

5.32 Intermediate Chemical Experimentation

EXPERIMENT #2: QUALITATIVE ORGANIC ANALYSIS

I. INTRODUCTION

The identification of the structure of chemical substances is an activity fundamental to all varieties of research in organic chemistry. Chemists may wish to establish that an unknown substance is identical to a previously reported compound, or they may need to deduce the structure of an entirely new compound. This experiment is designed to simulate these common research situations.

The aim of the experiment is to separate and identify the components of a binary organic mixture. Unknown samples will consist of approximately 2 g of a mixture of two organic compounds. One unknown in each sample is an organic acid (e.g. carboxylic acid, phenol or sulfonic acid). The other unknown in each sample is a neutral organic compound (e.g., ketone, ester, amide etc.).

- The unknowns will be separated by extraction (monitored by thin layer chromatography), and the separated compounds will be purified by recrystallization.
- The structure of the neutral unknown will be deduced by analysis of the 1D NMR (^1H NMR, ^{13}C NMR, DEPT¹), 2D NMR (gCOSY,² HETCOR³ or HMQC⁴), FT-IR and GC/MS spectral data together with analytical and data supplied by the teaching staff. UV-Vis analysis is optional.
- The acid unknown will be identified by infrared, ^1H NMR, ^{13}C NMR spectral data and comparison of its spectral characteristics with those of previously reported compounds, and by the preparation of a suitable derivative (amide).

II. REFERENCES

1. Zubrick, J. W.; *The Organic Lab Survival Manual*, 5th Ed.; Wiley: New York, 2001. (Z)
2. Pasto, D. J.; Johnson, C.R.; Miller, M.; *Experiments and Techniques in Organic Chemistry*; Prentice-Hall: Englewood Cliffs, NJ, 1992. (PJM)

¹ Distortionless Enhancement by Polarization (population) Transfer: display separate spectra for CH₃, CH₂ and CH carbon signals.

² Field gradient selection COrrrelation SpectroscopY: homonuclear (for example H vs H) correlation spectroscopy

³ HETeronuclear chemical shift COrrrelation by Polarization Transfer: carbon vs hydrogen chemical shift correlation.

⁴ Heteronuclear Multiple Quantum Coherence, carbon vs hydrogen chemical shift correlation.

3. Lambert, J. B.; Shurvell, H. F.; Lightner, D. A.; Cooks, R. G.; *Organic Structural Spectroscopy*; Prentice-Hall: Upper Saddle River, NJ, 1998. (LSLC)
4. Silverstein, R. M.; Bassler, G. C.; T.C. Morrill, T. C.; *Spectrometric Identification of Organic Compounds*, 5th edition; Wiley: New York, 1991.
5. McLafferty, F.; Tureen, F.; *Interpretation of Mass Spectra*; University Science Books: Mill Valley, CA, 1993.

Lists of acid unknowns are contained in S. Rappaport, *Handbook of Tables for Organic Compound Identification*, 3rd Edition, Chemical Rubber Co. (1967).

III. PROCEDURE

Review the following laboratory techniques in the **Digital Laboratory Techniques Manual**, which can be found on the 5.32 website, before beginning this section.

DLTM:

3. **TLC – the Basics**
4. **TLC – Advanced**
5. **Reaction Workup I**
6. **Reaction Workup II**
7. **Filtration**
9. **Recrystallization**

A. TLC ANALYSIS OF A MIXTURE

(read Z: Ch 26 or PJM: section 2.6.4; DLTM: 3 & 4)

Determine analytical TLC conditions that clearly resolve your unknown components. Be sure you are familiar with the effect of a compound's polarity on its mobility on a TLC plate. Your objective is to find a TLC solvent and stain that allow a quick and convenient analysis for the presence/absence of the two components of the mixture. The solvent system should elute one or both of the components near the middle of the plate.

1. TLC Plates - pre-coated plastic-backed silica gel plates containing a fluorescent indicator will be provided.
2. Eluents – find out what is the effective solvent to separate components. For example you can try a single solvent such as hexane, ether, dichloromethane, ethyl acetate, etc or mixtures of two or three components.
3. Spot the TLC plates with a glass capillary, using a solution of approximately 5 mg of unknown in 0.5 mL of a suitable solvent (Et₂O or Et₂O:CH₂Cl₂, etc.). Always spot a sample of the original mixture on the same plate as a test sample for a definitive comparison. To spot a neutralized aqueous solution, you must remove the water *in vacuo* prior to eluting the plate.
4. Visualization
 - a. Nondestructive - When the plate has developed, remove it from the chamber and allow the solvent to evaporate. Place the plate under the short-wave UV lamp.

Highly conjugated molecules, e.g. aromatic systems, will show up as dark spots on a green background. Beware that minor impurities can often show up rather strongly under UV light, while an unknown with no significant UV chromophore will be completely invisible.

b. Destructive - Staining with ethanolic *p*-anisaldehyde- $\text{H}_2\text{SO}_4/\text{CH}_3\text{COOH}$ solution is very effective for visualizing a variety of organic molecules. The plate is simply dipped in the solution and then placed on a hot plate (100-150 °C). Phosphomolybdic acid solution is another effective stain for most organic compounds. $\text{Ce}^{\text{IV}}(\text{SO}_4)_2$ solution in sulfuric acid may also be useful. Organic molecules will appear as a brown spot on a white background.

B. TESTING THE EXTRACTION PROCEDURE

Determine the acidic properties of your unknowns by conducting small-scale extractions of a solution of your mixture. In a small test tube, try to dissolve 5-10 mg of your mixture in approximately 0.5 mL of ether. If the mixture is not soluble, some dichloromethane can be added. Add a portion of 5% NaHCO_3 or 5% NaOH and swirl the test tube gently to mix. Ether alone or mixtures of ether and hexane are the best solvents for the extraction of neutral organic compounds from ionized forms of organic acids because these ionic species have very little, if any, solubility in these solvents. The addition of significant portions of dichloromethane or the use of dichloromethane alone may extract both the neutral compound and the organic salt. Determine by TLC analysis whether your unknown components are still present in the organic phase (you can remove a portion of the upper organic layer with a pipette). Pay particular attention to whether the products of the extraction are identical to the components of the original mixture. After removal of the ether layer, the aqueous layer can be washed with a fresh portion of solvent and then analyzed by TLC for the other component of the mixture (you must allow the water to dry from the plate for this to work.) Confirm that your acid component is not affected upon neutralization of aqueous extracts - reactions of organic compounds with acid or base are not uncommon. With some skill, the small-scale extraction is extremely quick and easy to perform (however a larger scale test extraction may also be performed using a small separatory funnel).

C. SEPARATION OF THE MIXTURE BY EXTRACTION

(read Z: Ch 15 or PJM: sections 2.1, 2.5; DLTM: 5, 6 & 7)

Based on the results of the small-scale extractions, separate the components of your mixture by an appropriate extraction procedure. A 60 mL or 125 mL separatory funnel should be effective for this purpose. Use no more than 2/3 of your mixture (save the remainder in case of an accident!). Two or three extractions with small quantities of solvent are more efficient than a single large extraction. Whether or not further extractions are necessary can be ascertained by analyzing the most recent extraction by TLC relative to the combined extractions. The organic solutions should be dried by filtration through anhydrous magnesium sulfate before evaporation using a Büchner funnel or fritted glass funnel (available from the stockroom). The rate that anhydrous magnesium sulfate will dry an organic solvent is extremely rapid. Remember to carefully

pour ethereal solutions out the top of the separatory funnel without allowing water to pour in. A particularly good practice involves the passage of each sequential organic extract through a fritted glass funnel containing anhydrous magnesium sulfate (later extractions serve to wash through the compound from the first portions). To separate 1 gram of your mixture, you will likely find that a total of 25 mL aqueous base and 150 mL of organic solvent is adequate for the entire operation. When removing the solvent on the rotary evaporator, use a dish of lukewarm water under the flask rather than steam because some of the unknowns may not be very stable. Upon confirming that the neutral compound has been effectively removed, neutralize the aqueous layer and isolate the acid component of your mixture. Record the weights of original and separated material.

D. PURIFICATION OF SOLID UNKNOWNNS

(read Z: Ch 13 or PJM: section 2.2; DLTM 9)

Purify both unknowns by recrystallization. Some of the unknowns will solidify upon concentration. For those that don't, save a small portion of the original solid mixture as seed crystals for recrystallization of one component. Good solvents for recrystallization include ether, hexane, dichloromethane, ethyl acetate and mixtures of these three solvents. An all-purpose procedure involves initially adding 1-2 mL of ether to the solid in a 50 mL flask. If the solid starts to dissolve rapidly, then add some hexane until it begins to crash out, then warm gently to dissolve. If the solid appears to dissolve slightly, then warm ether is probably the best choice. If the solid does not dissolve at all, then dichloromethane can be added and perhaps a more polar solvent such as ethyl acetate or ethanol. After the material has been dissolved in the optimum solvent, it can be cooled slowly to room temperature on the bench. At this time, add a very small seed crystal if crystallization has not already taken place. After crystal formation occurs at room temperature, place the mixture in the refrigerator or freezer to complete crystallization. Be warned: for many compounds, a fluffy crystalline product may be a lot less material than expected. Crystals that have been isolated on the filter can usually be effectively freed from solvents such as ether, hexane and dichloromethane by sucking air through for 10-15 minutes. Oven drying is not recommended because most of the unknowns will either melt or decompose. Record the amount of material recovered, the melting point and melting range for each of your unknowns. A sharp melting range is the best indication of purity and a prerequisite to investing a lot of time in spectral analysis.

E. NMR SPECTRAL ANALYSIS

Obtain a ^1H NMR spectra (in CDCl_3) of both of your unknowns. If the compounds are not soluble in CDCl_3 , try deuterated acetone or DMSO - ask your TA. Be sure that your sample is pure and not wet with solvent. Your NMR tube must also be free of any solvent that may have been used to wash it out. Such solvent peaks can obscure important peaks in the spectrum. After you obtain a good spectrum, print out all of the information that you will need to analyze the spectrum, including integrals, expansions and peak listings - having to redo spectra because of these two problems can lead to a great deal of congestion on the instruments. The TAs can give you a copy of the ^1H NMR spectrum of your *neutral (not acid)* unknown if you find that your spectrum contains some minor inadequacy or ambiguity. As soon as possible, check each ^1H NMR spectrum carefully for purity and try to assign a number of protons to each peak or cluster of peaks based on the integration. Check for the presence of common impurities such as ether, acetone, etc. as well as the deuterated solvent peak(s) and water (which appears at various places in different solvents). After you have determined which peaks belong to your compound, make a list of them in your worksheet. In the table, organize the chemical shift, multiplicities (singlet, doublet, triplet). Some of the peaks in the spectrum may appear as unmanageable multiplets (**m**); however, the more carefully and patiently you analyze the peaks, the more information you will glean from the spectrum. Such analysis of complex multiplets (**dd**, **ddd**, **dt**, etc.) and matching of the coupling constants allows one to trace out the spin system of the unknown. Read the section on analysis of ^1H NMR coupling patterns and make sure you understand how to do the practice problems on the First Report.

If the ^1H NMR of the neutral compound proves to be acceptable, then you can proceed to record the ^{13}C , DEPT, gCOSY and HETCOR NMRs.

F. IR ANALYSIS (Optional; read Z: chap. 32 or PJM: chapter 5)

Obtain an IR spectrum of each component of your unknown mixture. Using the FTIR spectrophotometer, IR spectra can be measured on solutions of solid samples and thin-films of liquid samples. Identify the principal absorptions in each spectrum in your notebook and determine which functional groups are most likely present.

G. UV SPECTRAL ANALYSIS (Optional)

H. GC-MASS SPECTRAL ANALYSIS

Obtain the high-resolution mass spectral data for your neutral unknown. This task will be accomplished by using the Hewlett-Packard 5890 Series II GC/MS. Sign up in advance to use this instrument in 4-474 and ask your instrument TA or Ray Dove for help with this delicate equipment. Because mass spectrometry is the most sensitive analytical tool available to the organic chemist, be careful to prepare a sample that is not too concentrated. Usually, a solution containing 1 mg of your neutral unknown dissolved in

0.5 mL of dichloromethane is sufficient to obtain a strong spectrum. Small disposable sample tubes are available for this purpose.

After you have completed parts E, F and H, you should prepare to show your TAs that your spectral characterization of your two unknowns is acceptable to proceed. You will then receive the elemental analysis of your neutral unknown.

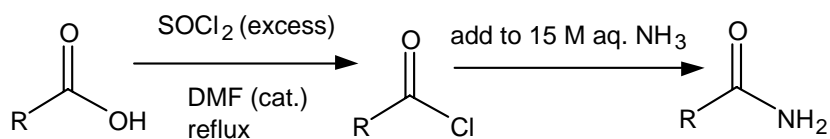
I. ORGANIC ACID UNKNOWN: PREPARATION OF DERIVATIVES (Optional)

Locate the m.p. of your organic acid unknown in the *Tables for the Identification of Organic Compounds*, keeping in mind that it could be a carboxylic acid, a phenol or a sulfonic acid. Examine the entries in the appropriate table within 6 °C of your measured melting point. Eliminate as many structures as possible on the basis of your NMR and IR data. Choose a derivative of your acid unknown which will allow you to confirm its identity or distinguish between two possibilities. Not all derivatives are listed for each entry in the *Tables for the Identification of Organic Compounds*. Procedures for preparation of common derivatives of almost any functional group are presented in PJM: chap. 9.

Preparation of Acid Chloride and conversion to Amide or Anilide (Optional)

Generally, various acids are converted to amides or anilides via the acid chloride. Thionyl chloride is used to convert the unknown acid to the acid chloride. However, the range of possible unknowns covers a very wide spectrum of reactivity (aliphatic, aromatic, sterically hindered/unhindered, etc.). Some acids will react rapidly with SOCl_2 alone with gentle warming, whereas sterically hindered or electron-deficient acids may only react after an hour or so at reflux or not at all. In either case, a couple of drops of N,N-dimethylformamide (DMF) serve as an efficient catalyst for the reaction between a carboxylic acid and thionyl chloride. This procedure allows most acids to be converted to the corresponding acid chloride at room temperature over 1-3 hours (as observed by evolution of SO_2 and HCl gas). Questions you might consider:

- how does DMF catalyze the first reaction?
- how much volume of gas evolution will you be looking for this reaction?



Caution: Thionyl chloride is corrosive and its vapors are highly noxious. This procedure must be carried out in an efficient fume hood.

Procedure: Weigh 50-150 mg of your unknown carboxylic acid into a 25 mL flask. Add 2-3 drops of N,N-dimethylformamide as a catalyst followed by 2.5-5 mL thionyl

chloride. Attach a reflux condenser and, by means of a piece of tygon tubing, attach the top of the reflux condenser to a mineral oil bubbler. Warm the mixture gently to reflux with a heating mantle and note any significant evolution of gases as an indication of the reaction. Based on your observations as well as the expected reactivity of your acid, decide how long to reflux the reaction.

Cool the acid chloride reaction to room temperature. In the fume hood, cautiously pour or pipette the mixture into 25 mL of concentrated ammonia solution contained in a 250 mL beaker. The amide will precipitate from the aqueous solution and can be collected by filtration. Obtain a m.p. and an IR spectrum of your amide derivative.

IV. A STEPWISE GUIDE TO STRUCTURE ELUCIDATION

To determine the structure of your neutral unknown, you will draw upon the several powerful spectroscopic and analytical methods described above. A systematic approach to structure elucidation is detailed below. While reading about each of the five stages of the structure elucidation process, follow the example to see how this is applied to a sample unknown.

- A. Determination of Molecular Formula (and Unsaturation number)
- B. Identification of Unsaturated Groups
- C. Analysis of ^1H NMR Coupling Patterns
- D. Assemblage of Molecular Subunits
- E. Confirmation of Structure

A. DETERMINATION OF MOLECULAR FORMULA (and unsaturation number)

The following data will be important for determining the molecular formula: **Elemental Analysis, Mass Spectrum Highest Ion Pattern, ^1H NMR (integration) and ^{13}C NMR (# of lines)**. The goal is to find a molecular formula that is consistent with *all* of these techniques.

1. Elemental Analysis.

In research, samples are sent to outside laboratories for the purpose of obtaining % composition of C and H (N, S are more expensive). Two to five milligrams of material are subject to complete combustion in an atmosphere of O_2 , and the resulting H_2O and CO_2 are accurately weighed to obtain %C and %H within $\pm 0.4\%$. In practice, stray solvents and impurities can lead to incorrect results. Your TA's have calculated an elemental analysis of your unknown for the elements C, H, N, S, which is within $\pm 0.4\%$ for each element. Because you are *guaranteed* the $\pm 0.4\%$ range, you can apply a high level of confidence in this data.

2. GC-MS Highest Ion Pattern.

A single large peak eluting from the gas chromatograph is evidence that your neutral unknown was pure and passed through the GC unharmed. Each molecule passing into the Mass Spec is first converted by electron ionization (EI) to the *molecular ion* ($\text{M}^{+\bullet}$) in which the molecule has simply lost one electron. For nearly all of the unknowns, some of the $\text{M}^{+\bullet}$ ions will survive and show up in the spectrum. If true for your compound, then the Elemental Analysis data and GC-MS may be used directly to determine the *number* of C, H, N and S atoms in the molecular formula (check out the example to see how easy this is).

If the Highest Ion Pattern of your mass spectrum is *not* $\text{M}^{+\bullet}$, then figuring out the molecular formula will be a fair amount more difficult. The following symptoms suggest that you don't have $\text{M}^{+\bullet}$:

1. The calculations using the elemental analysis and the Highest Ion Pattern do not span an integer for each element. (see example)

2. The mass of $M^{•+}$ does not agree with the Nitrogen Rule. (The Nitrogen Rule states that a molecule or $M^{•+}$ ion with an even number of nitrogen atoms *must* have an even mass. Similarly, a molecule or $M^{•+}$ ion with an odd number of nitrogen atoms *must* have an odd mass.)

The Highest Ion Pattern of the mass spectrum will generally display at least three peaks at consecutive m/e values if a sufficiently strong $M^{•+}$ signal has been detected. The ratio of intensities of $M^{•+}$, $(M+1)^{•+}$ and $(M+2)^{•+}$ result from the statistical presence of $(A+1)$ and $(A+2)$ isotopes of the individual elements. The $M+1$ peak % results primarily from the number of carbons in the organic molecule. The $M+2$ peak % will generally be 1% or less that of the M peak % for small to medium sized organic molecules containing only C,H,N and O. A sizeable $M+2$ peak indicates the presence of $(A+2)$ elements, most notably **Cl** and **Br**. Below are shown several possible isotopic patterns for the $M^{•+}$ region of the spectrum.

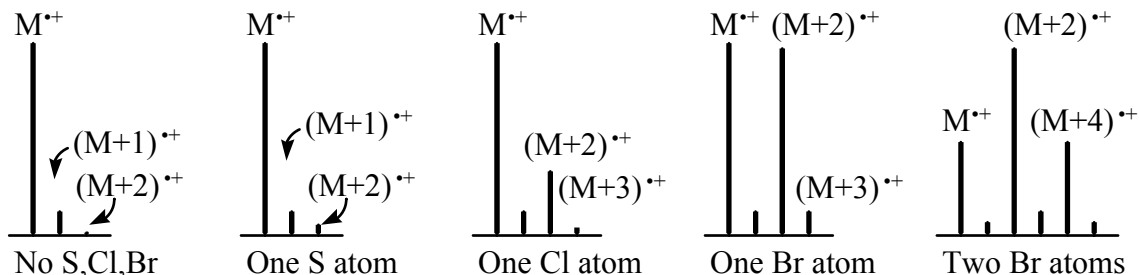
Isotopic Patterns for Selected Elements

A isotope =100%	A+1 (relative %)	A+2 (relative %)
^1H	^2D (0.015%)	---
^{12}C	^{13}C (1.1%)	---
^{14}N	^{15}N (0.37%)	---
^{16}O	^{17}O (0.04%)	^{18}O (0.20%)
^{19}F	---	---
^{28}Si	^{29}Si (5.1%)	^{30}Si (3.4%)
^{31}P	---	---
^{32}S	^{33}S (0.80%)	^{34}S (4.4%)
^{35}Cl	---	^{37}Cl (32.5%)
^{79}Br	---	^{81}Br (98%)
^{127}I	---	---

Isotopic Patterns for $\text{C}_x\text{H}_y\text{O}_n$

M peak=100%	M+1 peak %*	M+2 peak %
C_1	1.1 %	0.00%
C_2	2.2 %	0.01 %
C_3	3.3 %	0.04 %
C_4	4.4 %	0.07 %
C_5	5.5 %	0.12 %
C_6	6.6 %	0.18 %
C_7	7.7 %	0.25 %
C_8	8.8 %	0.34 %
C_9	9.9 %	0.44 %
C_{10}	11.0 %	0.54 %
C_{11}	12.1 %	0.67 %

*add 0.37% for each N



3. ^1H NMR (integration).

The integration of the ^1H NMR spectrum is the most reliable method for determining the number of protons in the molecular formula. Only two problems can interfere with this method:

1. Stray peaks in the ^1H NMR spectrum may be mistakenly counted.
2. Exchangeable protons (e.g., NH, OH) are broadened and may be exchanged with D_2O present in some deuterated solvents (e.g. d_6 -acetone), giving a lower integral. Occasionally, these peaks (e.g., $-\text{COOH}$) may be overlooked altogether.

4. ^{13}C NMR (# lines)

The number carbon atoms should be checked with the number of lines in the ^{13}C NMR. First, make sure that you know which peaks are from the deuterated solvent. Because ^{13}C NMR spectra are generally acquired with broadband ^1H decoupling, all resonances will appear as sharp singlets unless they are coupled to other nuclei such as ^2D ($I = 1$), ^{19}F ($I = 1/2$) or ^{31}P ($I = 1/2$). If # Lines > #C, your unknown has one or more of these atoms. # Lines < #C means that your unknown has groups for which pairs or sets of carbons are equivalent by virtue of symmetry or free rotation.

5. Unsaturation Number

For a molecule having a molecular formula $\text{C}_x\text{H}_y\text{N}_z\text{O}_n$, the number of double bonds + number of rings = $1 \cdot x - 0.5 \cdot y + 0.5 \cdot z + 0 \cdot n + 1$. This calculation is a direct result of the known valences of these elements ($\text{C}=4, \text{H}=1, \text{N}=3, \text{O}=2$). For other elements, all that is needed is to know the valence of the element. Halogens have a valence of 1 for the majority of organic compounds. However phosphorus may have valence = 3 or 5, and sulfur may have valence = 2, 4 or 6 (e.g., CH_3SCH_3 , CH_3SOCH_3 , PhSO_3H).

B. IDENTIFICATION OF UNSATURATED FUNCTIONAL GROUPS

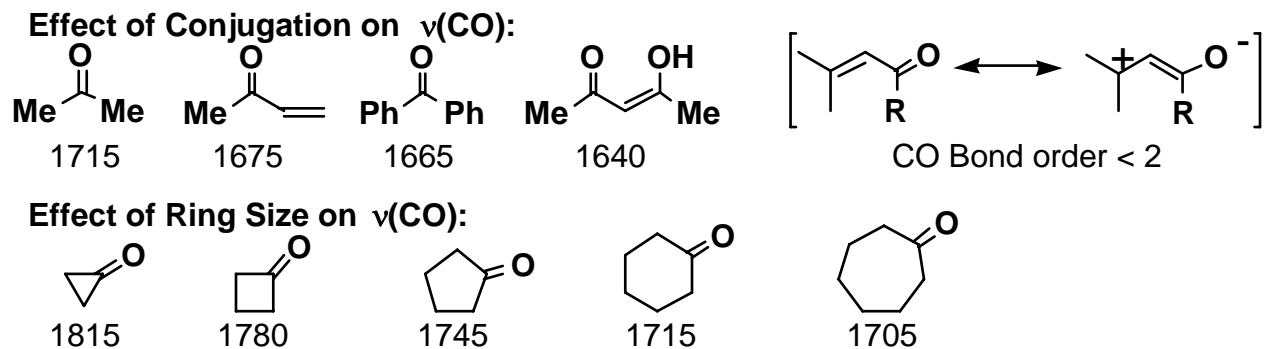
Careful examination of the IR, and ^{13}C NMR spectra will reveal all of the unsaturated functional groups that are present in your unknown. The first stage of this search involves examination of the IR spectrum. Although several regions of the IR spectrum can reveal useful information about the unknown, we are only interested in examining the two regions of the IR spectrum that will help us identify special unsaturated functional groups. In the 2300 to 2000 cm^{-1} region, *any* peak that is observed, no matter how small, is likely to be diagnostic of one of these functional groups. By contrast, in the 1800 to 1650 cm^{-1} region, a carbonyl peak will always be strong, very likely the strongest peak in the spectrum.

-C N, -C C-, >C=C=C< Region (2300 cm^{-1} to 2000 cm^{-1})			
cm^{-1}		comments	^{13}C ppm
2250	$\square(-\text{C}\square\text{N})$	sharp, weak to med intens, almost always observed	117-123 ppm

2150	$\nu(\text{RC}\equiv\text{CH})$	sharp, weak to med intens, check for $\nu(\text{C-H})$ at 3300	ppm
2260-2190	$\nu(\text{R-C}\equiv\text{C-R}')$	sharp, weak to med intens, obsd only for R,R' different	ppm
1950	$\nu(>\text{C}=\text{C}=\text{C}<)$	sharp, strong allene	200-210 ppm

>C=O Region (1800 cm^{-1} to 1650 cm^{-1})			
cm^{-1}		Comments	^{13}C ppm
1800	Acid Chloride	$\left[\text{R}-\overset{\text{O}}{\parallel}{\text{C}}-\text{Cl} \longleftrightarrow \text{R}-\overset{\text{O}^+}{\parallel}{\text{C}}-\text{Cl}^- \right]$ CO Bond Order >2	170-180 ppm
1820,1760	Anhydride	two peaks are observed ($\nu_s \nu_{as}$)	160-172 ppm
1735	Ester	$\text{RCO}_2\text{R}'$	165-175 ppm
1755	Carbonate	$\text{ROCO}_2\text{R}'$	150-155 ppm
1735	Urethane	ROCONR'_2	154-160 ppm
1720	Aldehyde/Ketone	aldehyde has $\nu(\text{CH})$ at 2750 cm^{-1}	190-210 ppm
1650	Amide	$\left[\text{R}-\overset{\text{O}}{\parallel}{\text{C}}-\text{NR}_2 \longleftrightarrow \text{R}-\overset{\text{O}^-}{\parallel}{\text{C}}^+-\text{NR}_2 \right]$ CO Bond Order <2	164-172 ppm
1630	Urea	$\text{R}_2\text{NCONR}'_2$	ppm

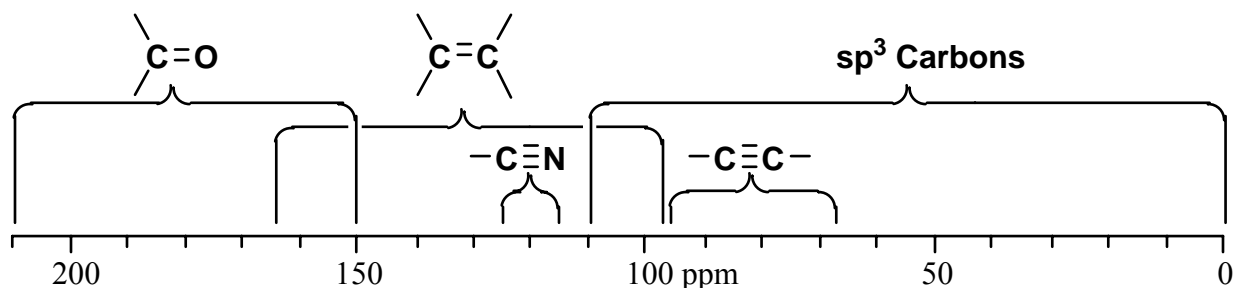
For each of the peaks listed in the table, a range of actual positions can be observed. Carbonyl groups, phenyl substituents or double bonds attached to the C=O group donate electron density to the carbonyl group and lower the C=O stretching frequency. A carbonyl group in a small ring has a higher stretching frequency.



Further evidence taken from the IR spectrum must be used with care. **If one peak is found** in the carbonyl region, perhaps only one C=O group is present. However, because the C=O region spans only 170 cm^{-1} and even the sharpest peaks are several cm^{-1} wide, two C=O groups could show up in the same position by coincidence. Alternatively, the molecule could have two C=O groups related by symmetry. **If two peaks are found** in the carbonyl region, perhaps the molecule contains two C=O groups which are not related by symmetry. However, exceptions to this assumption exist. Symmetrical anhydrides

display two peaks in the carbonyl region because of the close proximity of the two C=O groups. One peak represents the symmetrical stretching of the two C=O groups and the other peak represents antisymmetric stretching.

Each of the functional groups above may be confirmed by the presence of the appropriate peaks in the ^{13}C NMR spectrum, and the exact positions in the IR and ^{13}C NMR may be used later to help identify the exact nature of the functional group. The remaining downfield resonances in the ^{13}C NMR can then be used to identify C=C double bonds and aromatic rings present in the molecule. Below is shown a diagram of the expected chemical shift ranges of functional groups in the ^{13}C spectrum.



Although *nearly all* peaks beyond 100 ppm in the ^{13}C spectrum are sp^2 , sometimes sp^3 carbons bearing two or three electronegative atoms may be found this far downfield (chemical shift effects are more or less additive). If the molecule contains no symmetry, then each line represents a single carbon in the ^{13}C NMR spectrum and the number of sp^2 carbons can simply be counted.

An unknown that displays symmetry in the ^{13}C NMR requires determining which lines represent symmetrical carbons. Unfortunately, the number of carbons cannot be determined by integration of the ^{13}C spectrum (carbons bearing no hydrogen substituents will give smaller peaks). One way to approach this problem is to use the integration of the ^1H NMR spectrum to identify protons that are related by symmetry and then extrapolate this knowledge to the ^{13}C NMR spectrum.

The first thing you should do when you get your ^{13}C NMR spectrum is to make sure you know which peaks are from the deuterated solvent (CDCl_3 , CD_3COCD_3 , etc.) and possible impurities. If your ^{13}C NMR spectrum displays exactly the number of lines *equal* to the number of carbons, then your unknown has no symmetry. If your unknown displays *fewer* lines than number of carbons, then some of the carbons in your unknown are related by symmetry or free rotation.

C. ANALYSIS OF ^1H NMR COUPLING PATTERNS

1. Construction of Spin-Spin Coupling System.

The first stage of the analysis is to **match** up the coupling constants for all of the first order multiplets. For each multiplet in a proton NMR spectrum, another resonance that shares the same coupling constant must exist. For molecules that contain phosphorus or fluorine, many nearby protons may couple to these atoms. For molecules that do not contain these atoms, every coupling constant must be shared by another proton in the ^1H

NMR spectrum. Usually, coupling constants will match with each other to within approximately 0.2 Hz. Furthermore, if a methyl group is a doublet, then some other proton in the spectrum must appear as a 1:3:3:1 quartet or have this pattern combined with some other coupling constants. Multiplets be interpreted correctly as described in the Appendix.

After the coupling constants are matched between each other in the ^1H NMR spectrum, their relationships must be shown using a spin system. To accomplish this task, some decisions must be made about whether a given H-H coupling constant is **geminal, vicinal, or long-range**. If a coupling constant is large (>3 Hz), it may be assumed to be geminal or vicinal; these should be considered first. A series of vicinal coupling constants can be used to construct a chain of the organic compound.

The coupling information, if analyzed correctly, offers valuable and reliable information about the structure of a new compound.

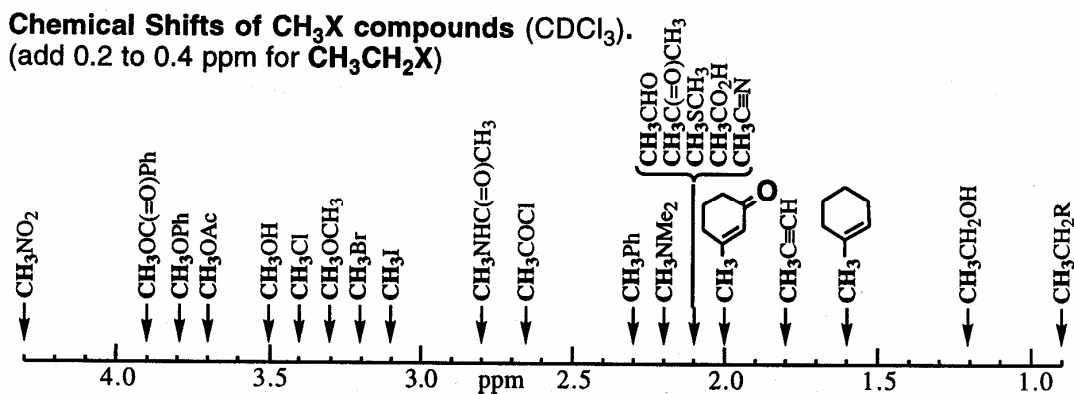
2. **DEPT** spectra provide the information necessary for distinguishing CH_3 , CH_2 and CH groups. The signals of quaternary carbon nuclei and CDCl_3 are missing.

3. **gCOSY** spectra provide information regarding coupled pairs of protons separated by two or three bonds. In the chart that correlates signals of a normal ^1H NMR with each other, cross-peaks appear if spin coupling is present. This technique is useful for finding connectivity and the chemical structure.

4. **HETCOR** is a two dimensional C, H correlation experiment that yields cross signals for all the protons and ^{13}C nuclei which are connected by a J_{CH} over one bond.

D. ASSEMBLAGE OF MOLECULAR SUBUNITS

Methyl and ethyl substituents in an unknown structure can reliably be assigned connectivity to different groups based on their chemical shift values. Because the chemical shifts of peaks in the ^1H NMR spectrum are subject to subtle effects of solvent and structure, chemical shifts of other protons can be difficult to interpret beyond the order of 0.5 to 1 ppm.

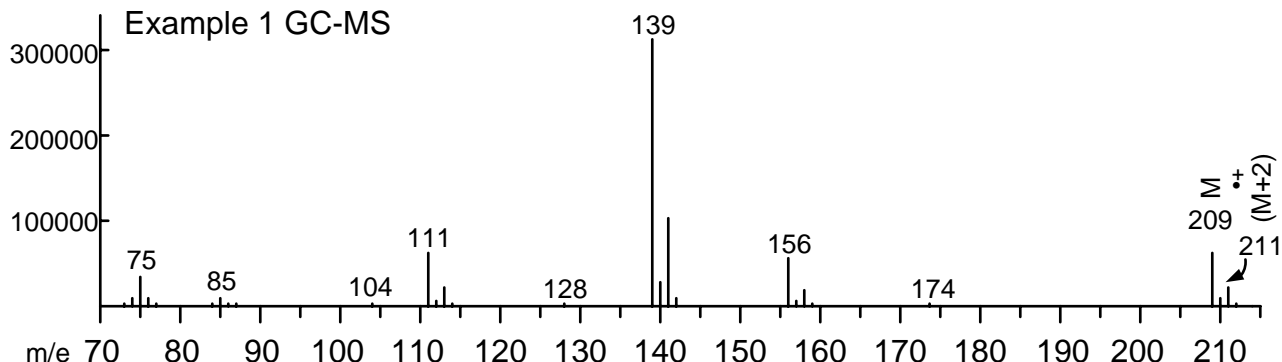


Peaks in the mass spectrum can be very helpful in piecing together the molecular subunits found above. For molecules that contain bromine or chlorine atoms, it becomes especially easy to follow the fragments that contain the halogen atom isotopic pattern.

E. CONFIRMATION OF STRUCTURE

The final stage of the analysis involves bringing together all of the data of the unknown compound and confirming that all of the data can be correctly rationalized for the structure proposed.

EXAMPLE UNKNOWN



1. Determination of Molecular Formula (and Unsaturation number)

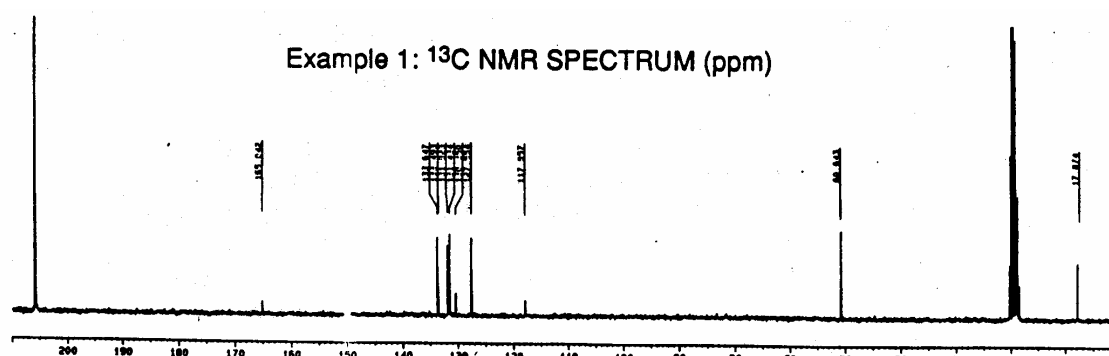
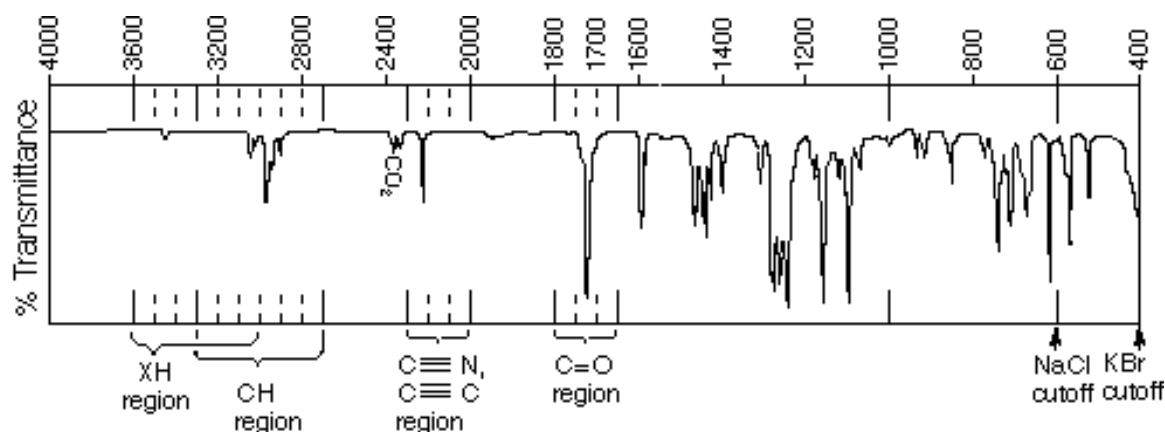
The GC-mass spectrum shows a highest ion pattern at m/e 209, which will be assumed to be M^{+} . From the intensity of the $(M+2)^{+}$ peak, one Cl is clearly present (^{35}Cl 76%, ^{37}Cl 24% nat. abund.). Using the elemental analysis data, the number of C,H,N,S atoms can be obtained:

56.94% x 209 ÷ 12.01 = 9.91	57.74% x 209 ÷ 12.01 = 10.05	10 C
3.84% x 209 ÷ 1.01 = 7.95	4.64% x 209 ÷ 1.01 = 9.60	8 or 9 H
5.94% x 209 ÷ 14.01 = 0.886	6.74% x 209 ÷ 14.01 = 1.005	1 N

The $\pm 0.4\%$ error does not allow the determination of the exact number of H atoms in the molecular formula. However, from the ^1H NMR integration, 8H are clearly present. The ^{13}C NMR spectrum shows 10 lines, which is consistent for 10 C if the molecule has no symmetry. Adding up the groups so far, $10 \times 12 + 8 \times 1 + 1 \times 14 + 1 \times 35 = 177$. $209 - 177 = 32$. (Note that 35 is used for the mass of the ^{35}Cl isotope corresponding to the larger peak at m/e 209.) The molecular formula is, therefore, **$\text{C}_{10}\text{H}_8\text{NO}_2\text{Cl}$** . Unsaturation # = $10 - 4.5 + 0.5 + 1 = 7$.

Molecular Formula & MW	Elemental Anal. calc. obsd.	GC-MS Highest Ion Pattern	^1H NMR integration	^{13}C NMR # of lines
$\text{C}_{10}\text{H}_8\text{NO}_2\text{Cl}$ MW 209.64	C 57.34% H 4.24% N 6.34% S 0.0% each $\pm 0.4\%$	100% 13.0% 36.1% 3.5% M^{+} 209 210 211 212	1:2:1:2:2	10 lines

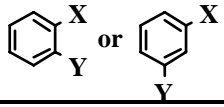
Unsaturation Number (# of double bonds or rings): 7

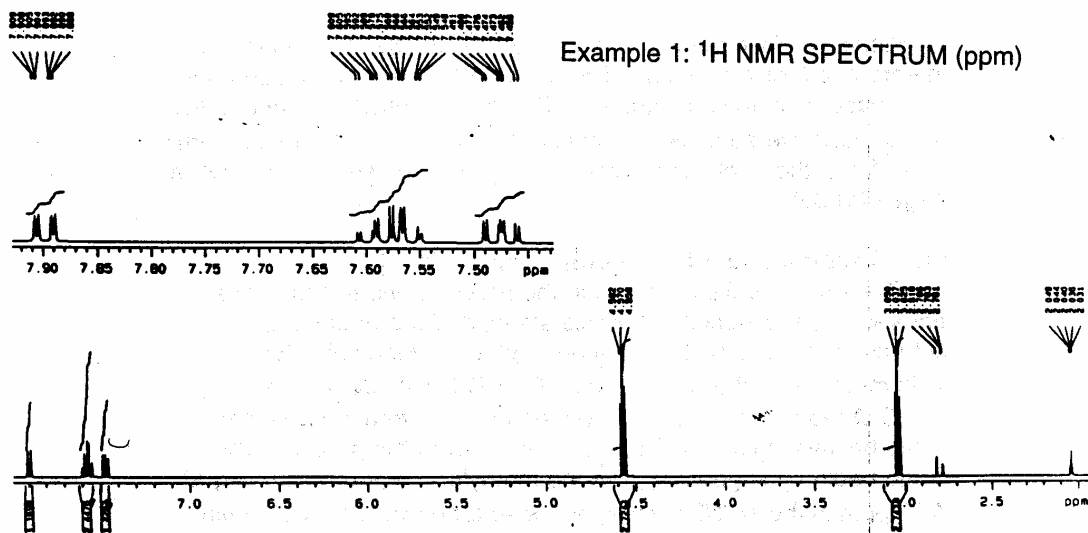


2. Identification of Unsaturated Functional Groups

A cyano group (2254 cm^{-1}) and a carbonyl group (1732 cm^{-1}) are clearly present in the IR spectrum and are entered into the worksheet. Because 10 lines are found in the ^{13}C NMR spectrum for 10 C, each line represents exactly one carbon of the structure. In this example, six peaks left in the 120-150 ppm region of the ^{13}C NMR represent six sp^2 carbons (formally three double bonds). Because four protons are found in the aromatic region of the ^1H NMR, a disubstituted benzene ring satisfies this data and accounts for the remaining 4 units of unsaturation. This aromatic ring must be either *ortho* or *meta* substituted, not *para*. (Free rotation would result in only four ^{13}C lines for a *para*-disubstituted aromatic ring.)

Unsaturated Functional Group	IR (cm ⁻¹)	^{13}C NMR		^1H NMR		Unsaturation No. of FG
		ppm	# of lines, #C	ppm	#H	

-C≡N	2254	118.0 ppm	1 line = 1 C	---	2
>C=O	1732	165.0 ppm	1 line = 1 C	---	1
	v(CH)	133.6, 133.5, 131.8, 131.4, 129.2, 127.6	6 lines = 6C	7.90, 7.59, 7.56, 7.47	4H
Remaining Molecular Symmetry: None.				Remaining Unsaturation: None.	



3. Analysis of ¹H NMR Coupling patterns

To determine whether the aromatic ring is *ortho* or *meta* disubstituted, the ¹H NMR spectrum must be analyzed carefully. The coupling constants between aromatic protons will tell the relationship of the two protons to each other ($J_{ortho} \approx 6-8$ Hz, $J_{meta} \approx 2$ Hz, $J_{para} < 1$ Hz). Careful analysis of the three ddd patterns leads to the numerical coupling constants shown in the table. Match as many of the coupling constants (within a ± 0.2 Hz error allowance) as possible. Protons that share a common coupling constant are assigned as *coupled to each other* by that coupling constant. H_b is coupled to H_d with $J = 7.0$ Hz so H_b and H_d are *ortho* to each other. Because H_b also has a $J = 8.0$ Hz coupling constant, another proton is also *ortho* to H_b , likewise for H_d . Therefore, the aromatic ring is *ortho*-disubstituted and the proton assignments are as shown below.

¹ H NMR (500 MHz, d ₆ -acetone solvent)			¹ H NMR Spin System	
label	δ (ppm)	(multiplicity, #H, J values)		

a	7.90	(ddd, 1H, $J_{ac}=0.5$, $J=2.0$, $J=8.0$ Hz)
b	7.59	(ddd, 1H, $J=2.0$, $J_{bd}=7.0$, $J=8.0$ Hz)
c	7.56	(ddd, 1H, $J_{ac}=0.5$, $J=2.0$, $J=8.0$ Hz)
d	7.47	(ddd, 1H, $J=2.0$, $J_{bd}=7.0$, $J=8.0$ Hz)
e	4.57	(t, 2H, $J_{ef}=6.0$ Hz)
f	3.04	(t, 2H, $J_{ef}=6.0$ Hz)

Similarly, resonances **e** and **f** are each 6.0 Hz triplets and integrate to 2 protons. This result is consistent with the assignment for adjacent methylene groups. For each methylene group, the two protons are equivalent to each other in the ^1H NMR spectrum. Therefore, the methylene groups are not diastereotopic.

4. Assemblage of Molecular Subunits

The four subunits identified in the previous two sections are entered into the worksheet at this stage. The remaining subunits are a chlorine substituent and an oxygen substituent. This molecule is therefore a chain, Cl-A-B-C-D-C-N, where A,B,C,D are the four disubstituted groups. Several approaches could be investigated to determine the order of the groups. One could begin by suggesting that the methylene group resonating at 4.57 ppm in the ^1H NMR spectrum is so far downfield that it must be attached to oxygen. Similarly, the values of other peaks in the IR, ^1H NMR and ^{13}C NMR spectra may be considered.

^1H NMR Subunits	Remaining ^{13}C NMR subunits	Remaining subunits
 	$\text{N}\equiv\text{C}-$ (26) $\text{O}=\text{C}$ (28)	$\text{Cl}-$ (35) $- \text{O} -$ (16)

One very effective method of determining the arrangement of groups in a molecule consisting of a chain of subunits is to analyze the fragmentation ions in the mass spectrum. Remember that each peak in the spectrum represents the detection of a stable positive ion, either a cation (A^+) or a radical cation ($\text{A}^{\bullet+}$), resulting from a single cleavage event. In this case, the presence of the Cl substituent is particularly easy to detect. The very small m/e 174 peak that is $(\text{M}-\text{Cl})^+$ does not reveal much about the structure. However, each of the fragment ions 156, 139 and 111, display the characteristic chlorine isotopic pattern and are very helpful.

m/e 111: The 111 fragment is the easiest fragment to identify. The isotopic pattern reveals that it bears the Cl atom (m/e 35). The Cl atom is connected to the benzene ring (m/e 76).

m/e 139: The next highest fragment bears the Cl group too and is 28 mass units larger than m/e 111. So, either the $>C=O$ group or the $-CH_2CH_2-$ group (m/e 28) is next in the chain.

m/e 156: The 156 peak bears the chlorine substituent and resulted from a cleavage of 53 mass units from M^+ . What mechanism results in the loss of 53 mass units from M^+ given the subunits listed above? Ans: A rearrangement occurred losing the cyano group (26 mass units) and the C_2H_3 portion (27 mass units) of the $-CH_2-CH_2-$ piece. (McLafferty Rearrangement)

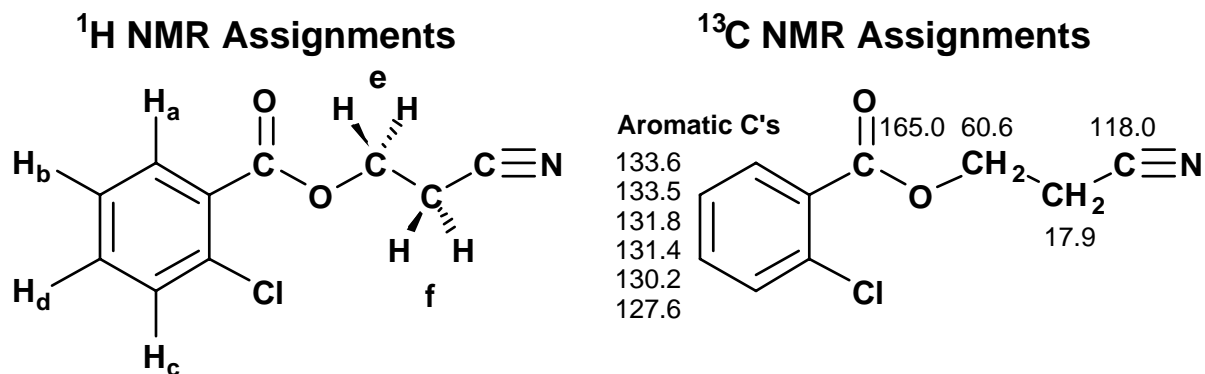
Important Mass Spectrum Fragment Ions							
m/e	(M+1) %	(M+2) %	Possible Assignments	m/e	(M+1) %	(M+2) %	Possible Assignments
209	13.0%	36.1%	M ^{•+}	139	8.7%	33.5%	35+76+28=139
174	---	---	(M-35) ⁺	128	---	---	?
156	7.8%	30.4%	(M-27-CN) ^{•+}	111	9.1%	34.5%	35+76=111

5. Confirmation of Structure

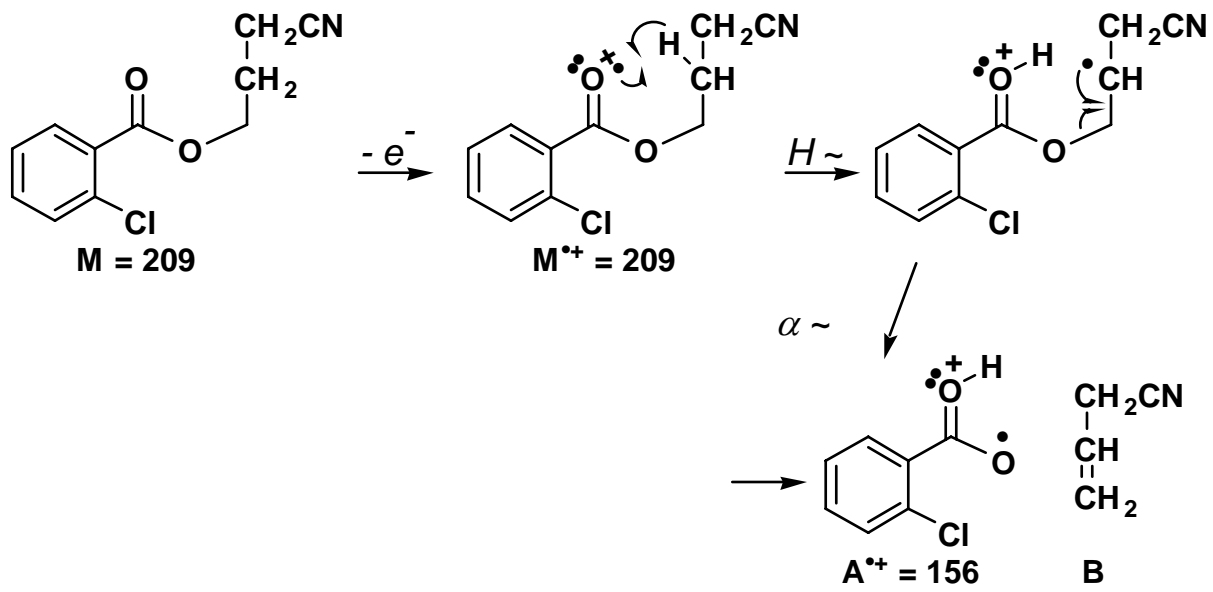
From the preceding analysis, the molecular subunits are assembled as follows: Cl - *ortho*-disubstituted aromatic ring - carbonyl - oxygen - CH₂CH₂ - and - C-N. The final step of the analysis involves drawing the structure carefully and bringing all remaining spectral data together for consideration.

In the ¹H NMR spectrum, because a mirror plane of symmetry runs down the length of the molecule, the protons denoted **e** are equivalent to each other and so are observed as a single resonance. Same for the protons labelled **f**. Although **e** and **f** are rigorously an AA'XX' system, free rotation of the bond between them apparently allows each to be observed as triplets.

In the ¹³C NMR spectrum, the table in the Appendix can be used to assign many of the carbon resonances in the spectrum. However, because the aromatic carbon resonances are bunched together, they cannot be individually assigned.



For the mass spectrum, drawing the mechanism for two or more of the prominent peaks in the spectrum is good practice. Shown below is the McLafferty Rearrangement that leads to the peak at m/e 156 and the ³⁷Cl isotope peak at m/e 158.



FIRST REPORT

RESULTS (5 points)

Discuss the relative polarity of the two compounds and any other information learned about the compounds based on TLC analysis and acid/base extraction. Briefly describe the separation of the two components of your mixture. Describe the conditions used for the purification of each of the compounds including the solvents for recrystallization, temperatures, etc. Report the melting point and melting range of each purified compound and use those numbers, as well as TLC, to assess the purity of the compounds.

QUESTIONS (5 points).

You will find out the structure of three compounds based on spectral information provided.

FINAL REPORT

Briefly describe the procedure used for the separation and purification of each of the two components of your unknown mixture. Include the conditions used for recrystallization as well as the % recovery from recrystallization. Discuss the evidence for purity based on TLC, melting point analysis, and ^1H NMR.

Identification of Acid Unknown

Present the spectral data obtained for your acid unknown. Tabulate the features of the ^1H NMR spectrum. Locate in reference (2), above the table of known compounds with the functional groups of your acid unknown, from the tables containing carboxylic acids or phenols. Tabulate the *names and structures* of all compounds with melting points within 6° of your acid or base unknown in the report. Eliminate as many structures as possible on the basis of your accumulated data (*explain your reasoning*). Describe the preparation of the derivative, which you carried out to confirm the structure of your unknown.

Identification of Neutral Unknown

Present the spectral data obtained for your neutral unknown. For each spectrum, note such important experimental conditions, such as the solvent in which they were taken, and point out any features that are believed to be artifacts or impurities. For the ^1H NMR spectrum, tabulate the features of the spectrum including integrations, multiplicities and coupling constants (where possible). For the ^{13}C NMR spectrum, tabulate the peaks for the spectrum and note the solvent and position of the solvent peaks. Similarly present the results of the IR, GC-MS spectra in a tabular fashion. Describe in a general sense how each of the spectroscopic methods is most powerful for the analysis of unknowns, i.e. detection of certain elements, determination of molecular weight, determination of molecular formula, sensitivity to symmetry, electronic effects, etc.

Proceed to determine the molecular formula of your neutral unknown. Describe how each of the methods ^1H NMR, ^{13}C NMR, DEPT, gCOSY, HETCOR, elemental

analysis and GC-MS can be valuable in this effort and what their possible shortcomings can be. Ultimately, your molecular formula must be compatible with all of these techniques.

Deduce the structure of your neutral unknown. *Discuss your reasoning in detail.* Show on separate structures of the unknown your assignments of the proton and ^{13}C resonances. Valuable insight concerning the interpretation of ^1H NMR spectra may be obtained by inspection of molecular models of proposed structures. Make assignments of the prominent peaks in the IR spectrum. Also, show how one or more of the fragmentation patterns in the mass spectrum are consistent with your structure. All of these methods will be valuable in determining the structure of your neutral unknown. If you performed a functional group classification test, describe how this supports your proposed structure.

Submit to your TA each purified unknown so that its purity, mp, etc. can be checked.

NEUTRAL UNKNOWN WORKSHEET

Molecular Formula & MW	Elemental Anal. calc. obsd.	GC-MS Highest Ion Pattern	¹ H NMR integration	¹³ C NMR # of lines

Unsaturation Number (# of double bonds or rings): _____

Unsaturated Functional Group	IR (cm ⁻¹)	¹³ C NMR ppm # of lines, #C	¹ H NMR ppm #H	Unsaturation No. of FG
Remaining Molecular Symmetry:			Remaining Unsaturation:	

¹ H NMR (MHz, solvent)		
label	δ (ppm)	(multiplicity, #H, J values)

¹ H NMR Spin System

Subunits	Remaining ¹³ C NMR subunits	Remaining subunits

Important Mass Spectrum Fragment Ions							
m/e	(M+1) %	(M+2) %	Possible Assignments	m/e	(M+1) %	(M+2) %	Possible Assignments

Assembled Subunits	Reasoning

Structure of Unknown showing ^1H NMR and ^{13}C NMR assignments	Mass Spectral Fragmentation Mechanisms