Experiment: Identification of Unknowns*

Introduction: Along with synthesis and the examination of the reaction mechanisms, an equally important part of organic chemistry has to do with the characterization and identification of compounds, which may be encountered in sources ranging from a laboratory reaction to exotic tropical plants. In any case, sufficient information must be accumulated to establish the identity of the compound in question with that of a previously described compound of known structure or else to determine from the beginning the structure of the unknown.

Qualitative organic analysis is an important part of organic chemistry. Every chemist must learn the appropriate methods for establishing the identity of a compound. Although it is often possible to establish the structure of a compound on the basis of spectra alone (IR, NMR, MS, etc.), the spectra typically must be supplemented with other information about the compound: physical state and properties (melting point, boiling point, solubility, odor, color, etc.), elemental analysis, and confirmatory tests for functional groups.

In this experiment you will be issued an unknown compound and asked to identify it through chemical and spectroscopic methods. You must first determine the class of compound to which the unknown belongs, that is, identify its main functional group; and then you must determine the specific compound in that class that corresponds to the unknown. The unknowns provided in this experiment are of the purity normally encountered in commercial organic chemicals, which are usually in the range of 95 to 99%. Minor impurities will generally not interfere in the identification procedure. If problems are encountered, recrystallization or distillation of a sample of the unknown may be desirable.

General instructions:

Part One
1. Preliminary classification by physical state, color, and odor
2. Melting-point or Boiling-point determination; other physical data
3. Determination of solubility behavior in water and in acids and bases
4. Simple preliminary tests: beilstein, ignition (combustion)
5. Application of relevant chemical classification tests

Part Two
6. Determination of IR and NMR spectra

Each of the above steps is discussed in detail in Appendix A.

Record all data using excel, word, or notebook paper. All data will be turned in with the laboratory experiment (ALL six parts). A combined report for your lab group will be submitted summarizing your data and the final identity of your unknown compound. Make sure you include the unknown identification on your report along with the compound name for your unknown (i.e. Unknown A is m-nitrotoluene).

*Adapted from Introduction to Organic Laboratory Techniques by Pavia, Lampman, and Kriz 3rd edition and Organic Chemistry Laboratory Standard and Microscale Experiments by Rodig, Bell, and Clark.
Appendix A. READ all instructions thoroughly BEFORE you conduct any experiment.

1. Preliminary Classification – Your group should note the physical characteristics of the unknown. These include its color, its odor, and its physical state (liquid, solid, crystalline form). Many compounds have characteristic colors or odors, or they crystallize with a specific crystal structure. This information can often be found in a handbook and can be checked later. Compounds with a high degree of conjugation are frequently yellow to red. Amines often have a fishlike odor. Esters have a pleasant fruity or floral odor. Acids have a sharp and pungent odor. As a note of caution, many compounds have distinctly unpleasant or nauseating odors. Some have corrosive vapors. Any unknown substance should be sniffed with the greatest caution. As a first step, open the container, hold it away from you, and using your hand, carefully waft the vapors toward your nose. If you get past this stage, a closer inspection will be possible.

2. Melting-Point or Boiling-Point Determination – The single most useful piece of information to have for an unknown compound is its melting point or boiling point. Either piece of data will drastically limit the compounds that are possible.

   Melting-point: to save time, you can often determine two separate melting points. The fist determination can be made rapidly to get an approximate value. Then, after the instrument has cooled off, you can determine the second melting point more carefully.

   Boiling-point: is easily obtained by a simple distillation of the unknown. The simple distillation has the advantage that it also purifies the compound. The smallest distilling flask available should be used if a simple distillation is performed, and you should be sure that the thermometer bulb is fully immersed in the vapor of the distilling liquid. For an accurate boiling-point value, the liquid should be distilled rapidly.

3. Solubility Behavior – Solubility tests should be performed on every unknown. The solubility of an organic compound in water, dilute acid, or dilute base can provide useful information about the presence or absence of certain functional groups. The tests are very simple and require only small amounts of the unknown. In addition, solubility tests reveal whether the compound is a strong base (amine), a weak acid (phenol), a strong acid (carboxylic acid), or a neutral substance (aldehyde, ketone, alcohol, ester). The common solvents used to determine solubility types are: water, 1.5M HCl, concentrated H₂SO₄, 0.6M NaHCO₃, 2.5M NaOH, and organic solvents. A flowchart showing the sequence of solubility tests along with the appropriate conclusions is shown in Figure 1. Further information about each of the solubility tests follows the procedure.

   Procedure. Place about 1mL of the solvent in a small test tube. Add one drop of an unknown liquid from an eyedropper, or a few crystals of an unknown solid from the end of a spatula, directly into the solvent. Gently tap the test tube with your finder to ensure mixing, and then observe whether any mixing lines appear in the solution. The disappearance of the liquid or solid, or the appearance of the mixing lines, indicates that solution is taking place. Add several more drops of the liquid or a few more crystals of the solid, to determine the extent of the compound’s solubility. A common mistake in determining the solubility of a compound is testing with a quantity of the unknown too
large to dissolve in the chosen solvent. **Use small amounts.** It may take several minutes to dissolve solids. Compounds in the form of large crystals will need more time to dissolve than powders or very small crystals. In some cases it is helpful to pulverize a compound with large crystals using a mortar and pestle. Sometimes gentle heating helps, but strong heating is discouraged, as it often leads to reaction. When colored compounds dissolve, the solution often assumes the color.

**Figure 1. Solubility Flowchart**

By the above procedure, the solubility of the unknown should be determined in each of the following solvents: water, 1.5M HCl, concentrated H₂SO₄, 0.6M NaHCO₃, and 2.5M NaOH (See Figure 1). With sulfuric acid, a color change may be observed rather than a solution. A color change should be regarded as a positive solubility test. Solid unknowns that do not dissolve in any of the test solvents may be inorganic substances. To eliminate this possibility, one must determine the solubility of the unknown in several organic solvents, like ether. If the compound is organic, a solvent that will dissolve it can usually be found.

If a compound is found to dissolve in water, the pH of the aqueous solution should be estimated with pH paper or litmus. Compounds soluble in water are usually soluble in all the aqueous solvents. If a compound is only slightly soluble in water, it may be more soluble in another aqueous solvent. For instance, a carboxylic acid may be only slightly...
soluble in water be very soluble in dilute base. It will often not be necessary to determine
the solubility of the unknown in every solvent.

**Solubility in water:** Most organic compounds are not soluble in water, except for low
molecular-weight amines and oxygen-containing compounds. Low molecular-weight
compounds are generally limited to those with fewer than five carbon atoms.

- Carboxylic acids with fewer than five carbon atoms are soluble in water and form
  solutions that give an acidic response (pH < 7) when tested with litmus paper.
- Amines with fewer than five carbons are also soluble in water, and their solutions
give a basic response (pH > 7) when tested with litmus paper.
- Ketones, aldehydes, and alcohols with fewer that five carbon atoms are soluble in
  water and form neutral solutions (pH = 7).

**Solubility in 1.5M HCl:** The possibility of an amine should be considered immediately
if a compound is soluble in dilute acid. Aliphatic amines (RNH₂, R₂NH, R₃N) are basic
compounds that readily dissolve in acid because they form hydrochloride salts that are
soluble in the aqueous medium: R-NH₂ + HCl → R-NH₃⁺ + Cl⁻
The substitution of an aromatic ring, Ar, or an alkyl group, R, reduces the basicity of the
amine somewhat, but the amine will still protonate, and it will still generally be soluble in
dilute acid. The reduction in basicity in an aromatic amine is due to the resonance
delocalization of the unshared electrons on the amino nitrogen of the free base. The
delocalization is lost on protonation, a problem that does not exist for aliphatic amines.
The substitution of two or three aromatic rings on an amine nitrogen reduces the basicity
of the amine even further. Diaryl and triaryl amines do not dissolve in dilute HCl since
they do not protonate easily. Thus, Ar₂NH and Ar₃N are insoluble in dilute acid. Some
amines of very high molecular weight, like triboromaniline (MW 330), may also be
insoluble in dilute acid.

**Solubility in 0.6M NaHCO₃ and 2.5M NaOH:** Compounds that dissolve in sodium
bicarbonate, a weak base, are strong acids. Compounds that dissolve in sodium
hydroxide, a strong base, may be either strong or weak acids. Thus, one can distinguish
weak and strong acids by determining their solubility in both strong (NaOH) and weak
(NaHCO₃) base. The classification of some functional groups as either weak or strong
acids is given in the accompanying table.

<table>
<thead>
<tr>
<th>STRONG ACIDS</th>
<th>WEAK ACIDS</th>
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<tbody>
<tr>
<td>(soluble in both NaOH and NaHCO₃)</td>
<td>(soluble in NaOH but not NaHCO₃)</td>
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<tr>
<td>Sulfonic acids</td>
<td>Phenols</td>
</tr>
<tr>
<td>Carboxylic acids</td>
<td>Nitroalkanes</td>
</tr>
<tr>
<td>Ortho- and para-substituted di- and</td>
<td>Beta-diketones</td>
</tr>
<tr>
<td>trinitrophenols</td>
<td>Beta-diesters</td>
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<td></td>
<td>Imides</td>
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<td></td>
<td>Sulfonamides</td>
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In this experiment, carboxylic acids (pKa~5) are generally indicated when a compound is soluble in both bases, while phenols (pKa~10) are indicated when it is soluble in NaOH only.

Compounds dissolve in base because they form sodium salts that are soluble in the aqueous medium. A carboxylic acid that is insoluble in pure water will be soluble in base due to the formation of the sodium salt of the acid as the base neutralizes the acid. In phenols, substitution of nitro groups in the ortho and para positions of the ring increases the acidity. Nitro groups in these positions provide additional delocalization in the conjugate anion. Phenols that have two or three nitro groups in the ortho and para positions often dissolve in both sodium hydroxide and sodium bicarbonate solutions.

**Solubility in concentrated sulfuric acid:** Many compounds are soluble in cold concentrated sulfuric acid. Of the compounds included in this experiment, alcohols, ketones, aldehydes, and esters are in this category. Other compounds that also dissolve include alkenes, alkynes, ethers, nitroaromatics, and amides. Since several different kinds of compounds are soluble in sulfuric acid, further chemical tests and spectroscopy will be needed to differentiate among them.

Compounds that are soluble in concentrated sulfuric acid but not in dilute acid are extremely weak bases. Almost any compound containing a nitrogen, an oxygen, or a sulfur atom can be protonated in concentrated sulfuric acid. The ions produced are soluble in the medium, $\text{R-OH} + \text{H}_2\text{SO}_4 \rightarrow \text{ROH}^+ + \text{HSO}_4^- \rightarrow \text{R}^+ + \text{H}_2\text{O} + \text{HSO}_4^-$

**Inert Compounds:** Compounds not soluble in concentrated sulfuric acid or any of the other solvents are said to be inert. Compounds not soluble in concentrated sulfuric acid include the alkanes, most simple aromatics, and the alkyl halides. Some examples of inert compounds are hexane, benzene, chlorobenzene, chlorohexane, and toluene.

4. **Simple preliminary tests** - In this experiment, methods are given for identifying the presence of a halogen or a nitro group in an unknown compound and also a test for the presence of an aromatic ring. The classification tests are given in the accompanying table.

<table>
<thead>
<tr>
<th>Halides</th>
<th>Nitro Groups</th>
<th>Aromatic hydrocarbon</th>
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<tbody>
<tr>
<td>Beilstein test</td>
<td>Ferrous hydroxide</td>
<td>Ignition test</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td></td>
<td></td>
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<tr>
<td>Sodium iodide/acetone</td>
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**Beilstein Test Procedure:** Bend a small loop in the end of a short length of copper wire. Heat the loop end of the wire in a Bunsen burner flame. After cooling, dip the wire directly into a small sample of the unknown. Now, heat the wire in the Bunsen burner flame again. The compound will first burn. After the burning a green flame will be produced if a halogen is present.

This test does not differentiate among chlorine, bromine, and iodine, any of which will give a positive test. A positive Beilstein test results from the production of a volatile copper
halide when an organic halide is heated with copper oxide. The copper halide imparts a blue-
green color to the flame. This test can be very sensitive to small amounts of halide impurities
in some compounds. Therefore, one should use caution in interpreting the results of the test,
especially when only a weak color has been obtained.

Silver Nitrate Test Procedure: Add 1 drop of a liquid or 5 drops of a concentrated ethanolic
solution of a solid unknown to 2 mL of a 2% ethanolic silver nitrate solution. If no reaction
is observed after 5 minutes at room temperature, heat the solution on a team bath and note
whether a precipitate forms. If a precipitate forms, add 2 drops of 5% nitric acid and note
whether the precipitate dissolves. Carboxylic acids give a false test by precipitating in silver
nitrate, but they dissolve when nitric acid is added. Silver halides, on the other hand, do not
dissolve in nitric acid.

This test depends on the formation of a white or an off-white precipitate of silver halide
when silver nitrate is allowed to react with a sufficiently reactive halide.

\[ RX + Ag^+NO_3^- \rightarrow AgX + R^+NO_3^- \rightarrow R-O-CH_2CH_3 \]

This test does not distinguish among chlorides, bromides, and iodides but does distinguish
labile (reactive) halides from halides that are unreactive. Halides substituted on an aromatic
ring will not usually give a positive silver nitrate test; however, alkyl halides of many types
will give a positive test. The most reactive compounds are those able to form stable
carbonium ions in solution and those equipped with good leaving groups (X = I, Br, Cl).
Benzyl, allyl, and tertiary halides give immediate reaction with silver nitrate. Secondary and
primary halides do not react at room temperature but readily react when heated. Aryl and
vinyl halides do not react at all, even at elevated temperatures. This pattern of reactivity fits
the stability order for various carbonium ions quite well.

Ferrous Hydroxide Procedure: Place 1.5 mL of freshly prepared 5% aqueous ferrous
ammonium sulfate in a small test tube and add about 10 mg of the unknown compound. Mix
the solution well and then add first one drop of 3N sulfuric acid, and then 1 mL of 2N
potassium hydroxide in methanol. Stopper the test tube and shake it vigorously. A positive
test is indicated by the formation of a red-brown precipitate, usually within 1 minute.
Most nitro compounds oxidize ferrous hydroxide to ferric hydroxide, which is a red-brown
solid. A precipitate indicates a positive test.

Ignition Test Procedure: Place a small amount of the compound on a spatula and place it in
the flame of a Bunsen burner. Observe whether a sooty flame is the result. Compounds
giving a sooty yellow flame have a high degree of unsaturation and may be aromatic.

The presence of an aromatic ring or other centers of unsaturation will lead to the
production of a sooty yellow flame in this test. Compounds that contain little oxygen, and
have a high carbon-to-hydrogen ratio, burn at a low temperature with a yellow flame. Much
carbon is produced when they are burned. Compounds that contain oxygen generally burn at
a higher temperature when a clean blue flame.
5. Chemical Classification Tests – A large variety of qualitative chemical tests for organic functional groups have been developed. Their use in structural analysis has been largely replaced by spectral methods, but a few simpler tests may be found useful in resolving ambiguities in other data. In each case the test should be run on known compounds similar to those suspected to be the unknown to insure that the reagent is correctly prepared and so as to be able to recognize the appearance of a positive test.

**Chromic acid test for 1° and 2° alcohols**: This test is a reliable method for confirming the presence of a primary or secondary hydroxyl group (aliphatic aldehydes also react). This test is based on the reduction of chromium (VI), which is orange, to chromium (III), which is green, when an alcohol is oxidized by the reagent. A rapid color change to dark green constitutes a positive test. With amines or phenols, a brown color and a dark precipitate are usually seen. Primary alcohols are oxidized by the reagent to carboxylic acids; secondary alcohols are oxidized to ketones; tertiary alcohols are not oxidized at all by the reagent.

**Procedure**: Dissolve 1 drop of a liquid or about 10 mg of a solid alcohol in 1 mL of reagent-grade acetone. Add 1 drop of the chromic acid reagent and note the result that occurs within 2 seconds. A positive test for a primary or secondary alcohol is the appearance of a blue-green color. Tertiary alcohols so not give the test within 2 seconds, and the solution remains orange. To make sure that the acetone solvent is pure and does not give a positive test, add 1 drop of chromic acid to 1mL of acetone that does not have an unknown dissolved in it. The orange color of the reagent should persist for at least 3 seconds. If it does not, a new bottle of acetone should be used.

**Ferric Chloride Test from Phenols**: The presence of phenolic or enolic hydroxyl groups in a compound is usually indicated by formation of a red or violet color due to an Fe(III) complex when treated with ferric chloride solution. Some phenols give negligible or very weak tests because of interference by substituents in the ring.

**Procedure 1 (water soluble)**: Add several drops of a 2.5% aqueous FeCl₃ solution to 1mL of a dilute aqueous solution (about 1-3% by weight of the phenol). Most phenols produce an intense red, blue, purple, or green color. Some colors are transient, and it may be necessary to observe the solution carefully just as the solutions are mixed. The formation of a color is usually immediate, but the color may not be permanent over any great period. Some phenols do not give a positive result in this test, so a negative test must not be taken as significant without other adequate evidence.

**Procedure 2 (water insoluble)**: Many phenols do not give a positive result when procedure 1 is used. Often procedure 2 will give a positive result with water insoluble phenols. Dissolve or suspend 20 mg of a solid phenol or 1 drop of a liquid phenol in 1mL of methylene chloride. Add one drop of pyridine and 3 to 5 drops of a 1% (w/v) solution of ferric chloride in methylene chloride. The colors observed in this test result from the formation of a complex of the phenols with Fe(III) ion. Carbonyl compounds that have a high enol content also give a positive result in this test.
Iodoform Test: Methyl ketones (RCOCH$_3$) and also methyl carbinols (RCHOHCH$_3$) react rapidly with iodine under basic conditions to give a carboxylic acid and iodoform.

Procedure: To carry out the test, dissolve approximately 20mg or 1 drop of the unknown in 0.5 mL of water (if insoluble in water, dissolve in 0.5 mL of methanol) and add 0.5mL of 10% aqueous NaOH. Add dropwise, with shaking, a solution of KI$_3$ until the dark iodine color persists (stopper the test tube to vigorously shake the tube). Warm the solution slightly, and if the color fades, add more KI$_3$ until the color remains for 1 to 2 minutes at 50°C. Then add a drop or two of NaOH solution to remove excess iodine, and dilute with water. Iodoform, if present, will separate as a dense, pale yellow solid, mp 119 to 121°C. To prove the identity of the yellow precipitate as iodoform, collect and dry the solid and determine its melting point.

2,4-dinitrophenylhydrazine test for aldehydes and ketones: This reagent is commonly used for preparing dinitrophenylhydrazones of aldehydes and ketones and can also be used as a qualitative test for these functional groups to distinguish them from other carbonyl compounds, particularly esters, that do not react. The formation of a red or orange precipitate constitutes a positive test. Since the reagent solution contains sulfuric acid, amines may give a heavy precipitate of the amine sulfate, which appears yellow in the solution and can be mistaken for a positive test.

Procedure: Place 1 drop of the liquid unknown in a small test tube and add 1mL of the 2,4-dinitrophenylhydrazine reagent. If the unknown is a solid, dissolve about 10 mg in a minimum amount of 95% ethanol before adding the reagent. Shake the mixture vigorously. Most aldehydes and ketones will give a yellow-to-red precipitate immediately. However, some compounds will require up to 15 minutes, or even gentle heating, to give a precipitate. A precipitate indicates a positive test.

Tollens Test for aldehydes: Tollens reagent, a solution of silver ammonia complexes Ag(NH$_3$)$_2^+$, provides a test to distinguish between aldehydes and ketones. Aldehydes react by undergoing oxidation, and silver ion is reduced to form a metallic silver mirror.

Procedure: In a clean test tube, place 1 mL of 5% AgNO$_3$ solution and add, dropwise, a dilute solution (1 to 2%) of aqueous ammonia, shaking after each drop, until the brown precipitate of silver oxide just dissolves (avoid excess ammonia). To this solution add 2 to 3 drops or a few crystals of the unknown. Formation of a silver mirror indicates a positive test.

Hinsberg Test for amines: Primary and secondary amines react with benzenesulfonyl chloride in the presence of sodium hydroxide to give solid benzenesulfonamides. The sulfonamide from a simple primary amine is usually soluble in excess hydroxide solution because of the acidity of the –NHSO$_2$Ar group. Tertiary amines do not react under mild conditions. The test can be ambiguous if the amine is insoluble in water or is a solid, since it is necessary to distinguish between unreacted amine in excess, unreacted benzenesulfonyl chloride, and an insoluble sulfonamide.

Procedure: To carry out the test, mix approximately 50mg or 1 drop of the amine and 0.2g of benzenesulfonyl chloride in 4mL of 10% aqueous NaOH solution, stopper the test tube and shake vigorously until the oily sulfonyl chloride has reacted (5 minutes or longer may be needed). If a clear or nearly clear solution is obtained, a primary amine is
indicated; acidification should give a precipitate of RNHSO₂C₆H₅. Formation of a significant amount of insoluble solid from a liquid amine indicates that the unknown is a secondary amine. To distinguish from the possibility of unreacted solid amine, acidify the mixture; an amine will be soluble. Note: the precipitate may also be unreacted p-toluenesulfonyl chloride, leading to confusing results.


The following database may prove useful, Organic Compounds Database
[http://www.colby.edu/chemistry/cmp/cmp.html](http://www.colby.edu/chemistry/cmp/cmp.html)