Interpretation of Experimental Data

When evaluating experimental data it is important to recognize what the data you are collecting is telling you, as well as the strengths and limitations of each method you are using. Additionally, it is important to be able to communicate your interpretations clearly since the validity of your interpretation is lost if someone else is unable to understand what it is that your are trying to say. This semester you will utilize a number of techniques to evaluate the results you obtained in the laboratory.

I. Thin Layer Chromatography:

Thin layer chromatography (TLC) is a useful method for the preliminary evaluation of reaction mixtures and for the preliminary identification of organic compounds. This method does not provide quantitative information, but when used in conjunction with other data can be of great utility. TLC can be used in the following ways:

1. Evaluation of a reaction mixture:

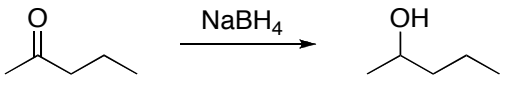
When spotting a reaction mixture, the disappearance of one spot (starting material) and the appearance of a different spot over time indicates that the original compound has been converted to something else. This is an indication that the reaction is proceeding or has gone to completion.

Generally speaking, the more polar a compound (e.g. the better it interacts with the stationary phase), the lower it's R_f value, and vice versa. Thus, for a given set of conditions, the R_f values of two spots on a TLC plate may provide some evidence as the identity of a compound, and the success (or failure) of a reaction.

Example:

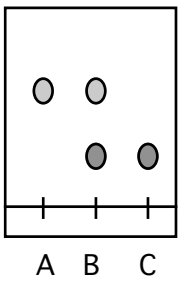
Upon treatment with $NaBH_4$, a ketone, 2-pentanone (**1**), is reduced to give the corresponding alcohol, 2-pentanol (**2**), as shown in the following equation. The progress of the reaction is monitored by TLC. The reaction mixture is sampled before the reaction begins (Lane A), after 30 minutes (Lane B), and again after 1 hour (Lane C). The single spot in Lane A represents that of the starting ketone. Evaluation of the reaction's progress after 30 min (Lane B) suggests that the reaction is proceeding, but not complete. After 1 hour (Lane C), TLC evaluation shows that all starting material has been consumed, and the reaction is finished. Note the relative R_f values in this case are as expected with the less polar ketone having a higher R_f value that of the (more polar) alcohol.

Reaction:



1 **2**

TLC:



2. As an indicator of purity:

A pure compound, when spotted and developed on a TLC plate should appear as a single spot. Two (or more) spots in a single "lane" indicate that the compound is impure. CAUTION! While TLC can show very clearly that a substance is impure, it is not an absolute indicator of purity. Different compounds (and impurities) may exhibit very similar behavior on TLC and thus may be impossible to distinguish using this method. As such, other experimental techniques should be used to confirm the purity of a substance even if it appears to be a single compound by TLC.

Example:

A laboratory sample, when analyzed by TLC, shows the presence of two components, the desired compound, **b**, and a higher R_f impurity **a**. The sample is purified by column chromatography, and the collected fractions analyzed by TLC. This analysis shows a good separation of the sample components. The desired compound is contained in Fractions 3-6. Of these, Fractions 4-6 are pure by TLC.

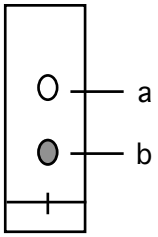


Figure 1: TLC plate showing the original laboratory sample. Compound **b** is the desired product; compound **a** an impurity

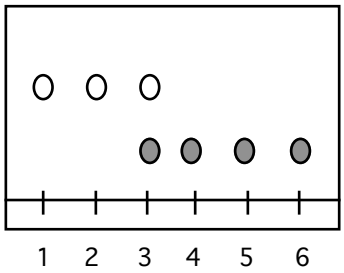


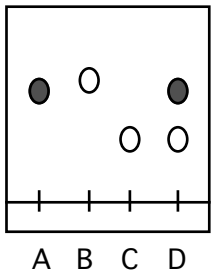
Figure 2: TLC plate showing the content of fractions collected by column chromatography. Pure **b** is contained in Fractions 4-6.

3. Identification of compounds:

For a given set of conditions, two compounds having different R_f values are different compounds, while those having identical R_f values may be the same. CAUTION! As noted above, different compounds may have identical R_f values. However, comparison of an unknown compound or reaction mixture with an authentic sample(s) can provide some insight as to the identity of that compound, or mixture components.

Example:

An unknown sample mixtures whose possible components are available in pure form is analyzed by TLC. The three known components (A - C) are spotted on a TLC plate along with the unknown mixture (D). The plate is developed and the unknown components identified as compounds A and C on the basis of their R_f values and physical characteristics (e.g. color). Compound B is not present in the unknown mixture.



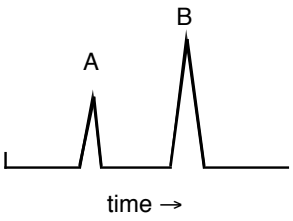
II. Gas Chromatography:

Gas chromatography (GC) is a useful tool for the analysis of volatile, thermally stable organic compounds. Unlike TLC, in certain cases GC can be used as a means of quantitative evaluation of organic mixtures. Gas chromatography is commonly used in the following ways:

1. To evaluate the components of a sample mixture

A mixed sample of known components can be evaluated quantitatively by gas chromatography. Since the detector response is proportional to the amount of compound passing through it, the area under a peak is proportional to the total amount of compound in the sample. As such, the ratio of areas in a single chromatogram is equal to the ratio of compounds in the mixture.

Example:



For the sample chromatogram at left, peak areas are calculated using the following equation:

Area = peak height x peak width at 1/2 height

By this method: Area A = 240mm^2
Area B = 600mm^2

$\therefore$ The ratio of components A:B in this sample is 1 : 2.5.

2. As an indicator of purity

When evaluating a compound by gas chromatography, the observation of a single, large peak under a variety of conditions (varied temperatures, columns, etc.) is a strong indication that the sample is pure.

Example:

By gas chromatography, the sample on the left is pure. The sample on the right is not.

3. Identification of an Unknown

GC can be utilized in the identification of unknown compounds. Preliminary information can be obtained by comparing the retention time of the unknown with those of authentic samples of known compounds, thereby narrowing down the list of possibilities and/or providing preliminary identification. While this process may be useful to identify components when evaluating mixtures of known compounds, stronger evidence as to the identity of an unknown can be obtained by co-injection. Thus, evidence as to the identity of the unknown is strong if, upon injection a mixture of the unknown and the suspected compound (authentic sample), gc analysis provides a single, sharp peak. The observation of a broadened peak or one with a "shoulder", or the presence of multiple peaks indicate that the unknown and authentic components are different.

III. Melting Point:

When a solid product is isolated in the laboratory, a melting point should always be obtained. The melting point of a compound is the temperature at which the first drop of liquid forms among the crystals to the temperature at which all the crystals have melted to give a clear liquid. The melting point is recorded as a *range* between these two temperatures. A pure substance will have a higher melting point and will melt over a narrower range than will the same substance that contains even a trace amount of an impurity. Note that there is often some inaccuracy associated with the thermometer used in a melting point determination (as much as 1-2°C). The melting point of a compound can be utilized as follows:

1. As an indicator of purity:

   For a known compound, comparison of the melting point value obtained with that reported in the literature for the pure substance provides any easy way to confirm the purity of the compound isolated. Generally speaking, the melting point of a compound will decrease in proportion to the amount of impurity present. Thus, a compound that melts over a narrow range at a temperature in good agreement with that found in the literature (for the pure substance) is considered to be pure. The further away the experimental melting point, and the broader the melting point range, the less pure the compound isolated.

   In cases where a new compound is prepared (e.g. no literature value is available) melting point can still be utilized as an indication of compound purity. Pure substances generally melt over a 1-2°C range. Thus, a new compound, melting over a broad range (5-10°C or more) would be considered impure.

2. To aid in the identification of an unknown solid

   Assuming that the sample is pure, melting point can be used to help distinguish between and/or identify unknown compounds that have been previously characterized in the literature. Preliminary identification can be made by comparing the melting point of an unknown with those of authentic samples of possible compounds. In this way, it is frequently possible to narrow substantially the list of possibilities.

   Mixed melting points (e.g. the sample contains equal amounts of unknown and authentic substance) can often be used to substantiate the identity of an unknown. If the two substances are identical then the melting point of the mixture will be unchanged relative to that of the pure compounds. If the substances are different the melting point of the mixture will be depressed.

IV. Boiling Point:

If sufficient quantities of material are available, the boiling point of a compound may be determined by simple distillation, and methods are also available for the determination of boiling points on a smaller scale. As the boiling point of a liquid will vary with atmospheric pressure, when comparing boiling point values it is important to know the pressure at which the individual determinations were made. Boiling point can be utilized in the following ways:

1. As an indicator of purity

   Boiling point is used less frequently in this course an indicator of purity. Generally speaking, however, a pure substance will distill at a constant temperature (±1-2°C), while a mixture will distill over a range of temperatures.

2. To aid in the preliminary identification of unknown liquids

   Boiling point can be used to help distinguish between and/or identify unknown compounds that have been previously characterized in the literature. Preliminary identification can be made by comparing the boiling point of an unknown with those of authentic samples of possible compounds.

V. Chemical Tests:

Periodically, you will use chemical tests to help identify the presence of functional groups in your compound or to identify the presence of unreacted reagents or starting materials. Any result in a chemical test is generally the consequence of a high yielding chemical reaction between the test reagents and a functional group on a compound, unused reagent or impurity to yield a new product that is readily visualized (e.g. color change, ppt forms, gas evolution, etc. However, for any test result to be meaningful, you must:

1. Understand the purpose of the test you are running.

   If you don't know why you're performing a chemical test it will be difficult for you to interpret your results. It usually helps to understand the chemical reaction that is occurring to give the visible test result, and by extension, the reason that a test reaction does not occur.

2. Know what to expect for both positive and negative test results.

   Chemical tests are often used to identify the presence of a general class of compounds that share a common functional group (e.g. ketones) or to differentiate between like functions that have different structural features (e.g. 1° vs. 3° alcohol). While chemical tests have been developed from reactions that occur with a wide variety of compounds, and anticipated test results are derived from results that are most frequently obtained. However, every compound is different, and the results of a test may vary with the specific compound being evaluated. In certain cases, the compound being tested will not react in the way that is expected (a false positive or false negative test is obtained) and sometimes the test results will be inconclusive. In these cases, it is important to look at all your data, and to consider possible reasons for the false result (it's often quite easily explained if you understand the chemical reaction). To avoid potential problems it is good practice to run any chemical test on both known positive and known negative compounds so as to observe the expected test result before trying to evaluate the outcome of a chemical test that is run on an unknown.

Reporting Data from Chemical Tests:

When discussing the outcome of a chemical test, unless otherwise specified, it is okay to indicate the test result (positive or negative) without going into detail re: your actual observations as this information is already recorded in your notebook. However, you must indicate exactly what the result you obtained means relative to the issue at hand (e.g. The starch iodide test gave a positive result indicating the presence of peroxides in the reaction mixture.).

VI. Infrared Spectroscopy:

Infrared (IR) spectroscopy is an excellent method for the identification of organic functional groups. It is often used to confirm the presence (or absence) of a specific functional group in a compound prepared in the laboratory as well as the compound's purity. Additionally, it can be used as a preliminary indicator of the types of functional groups that are present in an unknown sample.

Characterization of a Known Compound by IR:

1. Know the structure of the compound you are attempting to characterize.
2. Identify the functional groups you would expect to see in the IR.
3. Determine the position (cm^{-1}) at which you would expect to find each peak for a given functional group. Remember that for some functional groups (e.g. carboxylic acid, nitro group, etc.) you will expect to see several peaks.
4. Obtain a good, clear IR of your sample.
5. Considering your preliminary expectations, identify those peaks in the IR which support the identity of your compound.
6. Sometimes, unexpected peaks will be present in your IR spectrum that you will need to explain. Common impurities include unreacted starting materials, reagents, water, and solvents.

Evaluation of an Unknown Sample by IR:

- Preliminary Evaluation: Identification of Major Functional Groups

1. Determine what you would expect to see (peak position, # of peaks) for each of the possible major organic functional groups. Consider also any special circumstances that could affect what you see (e.g. hydrogen bonding, amine substitution, etc.)
2. Obtain a good clear IR of your sample. If the compound is a solid, it's a good idea to obtain both nujol mull and thin film spectra.
3. By inspection of your IR, determine which functional groups are potentially present in your sample. While you will probably not be able to narrow it down to a single group, you should be able to eliminate a number of options based on peaks that are not present (e.g. no C=O stretch? esters, carboxylic acids, aldehydes and ketones are eliminated from consideration).

- Subsequent Evaluation: Indications of Minor Functional Groups

1. Using the information you have obtained from functional group tests and other sources, reinspect your IR spectrum to gain additional support for your findings. Consider minor functional groups including double and triple bonds, halides, nitro groups, ethers, etc. Be very careful here. IR spectra are usually very complex. Don't try to read more into the spectrum than is actually there.

Reporting IR data:

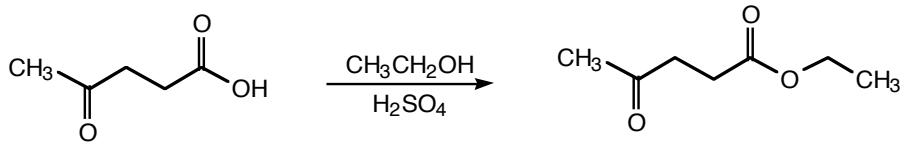
Report all information from your IR spectrum that is relevant to the identification and purity of the compound(s) you have prepared. IR data should be reported in table format as shown in the example below. Peak positions should be reported in cm^{-1} and their significance explained (e.g. OH, C=O, etc.) Also identify specifically the functional group each peak represents (e.g. The C=O stretch is found in esters, carboxylic acids, aldehydes and ketones. For full credit, you must specify which group this peak represents in your spectrum.).

Note that in some cases the absence of a peak may be significant. In these cases it is important to note within the body of your report that this peak is absent, and interpret what that absence represents.

Example: Ethyl levulinate

An esterification experiment is performed in the laboratory as shown below. As part of this process, students are asked to take IR spectra of the final product, ethyl levulinate (**4**).

Reaction:



3 **4**

The IR spectrum of ethyl levulinate shown below. For the laboratory report, the actual spectrum would be included in Appendix B.

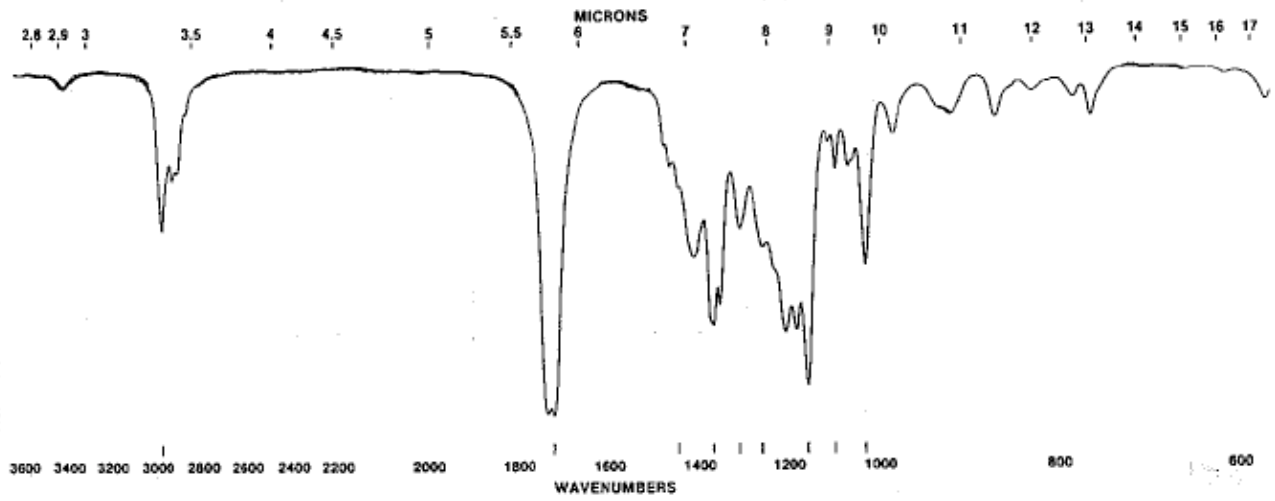


Figure 3: IR Spectrum of Ethyl Levulinate (**4**)

The spectrum is interpreted, and the data reported in the Results and Discussion section as shown below. The data presented is then discussed further in the body of the report as appropriate to the experiment at hand. In this case, in addition to discussing the absorbances that are present, it would be appropriate to note the absence of a broad OH stretch at about $3200cm^{-1}$ which would be expected from the CO_2H function in the starting material. The

absence of this absorbance indicates that the reaction was successful and has gone to completion. Additional interpretation of the IR spectrum is okay, but not absolutely necessary. Don't overdo it.

Table 1: IR data for ethyl levulinate (**4**):

position (cm^{-1})	**vibration**	**functional group**
1720 cm^{-1}	C=O	ketone
1740 cm^{-1}	C=O	ester
2950 cm^{-1}	C-H	alkane

VII. Nuclear Magnetic Resonance:

Nuclear magnetic resonance (NMR) is one of the most important diagnostic tools available to an organic chemist. This method provides information as to the relative positions and numbers of spin active nuclei (e.g. protons) in a compound, and as such can be used to identify the structural features of a molecule. In many cases, NMR can be used to determine a complete chemical structure in a very short period of time. NMR can also be used as an analytical tool to define product ratios, purity, etc.

Characterization of a Known Compound by Proton NMR:

1. Draw the structure of the compound you are trying to characterize. Count the total number of protons, and identify those which are equivalent.
2. Determine roughly what you would expect to see (peak position, # of peaks, multiplicity, etc.) for each set of equivalent protons. Consider also any special circumstances that could affect the spectrum (e.g. the vicinity of heteroatoms, proton exchange, etc.)
3. Take a look at your NMR spectrum. Identify reference solvents (TMS, Chloroform) and omit them from consideration. Note that water is also a potential impurity (*ca.* 1.5ppm).
4. Determine peak integration.
    a. Measure the line height of each integral plot in the spectrum.
    b. Divide through by the lowest value to get relative peak intensities.
    c. Verify that the total # of protons by integration agrees with the number you came up with in step 1. Remember the integral values from step 4b are relative intensities. If the compound is symmetrical it will be necessary to multiply through by an integer at that stage.
    d. Round integral values to nearest whole number.
5. Determine the chemical shift of each resonance in your spectrum.
6. Identify the multiplicity (splitting) of each resonance in the spectrum. You may also wish to determine coupling constants (J) for each set of resonances. This information can be used to determine which groups of protons are adjacent to each other.
7. Using the information obtained above, account for the resonances you see in the NMR spectrum. It may be helpful to consult a correlation table of chemical shifts.

Characterization of an Unknown Compound by Proton NMR:

- To Differentiate Between Several Known Possibilities

1. Draw structures for each of the possible compounds. In each case, count the total number of protons, and identify those which are equivalent.
2. Determine roughly what you would expect to see (peak position, # of peaks, multiplicity, etc.) for each set of equivalent protons. Note any characteristics that may help to distinguish one structure from another.
3. Take a look at your NMR spectrum. Identify reference solvents (TMS, Chloroform) and omit them from consideration. Note that water is also a potential impurity (*ca.* 1.5 ppm).

4. Determine the peak integration as described above.
5. Determine the chemical shift of each resonance in your spectrum.
6. Identify the multiplicity (splitting) of each resonance in the spectrum. A preliminary assessment of these patterns relative to your expectations in step 2 may allow you to eliminate one or more structures. You may also wish to determine coupling constants (J) for each set of resonances. This information can be used to determine which groups of protons are adjacent to each other.
7. Using the information obtained above, account for the resonances you see in the NMR spectrum. It may be helpful to consult a correlation table of chemical shifts.

- Starting From Scratch

1. Take a look at the your NMR spectrum. Identify reference solvents (TMS, Chloroform) and omit them from consideration. Note that water is also a potential impurity (*ca*. 1.5ppm).
2. Determine peak integration as described above. If the molecular formula is known, then the absolute number of protons can be verified.
3. Determine the chemical shift of each resonance in your spectrum.
4. Identify the multiplicity (splitting) of each resonance in the spectrum. You may also wish to determine coupling constants (J) for each set of resonances. This information can be used to determine which groups of protons are adjacent to each other.
5. Using the information obtained above, account for the resonances you see in the NMR spectrum. In this situation it is generally most helpful to put together small fragments first and proceed from there. It may also be helpful to consult a correlation table of chemical shifts.

Reporting ^{1}H NMR data:

A single NMR spectrum provides a great deal of information that must be clearly interpreted and reported in an organized manner. As such, NMR data must be reported in table format. Refer back to this table as needed to clarify you explanations. Include a drawing of your compound (number the atoms as necessary) in the Results and Discussion section to facilitate identification and interpretation of specific resonances.

When reporting NMR data you must include the position in ppm, the integration (# protons), and the peak multiplicity (splitting). In general, you must interpret every peak (excluding standards such as TMS). While you may not be able to assign every set of protons in your compound to a specific resonance (they may be grouped together or difficult to distinguish - two methyl singlets, for example) you must account for every proton. A possible exception: protons on heteroatoms are exchangeable and may not be seen.

Integration must be reported in whole numbers, and should reflect the actual number of protons represented by the resonance (not the relative intensity). The total number of protons reported should agree with the number present in the compound being evaluated. If these values do not agree, you must provide an explanation.

Example: Ethyl levulinate (**4**)

The proton NMR spectrum of ethyl levulinate is shown below (note the presence of the TMS reference at 0 ppm). For the laboratory report, the actual spectrum would be included in Appendix B.

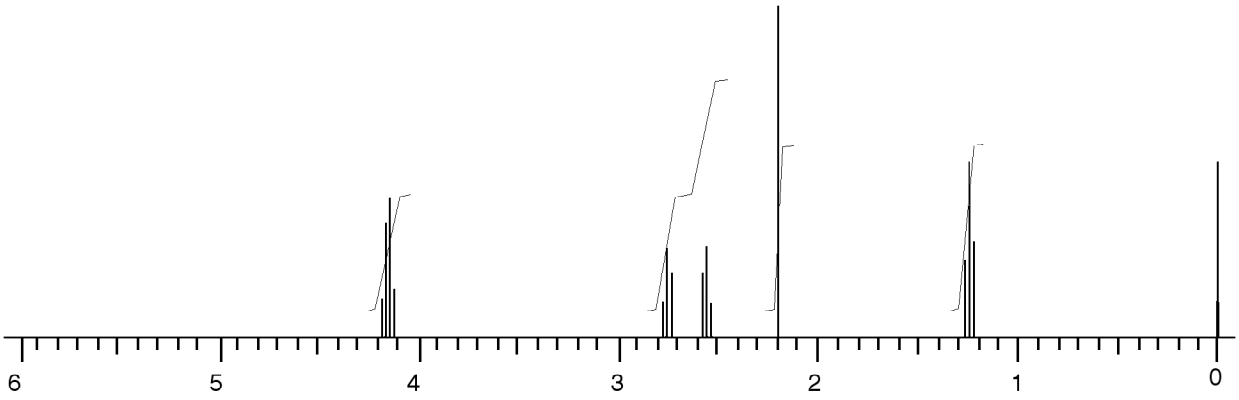
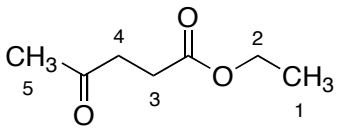


Figure 4: ^{1}H NMR spectrum of ethyl levulinate (**4**)

The spectrum is interpreted, and the data reported in the Results and Discussion section. Note that a labeled structure of ethyl levulinate is included as well as the tabulated data. The data presented is then discussed further in the body of the report as appropriate to the experiment at hand.



ethyl levulinate (**4**)

Table 2: ^{1}H NMR data for ethyl levulinate (**4**):

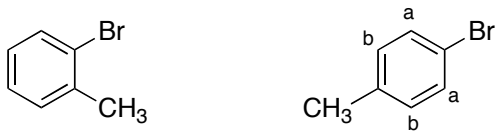
position (ppm)	integration	multiplicity	assignment	
1.25	3H	triplet	-CH_3	(1)
2.19	3H	singlet	-(C=O)CH_3	(5)
2.55	2H	triplet	-CH_2(C=O)	(3 or 4)
2.74	2H	triplet	-CH_2(C=O)	(3 or 4)
4.15	2H	quartet	-OCH_2-	(2)

B. ^{13}C NMR

Carbon-13 NMR provides direct structural information about the carbon skeleton of a molecule. In addition to the structural details provided by chemical shifts, ^{13}C NMR can be used to determine the symmetry of a molecule. Because ^{13}C chemical shifts are more sensitive to small changes in chemical environment, the number of resonances in a ^{13}C spectrum is frequently an indicator of the number of equivalent carbon types in a molecule.

Characterizing Compounds by Carbon NMR:

The process for characterizing compounds by ^{13}C NMR is similar to that described above for ^{1}H NMR, except that ^{13}C NMR spectra are typically decoupled so all the signals appear as singlets. Another difference is that ^{13}C spectra are not integrated; the key piece of information obtained is chemical shift. For a pure compound, the number of peaks observed will not exceed the total number of carbons present in the skeleton. If some carbon signals overlap (less common) or represent chemically equivalent carbons, the total number of peaks observed will be less. The actual number of carbon signals observed can often be used to rapidly differentiate closely related compounds. For example, compounds **5** and **6** both have the same molecular formula (C_7H_7Br). At first glance it may seem different to differentiate between the two by 13C NMR as both are expected to display peaks having similar chemical shifts. However, closer examination reveals that while compound **5** shows 7 distinct types of carbon (and hence is expected to show 7 peaks in the ^{13}C NMR), compound **6** shows some symmetry and thus will show only 5 peaks (equivalent sets of carbons are marked a and b).



5 **6**

7 peaks 5 peaks

Figure 6: Using symmetry to differentiate similar compounds by ^{13}C NMR

Reporting ^{13}C NMR data:

When reporting ^{13}C NMR data you must include only the position in ppm. Include a drawing of your compound (number the atoms as necessary) in the Results and Discussion section to facilitate identification and interpretation of specific resonances. Present your data in table format.

The spectrum is interpreted, and the data reported in the Results and Discussion section. Note that a labeled structure of ethyl levulinate is included as well as the tabulated data. The data presented is then discussed further in the body of the report as appropriate to the experiment at hand. For the laboratory report, the actual spectrum would be included in Appendix B.

Example: Ethyl levulinate (**4**)

The carbon NMR spectrum of ethyl levulinate is shown below.

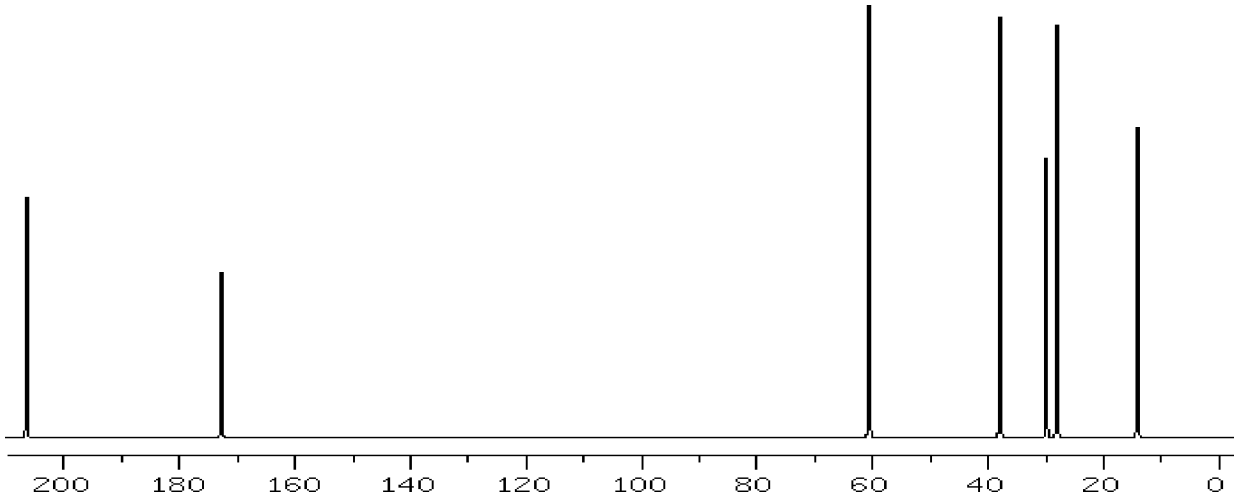
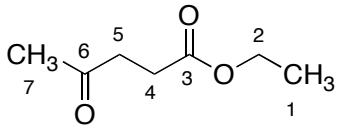


Figure 5: ^{13}C NMR spectrum of ethyl levulinate (**4**)

As above, you must interpret every peak (excluding standards such as $CHCl_3$). While you may not be able to assign every carbon in your compound to a specific resonance you must account for every carbon. Remember that chemically equivalent carbons will appear as a single peak.



ethyl levulinate (**4**)

Table 3: ^{13}C NMR data for ethyl levulinate (**4**):

position (ppm)	**assignment**	
205.6	-(C=O)	(6)
172.7	-(C=O)	(3)
60.5	-OCH_2-	(2)
38.0	-CH_2(C=O)R	(5)
29.8	-CH_3	(7)
28.1	-CH_2(C=O)OEt	(4)
14.2	-CH_3	(1)