so they cannot be separated by chemically active extraction either. The third technique, TLC, is typically used only as an analytical method, not for the separation of large amounts of compounds (although it is still a separation technique). The data you can collect from TLC analysis, however, can be used in conjunction with the last technique, column chromatography. If you recall the Microscale Column Chromatography experiment, you were able to separate the various ferrocene derivatives based on their varying polarities. In this case you can do the same thing, as alcohols and ketones vary significantly in polarity. For this experiment, column chromatography will be the method of choice.

The column chromatography method which you will use is referred to as flash column chromatography. In order to separate the two compounds by this method, you must first use TLC to determine a solvent system that will separate them. Do this by performing TLC analysis of your unknown mixture in a series of solvent systems which vary in the ratios of two solvents: hexanes and ethyl acetate. The ideal solvent system for column chromatography is the one that moves the faster-moving component to a relatively low R_f of 0.25–0.35 on a silica gel TLC plate and also gives a good separation between the faster-moving compound and the slower-moving compound. During this experiment you will run TLC using four pre-mixed solvent systems. Once you have determined the ideal system, you will use this system to elute the flash chromatography column and separating the two components of your unknown mixture.

After the two compounds have been separated and dry solids obtained, you will identify them by three physical properties: melting point, IR spectrum, and ^{1}H NMR spectrum. Use what you know about NMR and IR to make sense of your obtained spectra, and compare the melting points with those of the possible unknown compounds (Figure 15-1).

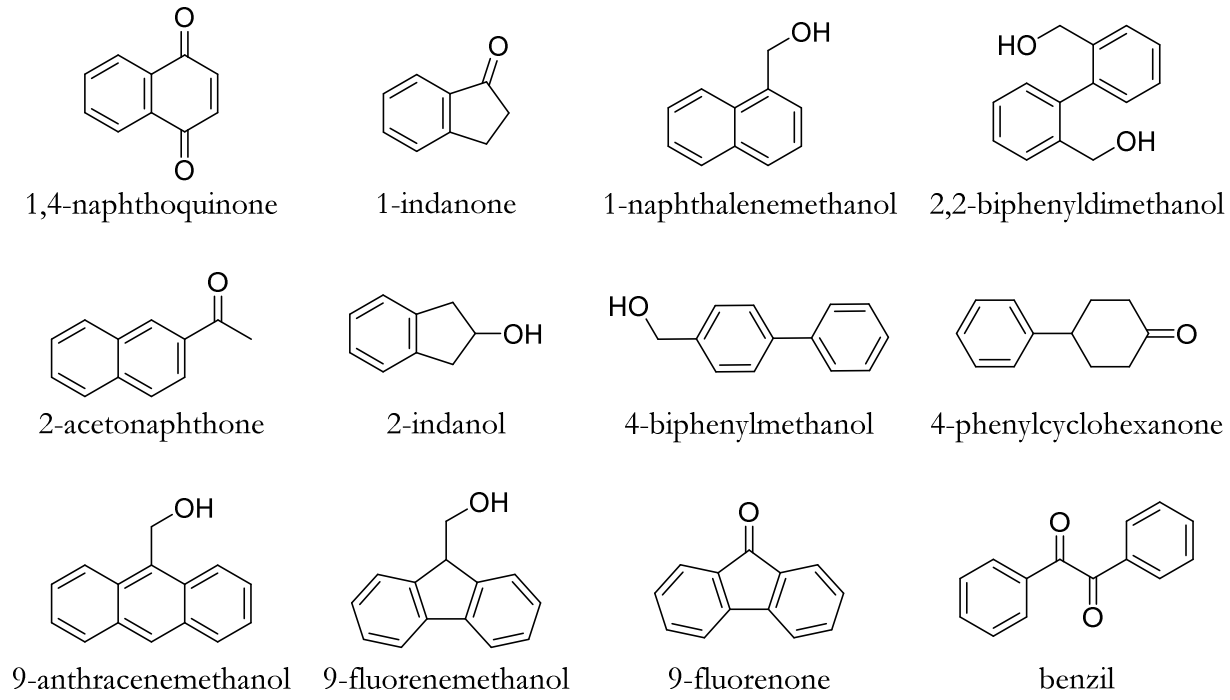


Figure 15-1: The possible unknowns for this lab.

For this lab you are given two lab periods for the TLC analysis and column chromatography. The lab report is due at the third lab meeting after the last ID of Unknowns session. This allows adequate time for you to finish characterizing the compounds. Proper technique is crucial to making this experiment work well, so make sure to reread the Handbook chapters on TLC and Column Chromatography.

Safety Precautions

Hexanes and ethyl acetate are highly flammable.

You will not know the identity or the hazards of the unknown compounds until the end of the experiment; please be assured that we will not give any student a compound that is carcinogenic or highly toxic. Nonetheless, some of them might pose moderate health risks and like any substance, known or unknown, should be handled with great care. Always wear gloves, goggles and appropriate clothing.

Procedure

Your TA will give you a vial containing a unique unknown mixture. The mixture contains two solid compounds: one alcohol and one ketone. This vial is only for you. You may not use the same vial as any other student. **Keep this vial until the end of the semester**; you never know if you will need the extra material it contains. Note that each lab section is assigned a certain range of vial numbers, so if you are making up this experiment in a lab section other than your normal one, you should speak to the lab director to make sure you are given a vial from the correct group.

Two lab periods are scheduled for this experiment. During the first lab period, use thin layer chromatography (TLC) to determine which solvent system you should use for the separation and to prepare your slurry of the unknown mixture and silica gel. During the second lab period, you will separate the components of your mixture by column chromatography.

Thin Layer Chromatography

Follow the procedure in this section to determine a solvent system that satisfies the stipulations outlined in the introduction. The different solvent systems available to you are summarized in the table below. You should make up 20 mL of a solvent system at a time in a stoppered Erlenmeyer flask.

System #	Solvents	%	Polarity
1	Hexanes-ethyl acetate	95:5	Least polar
2	Hexanes-ethyl acetate	90:10	…
3	Hexanes-ethyl acetate	85:15	…
4	Hexanes-ethyl acetate	80:20	Most polar

Dissolve a small spatula tip of your unknown mixture in about 1 mL of acetone (about 2/3 of a Pasteur pipet). Save this solution in a tightly stoppered vial and label it "unknown standard". This will be the solution you spot to determine the TLC solvent system and you will also use it as a standard when you run TLCs of the column fractions in the second part of this laboratory experiment.

Next, you should prepare a solvent mixture. If you team up with three other nearby students, then each of you can choose one of the solvent mixtures and make up 20 mL of it in a stoppered Erlenmeyer flask. Then you can take turns using each other's developing chambers. This way, you can avoid having to make up all four solvent systems yourself.

Using your "unknown standard", spot four small TLC plates and run each plate in a different solvent system. Observe the developed plates under a UV lamp and mark the spots lightly with a pencil. Once you have developed all four TLCs and calculated the R_f values for each, you can make an educated decision about which solvent system to use during the column chromatography step. Remember, the system which gives an R_f of 0.25–0.35 for the faster-moving spot, with a difference of at least 0.1 between the two spots, will be optimal. This relatively slow pace of travel will give the mixture adequate time to separate before each compound elutes from the column.

At this point you should show your TA the developed TLC plates and explain to them how you have arrived at your decision.

If you see only a single spot on your TLC, one of your compounds might not be very visible under UV. In this case, you will need to stain your plate, either with PAA or iodine. Alternatively, the contents of your vial might not have been mixed thoroughly. Look in your vial - if you see any clumps, break them up with your spatula and stir them in, then try running another TLC. Another possibility is just that your sample is not spotted heavily enough – try putting two to three times as much sample on a new plate and developing it again.

If you see more than two spots on your unknown-mixture TLCs, consult your TA to determine which spots correspond to your assigned unknowns. There is always a chance that your sample may have a trace impurity, or that the vial you used to make your "unknown standard" was contaminated with something else before you used it. The darkest two spots will always be your two unknown compounds, however. Keep a careful record of your observations so that you know which spot is the desired spot during the second part of this experiment.

In preparation for the column chromatography step (next lab period), dissolve 70-80 mg (no more!) of your unknown mixture in as few drops of DCM as possible. Mix this with 70-80 mg of silica and stir them

together to make a slurry. Store the mixture in an open or loosely-covered container in your lab drawer so it will be dry by the next lab period.

During the following lab period, you will use the procedure shown in the Handbook for microscale column chromatography. Since this procedure is usually time-intensive, you can speed things up by following the first few steps to dry-pack your column now. You should add cotton and silica to the column, but do not add any solvent to it yet – this would result in it drying out and cracking, making it unusable. When storing the column in your drawer, prop it up at one end to make sure that the silica will not fall out.

You can also speed things up by 20-30 minutes if you make sure all the glassware you will need next week (your round-bottom flasks, all your vials, and enough beakers to run TLCs) are clean and ready to go now. Label your vials with numbers 1-20, for the fractions you will collect next week.

Column Chromatography

At the start of the second lab period, pre-elute your dry-packed column with hexanes, then add your pre-loaded silica mixture. It should be completely dry.

Number the vials from your lab drawer, if you didn't already do this. If you need more vials during the lab, you will have to reuse your vials by either discarding the contents (if they contain no compound or both compounds) or by combining them into a round-bottom flask (if they contain a single pure compound). Make sure to rinse the vials out with acetone before using them, so they are clean and will not contaminate your pure compounds.

Fill the pipet with the solvent system you determined in the first part of this experiment, then elute the column and collect the eluent in a vial. Repeat this process, collecting approximately 1mL of solvent in each fraction before changing to the next vial. (You may wish to measure out 1 mL of solvent into a vial, to use as a reference on how much volume to collect for each fraction.) Continue this process until you have collected 10 individual fractions. Do not let the column run dry at this point, in case you need to collect more fractions later.

Cut two TLC plates, each 4-5 cm wide and 5 cm tall, and mark them with six lanes. Spot one plate with fractions 1-5, and the other with fractions 5-10. Be sure to spot one of the side lanes on each plate with your unknown standard (you can label it as "UNK") for reference. Make sure you rinse the microcap thoroughly after spotting the unknown standard – if traces of this solution remain it will seriously confuse your results. Develop the TLC plates using solvent system #4.

Observe the developed TLC plates under a UV lamp. If you needed to use either iodine or PAA to see both spots in your "unknown standard", you should use this method for all column fraction TLCs that you take also. If the faster-moving compound is still eluting from the column (meaning that you see it in fractions 9 and 10), collect another five fractions and re-evaluate. If the faster-moving compound is off the plate, switch to a more polar solvent system (50:50 hexanes-ethyl acetate) and collect five additional fractions. Analyze all of the newly collected fractions by TLC to determine if the slower moving compound has eluted from the column. Continue in this fashion until you have TLC evidence that you have separated and collected both of your desired compounds. If the slower-moving spot is taking a very long time to elute, you can switch to a very polar solvent (pure ethyl acetate) to force it off the column.

Using your TLC analysis of your fractions, determine which fractions contain pure samples of each unknown. Ask your TA to confirm your choice and then combine your pure fractions in clean round-bottom flasks (one for each compound) and remove the solvent by rotary evaporation. The samples may

be gummy at first, but leaving them in the unstoppered flasks in your drawer until the following lab period should give them time to solidify. Perform these characterizations over the next couple of lab periods:

- Submit a sample of each unknown for NMR. This is the most important characterization method. If you only have enough compound for a thin coating on the inside of your round-bottom flask, you can still take an NMR sample by rinsing your flask with 0.5 mL of $CDCl_3$ (about 20 drops), then transferring this rinse into your NMR tube. If you don't have much sample, the sample peaks in your spectrum might be very short in relation to the solvent peaks. If so, increase the vertical zoom by scrolling up until the peaks are taller. The easiest peaks to characterize are the ones in the aromatic region, because only one solvent ($CHCl_3$) tends to show up here. If you integrate the aromatic peaks and compare their integrals and locations to literature spectra, you should find a match. Not all the compounds are in online spectral databases, so spectra are also posted on the orgchem website (on your lab course page, in the calendar entry corresponding to this experiment).
- Run an IR spectrum of each unknown, if you have enough sample to do so. You can compare the fingerprint region of the spectrum to literature IR spectra, once you have used NMR to determine your compound. This will provide good supporting evidence of your compound's identity.
- Take a melting point of each compound, if you have enough sample to do so. Compare the melting points with values of the possible unknowns in the table at the end of this experiment. This will also help narrow down possibilities, but remember that any remaining impurities (such as traces of ketone mixed with your alcohol, or vice versa) might make this unreliable.
- If you managed to purify one of your compounds but not the other, you may be able to take an NMR spectrum of the mixture, and then subtract out the peaks that you know are from the pure compound – this may allow you to determine the identity of the other compound.
- If you still cannot identify one or both of your compounds, you can obtain a vial of the pure compound from the lab director for a deduction of a few points. Then you can use this vial to run IR, MP, and NMR. Note that if you already know the identity of your compounds from NMR, this will probably not be worthwhile – you will lose more points for taking pure vials than for not having IR/MP data. **If you do this, it must be before the ID of Unknowns paper is due for your lab section.**

Wastes

Organic Waste: Column eluents and TLC solvents.

Solid Chemical Waste: Used TLC plates, pipets, flash columns and melting point capillaries.

Lab Report and Paper

The breakdown of points for this lab is different from the other labs. Your prelab should follow the normal structure for a prelab, including physical data for all possible compounds. Instead of a postlab, you will write a paper detailing your findings. This experiment uses a different grading rubric, which is available on the course website.

General: Type the paper using 10- or 12-point font, 1.5- or 2-spaced. It should be no longer than three pages (not counting spectra); if it is longer, points might be deducted. Spelling and grammar will be taken into account when grading. Failure to get a correct match will result in a loss of points for that unknown.

Introduction: Provide your unknown mixture number in the first paragraph (the number on the vial). The first paragraph should also contain a brief explanation of how the unknowns were isolated. Do not give a step by step run through of the procedure. Include in the introduction the information that helped

you determine the best solvent system to use for your column chromatography (i.e., R_f values and ΔR_f values). Explain (using TLC data) how you selected which column fractions to combine for each unknown.

Body of Paper: In this section of the paper provide a detailed description of how the data that you collected (TLC, MP, IR, and NMR) lead you to identify each compound. Use one paragraph for the alcohol and one paragraph for the ketone.

Conclusion: Briefly summarize your conclusions (supported by your discussion in the body section) and make sure that you have included the name of both your ketone and your alcohol.

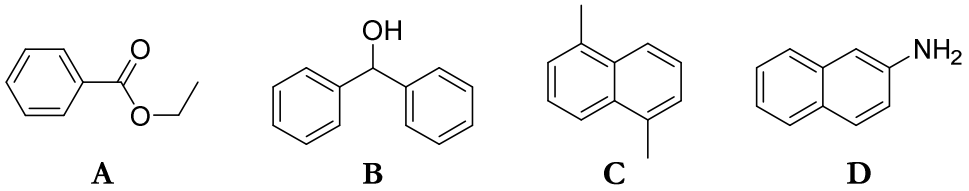
Observations: Turn in your observations with your final report: if you do not, you will lose points. Observations are as in all other labs: recorded in your carbon laboratory notebook as you do the experiment. Include drawings of your TLC plates and calculation of R_f values.

Spectra: Include with the paper all IR and NMR spectra: if you do not, you will lose points. Label all appropriate peaks for full credit and do not talk about peaks in your spectra that you have not labeled.

Study Questions

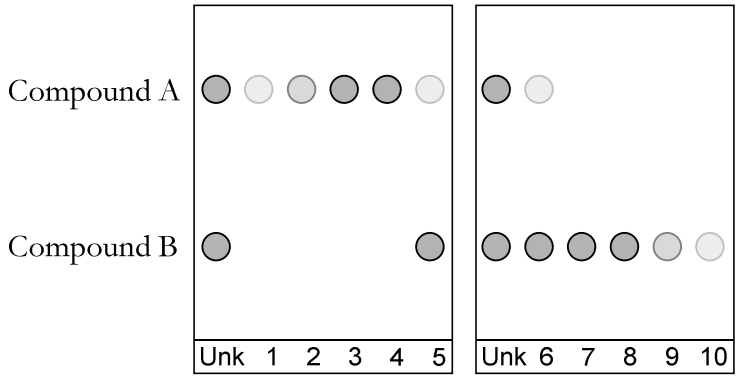
1) List the following solvents from most polar to least polar: hexanes, methanol, dichloromethane, acetic acid, ethyl acetate.

2) Order the following compounds from most polar to least polar. Which would you predict would have the highest R_f value on a TLC plate?



A **B** **C** **D**

A mixture of two compounds is processed by flash chromatography; fractions 1–10 are collected and spotted on TLC plates. Consider the developed TLC plates below:



a. Which fractions would you combine to yield pure compound A?
b. Which fractions would you combine to yield pure compound B?

4) A student combines her fractions properly according to her TLC analysis. However, the melting point of the combined sample is 131-145° C. What should the student do?