APPENDIX 1

INSTRUMENTATION

A section of this laboratory manual details the operation and theory associated with the instruments that you will be using in 5.32. Please study the instructions described in for each instrument before you embark on using them for the first time. Use of any instrument requires reading this section, learning from one of the TAs how to operate this instrument, and demonstrating to the TA's satisfaction that you are capable of handling the instrument properly.

INSTRUMENT SIGN-UP RULES

Sign-up schedules are posted for all major instruments (NMR, IR, UV, and GCMS). Schedules for use of these instruments are very tight; therefore, significant abuses of sign-up privileges cannot be tolerated. In each case, note the following **Sign-Up Rules**:

1. Sign up for time on instruments no more than two days in advance.
2. If you discover that you are not able to use the time that you have signed up for, then be sure to erase your name in advance so that someone else can use it. The TA's will let you know if you violate this rule.
3. Show up promptly for time for which you have signed up.
4. For your first NMR spectrum, you will need a full 30 minute slot and should bring **only one** sample. Once you are proficient in using the instrument, a normal NMR spectrum will require 15-25 minutes (one 30 minute slot should be more than sufficient for one sample and probably two samples could be done). You are not allowed to sign up for more than two 30-minute slots on a single day.

FTIR SPECTROPHOTOMETER

The Instrument

In a conventional IR spectrophotomer, a sample IR beam is directed through the sample chamber and measured against a reference beam at each wavelength of the spectrum. The entire spectral region must be scanned slowly to produce good quality spectrum. In 5.32, we will be using a Nicolet FTIR Spectrophotometer (Nicolet was heavily involved in the design of the Hubble telescope!). IR spectroscopy has been dramatically improved by the development of the Fourier Transform method in much the same way as NMR has been revolutionized by this method.

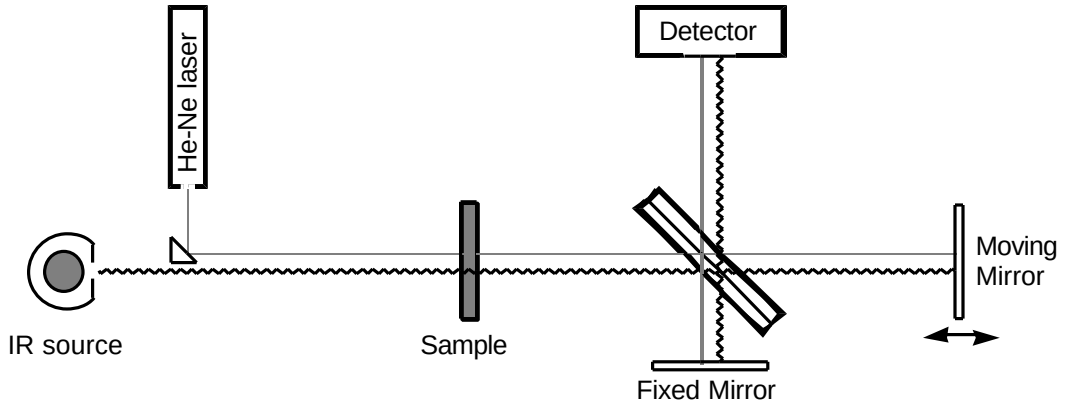


Diagram of the Michelson Interferometer used in an FTIR Spectrophotometer.

The heart of an FTIR Spectrophotometer is a Michelson Interferometer built around the sample chamber. Radiation from an IR source is directed through the sample cell to a beam splitter. Half of the radiation is reflected from a fixed mirror while the other half is reflected from a mirror which moved continuously over a distance of about 2.5 micrometers. When the two beams are recombined at the detector, an interference pattern is produced. A single scan of the entire distance takes about 2 seconds and is stored in the computer. In order that several scans may be added, they must coincide exactly. Obviously, this would be impossible considering the thermal fluctuations and vibrations in the laboratory. In order to solve this problem, a helium-neon laser is simultaneously directed through the Michelson Interferometer and the interference pattern of the laser is used as a frequency reference.

The performance of an FTIR is dramatically superior to that of conventional instruments. Generally, only a small amount of sample will produce an excellent spectrum in a fraction of the time.

Preparation of the sample: Due to the sensitivity of the FTIR instrument, the most convenient and satisfactory method involves simple evaporation of a solution of the sample (chloroform, ether, dichloromethane; or even a $CDCl_3$ NMR sample may be used) onto a KBr salt plate and acquisition of the spectrum from the thin film remaining. This method provides excellent spectra with flat baseline unless the thin film is too powdery in which case excessive scattering of the light leads to an irregular baseline. The sample may alternatively be prepared as a nujol mull (mull accessories: agate mortar and pestle, nujol and NaCl discs may be obtained from LS).

Preparation of Instrument: If the instrument has just been turned on, then it is necessary to run a TEST (**F10**) to be sure that all components are ON. If the instrument is not turned on or does not check out when the TEST is performed, then ask the instrument TA for help. In addition, it is **important** that N_2 is flowing through the chamber so that most of the CO_2 and H_2O are flushed from the chamber and from inside the instrument.

F4 SCAN BACKGROUND is performed with a *blank* IR plate in the chamber.

F8 then **F4** DISPLAY BACKGROUND will show the spectrum of CO_2 and H_2O that remain in the chamber. If the background shows excessive CO_2 and H_2O, then be sure the N_2 is flowing briskly, wait a minute or two and try again. Once a good background has been obtained, several students in succession can use the same background.

Scanning the Sample: Place the sample plate in the FTIR and wait for N_2 to purge out the air.

F5 SCAN SAMPLE. Wait until the scan and Fourier transform are completed.

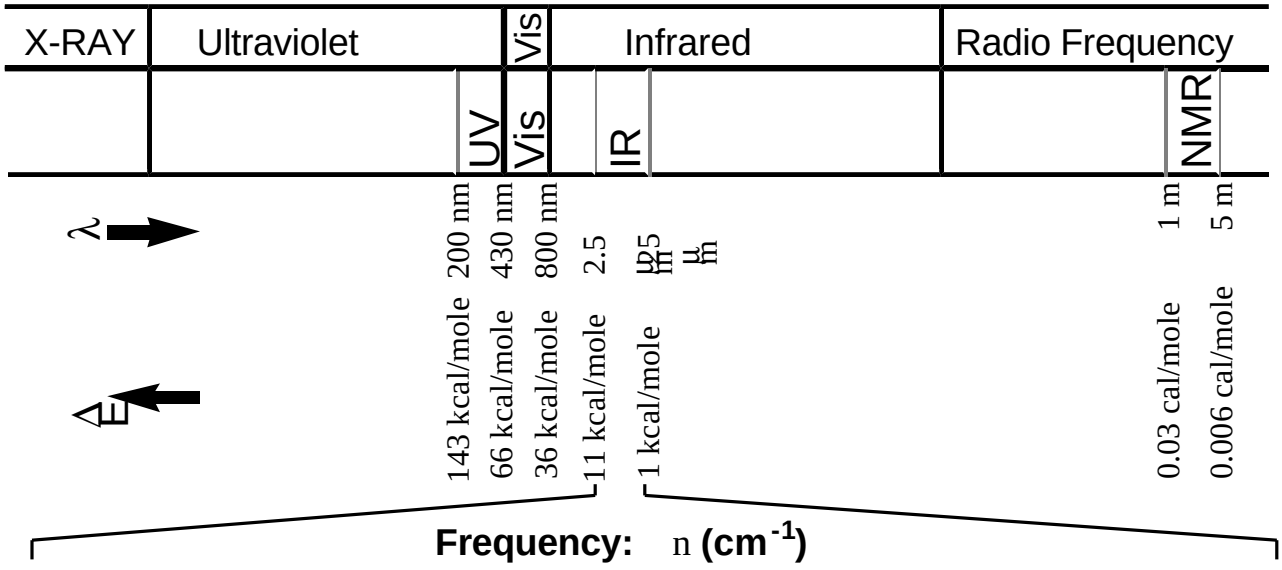
F8 then **F1** DISPLAY SPECTRUM will automatically subtract the stored background and display the spectrum.

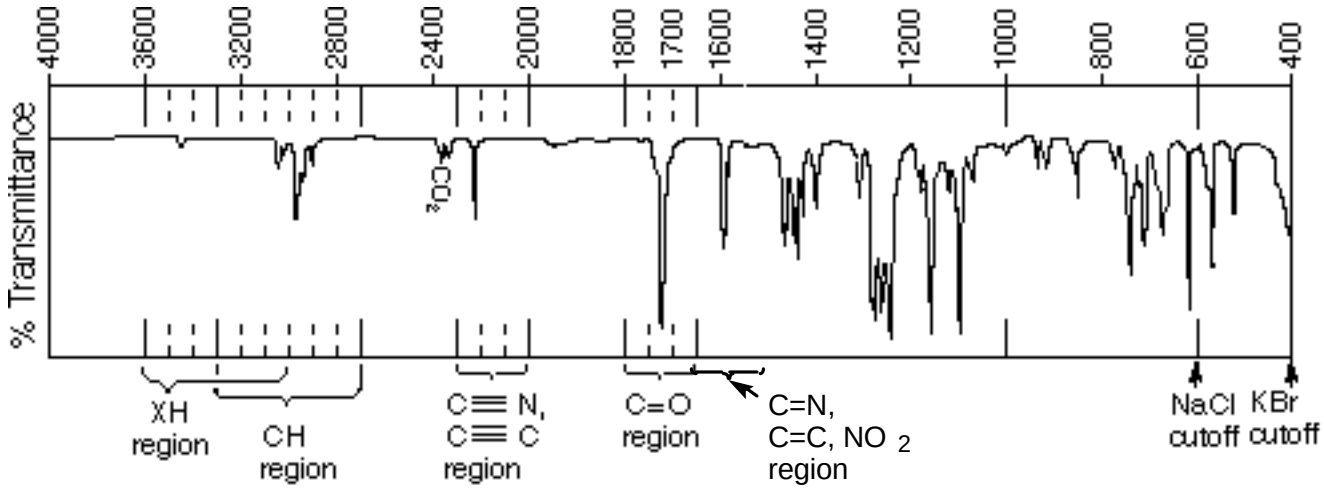
F7 PRINT. **Important:** Make sure that the printer is on-line before pressing F7.

Type **PEAKPICK S 4000 600** to find the peaks in the spectrum. This data is printed by pressing **F7** . If no one else is using the instrument next, please turn off the nitrogen purge.

PRINCIPLES OF IR SPECTROSCOPY

The different regions of the electromagnetic spectrum will be used in this section to learn about the structure and reactions of organic molecules. For each spectroscopic method, it is helpful to understand *how much* energy corresponds to each wavelength and how this relates to the physical process after absorption of radiation. Organic molecules can absorb IR radiation between 4000 cm^{-1} and 400 cm^{-1} which corresponds to an absorption of energy between 11 kcal/mole and 1 kcal/mole. This amount of energy initiates transitions between vibrational states of bonds contained within the molecule.

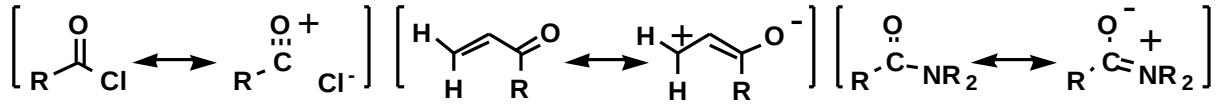




IR spectroscopy is a very powerful method for the identification of functional groups. The most important regions of the IR spectrum are >1650 cm^{-1}, whereas the fingerprint region (600 - 1500 cm^{-1}) of the spectrum cannot easily be used for identification of unknown compounds. Many references exist which tabulate the IR frequencies for various functional groups and organic compounds (a short table appears at the end of this section). However, **the most valuable** resource available to you for the interpretation of IR spectra is understanding the **five basic principles** of IR spectroscopy. Transitions between vibrational energy levels follow the same equation as for a classical harmonic oscillator:

Equation for the Classical Harmonic Oscillator: $\bar{n} = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$ $\qquad \mu = \frac{m_1 m_2}{m_1 + m_2}$

1) **k is the force constant.** **k** is proportional to bond strength or bond order. C=O vibrates at a higher frequency than C-O. Furthermore, the change in the force constant of different carbonyl groups can be understood based on the contribution of resonance structures. The base value for the stretching frequency of a carbonyl (e.g., acetone) is ν_{CO} ~ 1715 cm^{-1}. Acid chlorides have bond order slightly greater than 2 because an acylium ion resonance structure may be drawn (ν_{CO} 1800 cm^{-1}). Alternatively, Phenyl ketones, vinyl ketones and amides have a CO bond order slightly less than 2 and display a lower energy ν_{CO}.



Acid Chloride
ν_{CO}= 1800 cm^{-1}

Methyl Vinyl Ketone
ν_{CO}= 1675 cm^{-1}

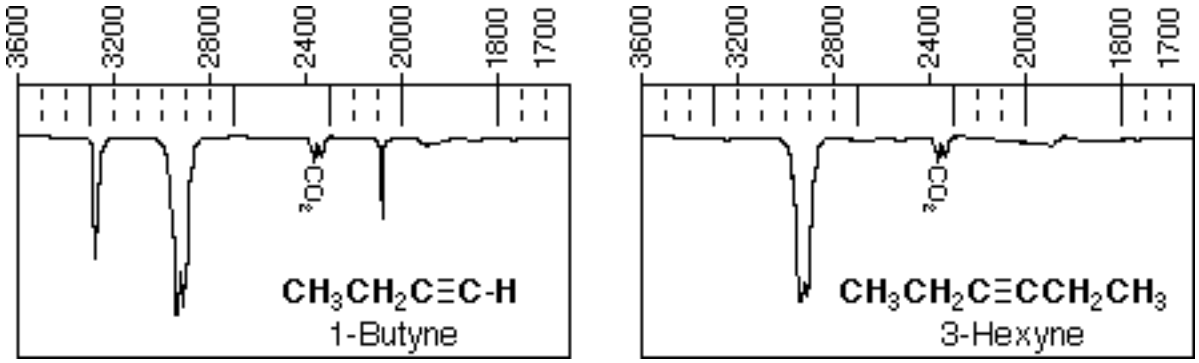
Amide
ν_{CO}= 1650 cm^{-1}

2) μ **is the reduced mass.** Heavier atoms slower vibration, lower energy. Compare C-O vs. H-O or H-O vs H-S.

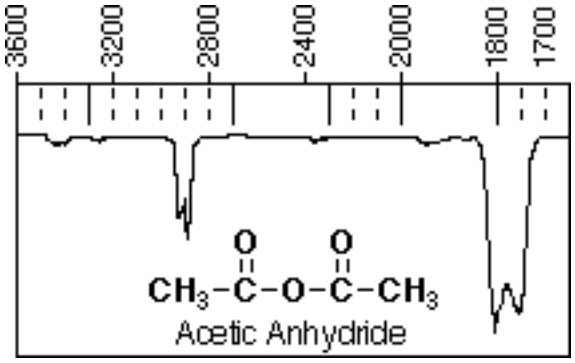
3) **Overtone Peaks.** Notice in the above spectrum that a small peak is found at 3450 cm^{-1}, even though the compound does not contain any O-H or C-H bonds. This peak is the overtone of the C=O vibration (at 1735 cm^{-1}). It corresponds to the transition from the ground vibrational state (n=0) to the second vibrationally excited state

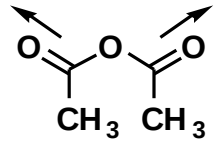
(n=2) rather than the first. Carbonyl overtones are always small and are easily found at slightly less than twice the normal C=O frequency.

4) **Dipole moment.** The strength of an IR peak is roughly dependent on the change in dipole moment during vibration. C=O bonds are very polar because of the greater electronegativity of oxygen and so give very intense bands. Also note that if a molecule is so symmetrical that the stretching of a bond does not produce any change in dipole moment, then *no* IR peak will be found in the spectrum. Compare the spectra of 1-butyne and 3-hexyne. 1-butyne shows an alkyne C-H stretch at 3280 cm^{-1} and an alkyne C≡ C stretch at 2080 cm^{-1}. -hexyne shows no C≡ C stretching peak.

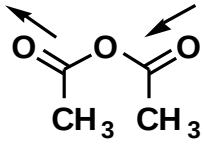


5) **Vibrational Modes.** The vibrations of two neighboring bonds can be coupled into *symmetric* and *antisymmetric* vibrational modes. One example is the vibration of CH_2 groups within an alkane (or the NH_2 group of a primary amine). The symmetric stretch requires slightly more energy (2925 cm^{-1}) for a transition while an antisymmetric stretch requires slightly less energy (2850 cm^{-1}). For acetic anhydride, notice that although the two C=O groups are identical by symmetry, two peaks are found in the C=O region of the IR spectrum.





Symmetric Stretch
(v = 1810 cm^{-1})



Antisymmetric Stretch
(v = 1750 cm^{-1})

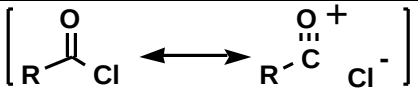
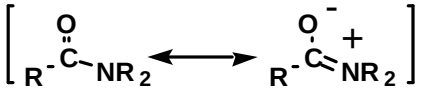
If a functional group's normal vibrational frequency happens to coincide in frequency with a weak overtone peak of a neighboring bond, then the peak will be observed as a *Fermi doublet*. In the case of aliphatic aldehydes, the aldehydic C-H stretching frequency at 2720 cm^{-1} couples with the overtone of the C-H bending transition at 1380 cm^{-1}. Fermi coupling also explains the observation of two peaks near 2300 cm^{-1} in the spectrum of CO_2.

CHARACTERISTIC IR FREQUENCIES

XH Region (3600 cm^{-1} to 2400 cm^{-1})				
cm^{-1}		**comments**		
3600	ν(free OH)	Sharp peak	Alcohol or Phenol free OH	
3600-2800	ν(H-bonded OH)	Very Broad peak:	Alcohol:	3400 to 3200 cm-1
			Phenol:	3600 to 3000 cm-1
			Carboxylic Acid:	3600 to 2400 cm-1
3500-3300	ν(NH)	Amines show broad peaks, Amides show sharp peaks Primary Amines display two peaks (ν_s and ν_{as})		

CH Region (3300 cm^{-1} to 2700 cm^{-1})		
cm^{-1}		**comments**
3300	Alkyne ν(CH)	strong, sharp
3150-3000	Alkene or Phenyl ν(CH)	medium intensity
3050	Cyclopropane or Epoxide ν(CH)	weak
2960,2870	Alkane ν(CH)	ν_s(CH), ν_{as}(CH) observed for CH_2 or CH_3 groups
2750	Aldehyde ν(CH)	sharp, medium intensity

-C°N, -C°C-, >C=C=C< Region (2300 cm^{-1} to 2000 cm^{-1})		
cm^{-1}		**comments**
2250	ν(-C≡N)	sharp, weak to med intens, almost always observed
2150	ν(RC≡CH)	sharp, weak to med intens, check for ν(C-H) at 3300
2260-2190	ν(R-C≡C-R')	sharp, weak to med intens, obsd only for R,R' different
1950	ν(>C=**C**=C<)	sharp, strong allene

>C=O Region (1800 cm^{-1} to 1650 cm^{-1})		
cm^{-1}		**comments**
1800	Acid Chloride	 CO Bond Order >2
1820,1760	Anhydride	two peaks are observed (ν_s ν_{as})
1735	Ester	RCO_2R'
1755	Carbonate	$ROCO_2R'$
1735	Urethane	$ROCONR'_2$
1720	Aldehyde/Ketone	aldehyde has ν(CH) at 2750 cm^{-1}
1650	Amide	 CO Bond Order <2
1630	Urea	$R_2NCONR'_2$

C=N, C=C, NO_2 Region (1660 cm^{-1} to 1500 cm^{-1})		
cm^{-1}		**comments**
1690-1640	ν(C=N)	weak to med intensity, sharp
1660-1640	ν(C=C)	weak to med intensity, sharp
1590	ν(NO_2)	strong, sharp

PRINCIPLES OF NMR SPECTROSCOPY

One of the first things to do after obtaining your ^{1}H and ^{13}C NMR spectra is to identify the resonances associated with the solvent used for the NMR sample using the table below.

Properties of Deuterated NMR Solvents

	$$$ per sample	**^{1}H NMR**	**^{13}C NMR**
$CDCl_3$	$ 0.20	7.26 (1 peak)	77.01 (3 peaks)
CD_3COCD_3	$ 1.54	2.04 (5 peaks)	206.2 (1 peak) 29.8 (7 peaks)
CD_3SOCD_3	$ 2.76	2.49 (5 peaks)	39.6 (7 peaks)
C_6D_6	$ 1.73	7.15 (broad)	128.0 (3 peaks)

Peaks in the ^{13}C NMR spectra corresponding to the deuterated solvent molecules show unique or peculiar spin coupling patterns, making these especially easy to identify. This is particularly obvious for the ^{13}C NMR spectrum of $CDCl_3$ - coupling to one detuerium atom produces a 1:1:1 triplet. A deuterium atom has nuclear spin quantum number I=1 and so its possible spin states are +1, 0, -1 each leading to one peak of the multiplet. The ^{13}C NMR spectrum of CD_3COCD_3 displays a seven peak multiplet pattern characteristic for the -CD_3 group

coupling to one D atom | coupling to two D atoms | coupling to three D atoms

The appearance of the solvent resonances in the ^{1}H NMR spectrum arise from the *residual solvent molecules* which contain one H atom. The cost of deuterated NMR solvents is proportional to their level of isotopic purity and inevitably, some percentage of molecules will contain one H atom. The solvent $CDCl_3$ has a small amount of $CHCl_3$ present, so a singlet is found in the ^{1}H NMR spectrum at 7.26 ppm. The solvent CD_3COCD_3 contains a small amount of the contaminant CD_2HCOCD_3. The hydrogen of a -CD_2H group will show up at the same chemical shift as for a CH_3 group in acetone, but it will be spin coupled to two deuterium atoms (each spin 1). The result is the pattern above having five peaks.

For 5.32, all the organic compounds which we will work with will involve only the elements listed on the following page in their natural abundances. The spin-spin coupling behavior of these elements in ^{1}H and ^{13}C NMR spectra are easily understood. The important determinant of this feature is the spin (**I**) of the element involved.

I = 0 No spin-spin coupling effect is observed. Examples: ^{12}C, ^{16}O, ^{28}Si, ^{32}S.

I = 1/2 Spin-spin coupling with neighboring ^{1}H and ^{13}C atoms is always operative. Examples: ^{1}H, ^{31}P and ^{19}F. The magnitude of the coupling depends on the separation between atoms. Spin coupling with **I=1/2** nuclei is discussed in the next section.

I = 1 Quadrupolar Nuclei. Different effects are possible.
Deuterium is a weak quadrupolar nucleus. **I=1** multiplets observed

^{14}N is a stronger quadrupolar nucleus. Broad peaks are observed for NH protons. Cl,Br,I are strong quadrupolar nuclei. No coupling effect is observed.

Spin Coupling Effects of Elements found in Organic Compounds

		Natural Abund.	Nuclear Spin (I)	Observed Effect in ^{1}H and ^{13}C Spectra
H	^{1}H	99.98%	1/2	$^{2}J_{HH}$ (0-25 Hz), $^{3}J_{HH}$ (0-18 Hz), $^{4}J_{HH}$ (usually <2 Hz) $^{1}J_{CH}$ = 115 to 250 Hz, but is not observed in the standard (^{1}H decoupled) ^{13}C spectrum.
	^{2}H(D)	0.015%	1	$^{2}J_{HD}$ (2-4 Hz) $^{1}J_{CD}$ (15 to 35 Hz)
C	^{12}C	98.9%	0	No Effect (I = 0)
	^{13}C	1.1%	1/2	very small ^{13}C sattelites are observed in ^{1}H NMR
N	^{14}N	100%	1	^{14}N nucleus is strongly quadrupolar (I>1/2). This feature allows its spin state to change rapidly for most N-containing compounds. Therefore, protons bonded to Nitrogen appear as broadened peaks rather than triplets.
	^{15}N	0.37%	1/2	
O	^{16}O	99.8%	0	No Effect (I = 0)
	^{17}O	0.04%	5/2	
	^{18}O	0.20%	0	
F	^{19}F	100%	1/2	$^{2}J_{HF}$ (40-90 Hz), $^{3}J_{HF}$ (5-50 Hz) $^{1}J_{CF}$ (200-300 Hz)
Si	^{28}Si	92.2%	0	No Effect (I = 0)
	^{29}Si	4.7%	1/2	
	^{30}Si	3.1%	0	
P	^{31}P	100%	1/2	$^{2}J_{HP}$ (10-20 Hz), $^{3}J_{HP}$ (5-10 Hz), $^{4}J_{HP}$ (0-3 Hz) $^{1}J_{CP}$ (50-100 Hz), $^{2}J_{CP}$ (5-10 Hz), $^{3}J_{CP}$, $^{4}J_{CP}$
S	^{32}S	95%	0	No Effect (I = 0)
	^{33}S	0.8%	3/2	
	^{34}S	4.2%	0	
	^{36}S	0.02%	0	
Cl	^{35}Cl	76%	3/2	
	^{37}Cl	24%	3/2	Strong quadrupolar nuclei (I = 1)
Br	^{79}Br	50.7%	3/2	
	^{81}Br	49.3%	3/2	Spin state of Cl, Br or I changes so rapidly that the effect on other nuclei is averaged
I	^{127}I	100%	5/2	(No coupling observed in ^{1}H ^{13}C NMR)

ANALYSIS OF MOLECULAR SYMMETRY USING ^{13}C (AND ^{1}H) NMR SPECTRA

For most organic compounds, all peaks in the ^{13}C NMR spectrum are usually singlets. Why are multiplets not produced by spin coupling with ^{13}C and 1H nuclei (each have I=1/2)?

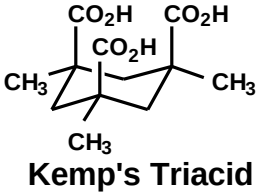
1) The low natural abundance of ^{13}C (only 1.1% compared to 98.9% ^{12}C) means that most individual ^{13}C atoms will have only ^{12}C atoms nearby which do not exhibit spin-spin coupling.
2) Although H atoms attached to each ^{13}C atom would normally be expected to couple with ^{13}C atoms, the acquisition of ^{13}C spectra is actually performed with deliberate decoupling of the ^{1}H nuclei by the instrument.

For these reasons, the **^{13}C NMR spectra** of most ordinary organic compounds exhibit **only singlet resonances** for each carbon in the ^{13}C NMR spectrum. This offers an exceptional opportunity to learn about the **symmetry** of a molecule by analyzing the ^{13}C NMR spectrum. Only compounds which contain D, F or P atoms will show multiplet peaks in the ^{13}C NMR.

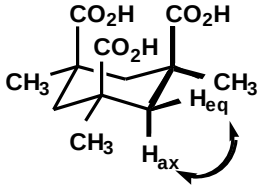
Due to the sharpness of singlet resonances in the ^{13}C NMR and the large chemical shift range (200 ppm), it is very unlikely that two carbons which are in *different* environments will display *one* singlet. If a molecule contains symmetry, then atoms or groups related by that symmetry element experience *identical* environments and one peak represents the two carbons. Unfortunately, the ^{13}C NMR cannot be integrated to tell how many carbon atoms are represented by each resonance. So you often have to figure out in a logical fashion which lines are representing equivalent carbon atoms. Since the ^{1}H NMR spectrum *can* be integrated to determine how many protons are represented by each resonance, one can use the integration of the ^{1}H NMR spectrum in a logical fashion to help analysis of the ^{13}C NMR. The examples which follow demonstrate different cases of molecular symmetry.

Case 1. Molecular Symmetry - Kemp's Triacid.

The structure of Kemp's triacid has three-fold rotational symmetry. Therefore, in the ^{13}C NMR spectrum only four lines are observed. In the ^{13}C NMR spectrum the three methyl groups are equivalent and are represented by one singlet and each of the three CO_2H groups are identical by symmetry. Similarly, each of the three CH_2 groups are identical to each other. But in the ^{1}H NMR, the three equatorial hydrogens (on the same side of the ring as the $-CO_2H$ groups) are in a different environment from the three axial hydrogens (on the same side as the methyl groups). Therefore, one doublet represents the equatorial hydrogens and the other doublet represents the axial hydrogens. Why are the CH_2 protons represented by a pair of doublets? because each of these protons is different from its geminal partner, it couples with it to form a doublet.

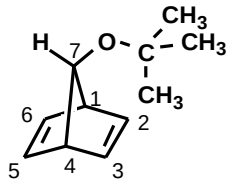


Kemp's Triacid

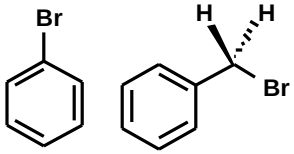


J_{gem} = 12.4 Hz

Here are some other symmetrical molecules: In the first case, a plane of symmetry parallel to the page bisects the bicyclic framework. Of the seven carbons of the bicyclic framework, C(1) and C(4) are identical environments. Similarly, C(2) and C(3) are identical. C(5) and C(6) are identical. C(2) is on the same side of the ring system as the hydrogen so is not identical to C(5) or C(6). Four ^{13}C resonances are observed for these seven carbons. The *tert*-butyl substituent will be discussed below.

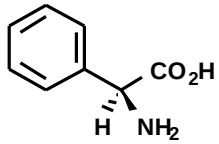


Bromobenzene also possess a plane of symmetry which renders the ortho carbons equivalent to one another and the meta carbons equivalent to one another Four ^{13}C peaks are found. Benzyl bromide possesses a plane of symmetry which renders the protons of the CH_2 group equivalent to one another in the ^{1}H NMR.



Case 2. Freely Rotating Substituents.

The compound phenylglycine displays **four aromatic carbon** signals in its ^{13}C NMR spectrum. This molecule contains no plane or axis of symmetry, but the phenyl group gives a symmetrical pattern. The phenyl group *freely rotates* about the single bond by which it is attached to the substituent. The equivalency of the two ortho carbons results because they experience *identical environments* over the course of this free rotation. For ordinary organic compounds, phenyl groups will always display this symmetry. In addition, *tert*-butyl groups can freely rotate and will render the three methyl groups equivalent in NMR spectra.

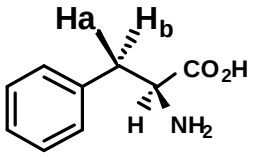


Phenylglycine

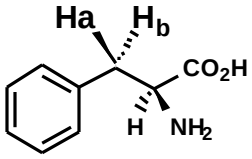
Case 3. Diasterotopic Groups. Phenylalanine and acetal

Let's look at the molecule phenylalanine. From the above discussion, a monosubstitued phenyl group rotates freely and therefore four aromatic peaks are expected in the ^{13}C NMR. We might be tempted to suggest that the two protons of the CH_2 group would be equivalent in the ^{1}H NMR spectrum. Both bonds attached to the CH_2 group can rotate freely and we already learned that the two protons of benzyl bromide are equivalent. In the spectrum of phenylalanine below, **two** resonances are observed for the two protons of the CH_2 group!

The two protons of phenylalanine are **not equivalent** even though free rotation is occurring. In order to understand this result, it is helpful to examine carefully the Newman projections of each of three staggered rotamers. H_a is between the CO_2H and NH_2 groups in rotamer A and H_b is between these groups in rotamer B. In rotamer A, H_a sees the phenyl group next to the NH_2 group. This is not the *same environment* that H_b sees when it is between the CO_2H and NH_2 groups in rotamer B (it sees the phenyl group next to the CO_2H group). Free rotation does *not* make H_a and H_b experience *identical environments*. The protons H_a and H_b are called diastereotopic protons and they experience diastereomeric environments.



Phenylalanine
H_a and H_b are diastereotopic



Phenylalanine
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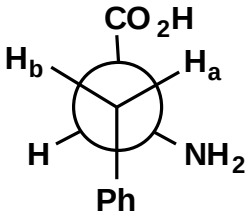
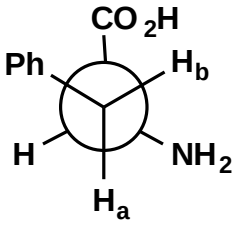
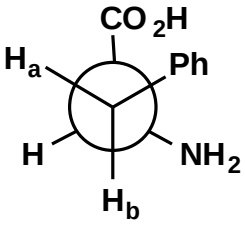


A



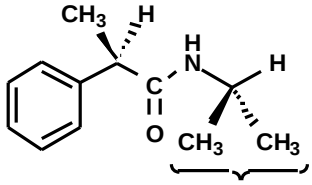
B



C

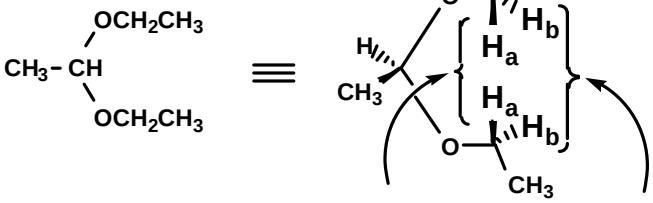
Benzyl bromide has equivalent protons for its CH_2 group because the entire molecule contains a mirror plane - it is achiral. Phenylalanine is a chiral molecule and so contains no mirror plane. As a rule, if a molecule is chiral, then all of its CR_2 substituents will be diastereotopic.

This can be seen for the amide compound shown below. Even though the carbon atom denoted by * is three bonds removed from the $C(CH_3)_2$ unit of the isopropyl group, two doublets are observed in the ^1H NMR spectrum for these two methyl groups.



Methyl groups are diastereotopic.
Two doublets are observed.

The last example of diastereotopic groups is acetal which is especially peculiar. Acetal is achiral. A mirror plane renders the two ethoxy groups equivalent to one another. However, the CH_2 protons of acetal are diastereotopic and two complex multiplets are observed in the ^1H NMR spectrum.



Diethyl Acetal (achiral)

H_a and H_b are diatereotopic.
J_{ab} = 9.4 Hz, J_{CH2CH3} = 7.1 Hz

^{1}H NMR Spectrum

ANALYSIS OF ^{1}H NMR COUPLING PATTERNS

First Order Coupling Patterns. Given the values of the coupling constants, it is a straightforward task to predict the appearance of the multiplet (Figure 1). A proton which couples to another proton with a 4.0 Hz coupling constant will be observed as a doublet with a 4.0 Hz separation of the peaks. If the proton is coupled to two protons with J = 4.0 Hz, it will appear as a 1:2:1 triplet, with each outer line separated from the taller center line by 4.0 Hz. (Similarly, coupling to three protons with J = 4.0 Hz is observed as a 1:3:3:1 quartet, see Pascal's triangle below.) In the case that the two coupling constants are different (J = 4.0, 12.0 Hz), four lines are observed. Although the appearance of the multiplets becomes more complicated as more spin 1/2 nuclei are added, the same procedure can be used to simulate any first order multiplet.

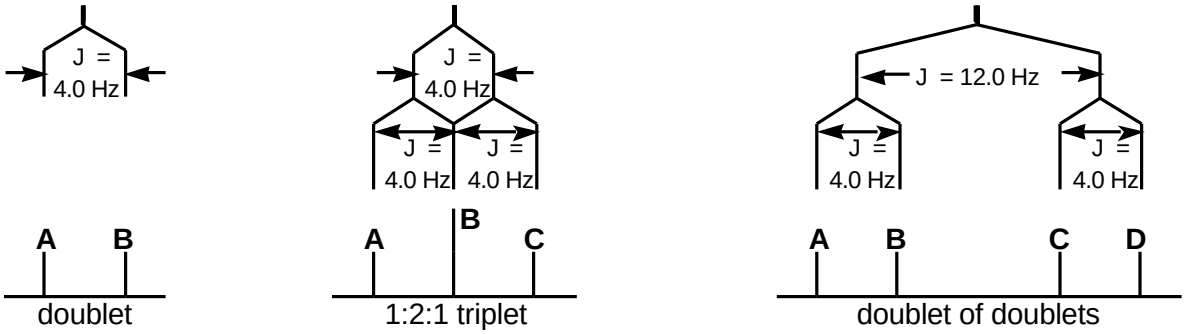
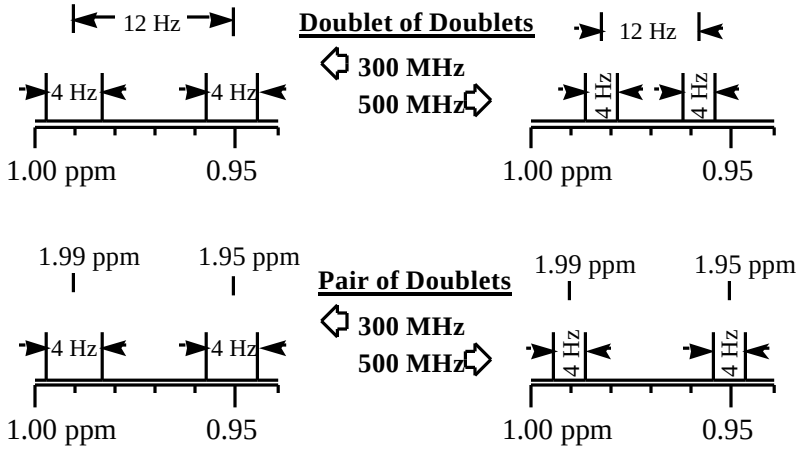


Figure 1. The doublet, triplet and dd patterns.

Extracting Coupling Constants from Simple First Order Spectra. The more difficult task is to extract the coupling constants from the appearance of a multiplet found in a spectrum. For the doublet of doublets (dd) just described, the smaller coupling constant J_1 = (line A - line B) = (line C-line D) and the larger coupling constant J_2 = (line A - line C) = (line B-line D). However, line B - line C is *not* a coupling constant of this multiplet, it is the difference between the two coupling constants, J_2-J_1 (similarly, line A - line D = J_1+J_2).

Note also that *not all* four line patterns can be analyzed as a doublet of doublets. Sometimes, such a pattern could be just two separate doublets (or even four singlets). The possibilities may be easily distinguished by comparing the spectra obtained at different magnetic fields and noting that coupling constants are constant in Hz, whereas chemical shifts are constant in ppm.



Coupling to n Equivalent Protons: Pascal's Triangle. When a proton couples to several (**n**) other protons with equal coupling constant J, **n+1** lines will be observed in relative intensities as shown in Pascal's triangle. As more adjacent protons are attached, the relative intensity of the outer lines diminish exponentially to the point where they may be lost in the baseline or mistaken for impurities. For example, $(CH_3)_2C\mathbf{H}CH_2Br$ has one methine proton which is coupled *equally*

to each of the eight adjacent protons. Although the observed ratio is expected to be 1:8:28:56:70:56:28:8:1, if the outer lines were not detected, the ratio of the remaining seven lines would be 1:3.5:7:8.75:7:3.5:1 and this might be mistaken for a simple quartet of quartets. Clearly, the outer lines of the multiplet are often the most important for correct recognition and this highlights the necessity for obtaining a ^{1}H NMR spectrum devoid of stray impurity peaks.

	All J's equal (Coupling to equivalent protons)			All J's different	
# of spin 1/2 nuclei	# of lines	Pascal's Triangle	2^n = Sum of intensities	maximum # of lines	possible multiplets
0	1	1	1		
1	2	1 1	2		
2	3	1 2 1	4	4	dd
3	4	1 3 3 1	8	8	ddd,dt
4	5	1 4 6 4 1	16	16	dddd,tt,...
5	6	1 5 10 10 5 1	32	32	ddddd,ddq,
6	7	1 6 15 20 15 6 1	64	64	dddq,qq,...
7	8	1 7 21 35 35 21 7 1	128	128	dqq,ttq
8	9	1 8 28 56 70 56 28 8 1	256	256	tqq

Coupling to Three Inequivalent Protons: The ddd Patterns. Several different ddd patterns are possible depending on the values of the coupling constants J_1,J_2 and J_3 (Figure 2). The maximum number of lines possible for coupling to three spin 1/2 nuclei is 8 (2^n) if no lines coincide (all lines would be expected to have equal intensity). Even when some of the lines coincide, the sum of the intensities of each line relative to that of the smallest (outer lines) will equal 8. The two cases below demonstrate that in order to extract the coupling constants from a ddd pattern, it is necessary to figure out if $J_3>J_1+J_2$ or if $J_3<J_1+J_2$ (J_1 is smallest, J_3 is largest).

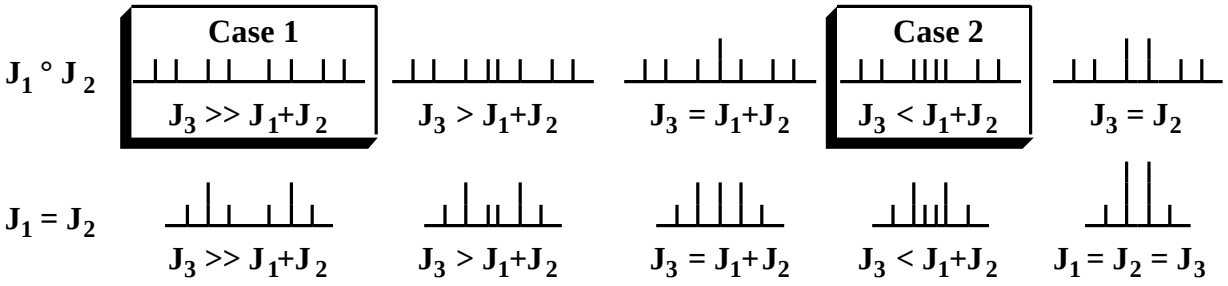


Figure 2. Possible appearances of the ddd pattern.

Case 1. J_1 = (line A - line B) = **2.8 Hz**, J_2 = (line A - line C) = **4.8 Hz**. In this case the portions of the multiplet are separate so that (line A - line D) = 7.7 Hz is *not* a coupling constant, it is J_1+J_2. J_3 is (line A - line E) = **12.9 Hz**. As a check, $J_1 + J_2 + J_3$ = (line A - line H) = 20.6 Hz.

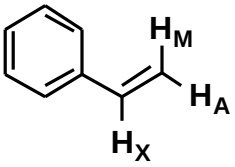
Case 2. J_1 = (line A - line B) = **8.7 Hz**, J_2 = (line A - line C) = **11.1 Hz**. In this case, the portions of the multiplet cross over one another so that (line A - line D) = **12.6 Hz**, this is J_3. J_1+J_2 is given by the value of (line A - line E) = 19.9 Hz. As a check, $J_1 + J_2 + J_3$ = (line A - line H) = 31.8 Hz.

Case 1 **Case 2**

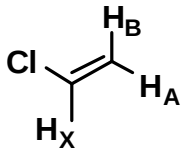
In analyzing a multiplet, keep in mind that (a) a multiplet which is not subject to second-order effects will be symmetrical and (b) the sum of the coupling constants = the spread of the multiplet.

Minor Deviations from First Order Spectra. In many spectra, the recognition of a multiplet and correct analysis is rendered more difficult due to minor deviations from the first order nature. As the multiplets representing two protons coupled to one another become closer to one another in frequency, the multiplets "lean" toward one another meaning that the intensities are greater for the inside lines than for the outside lines. A special nature to this leaning effect is that the multiplets lean toward each other with the coupling constants shared between them. This helps to identify the multiplets which are coupled to one another. If the leaning is not too severe, the multiplet can still be treated as first order.

Effects of Strong Coupling. When two multiplets which are coupled to each other overlap severely, they obviously can not be treated as first order patterns. Unfortunately, the effects on other resonances can also be very peculiar. In the first spectrum (styrene), the notations H_A,H_M and H_X represent that the resonances are well separated. Each of the resonances appears as a doublet of doublets (four lines) - this spectrum can be treated as first order. In the second spectrum (vinyl chloride), the notations H_A, H_B represent the two protons on the double bond which are strongly overlapping. The H_X resonance appears as a *six* line pattern - this spectrum cannot be treated as first order (the two smaller lines are called combination lines).

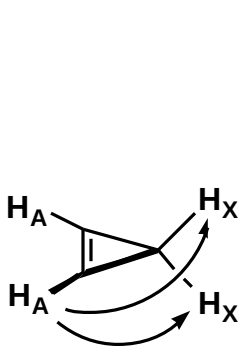


$^3J_{MX}$ = 17.5 Hz
$^3J_{AX}$ = 11.7 Hz
$^2J_{AM}$ = 1.7 Hz



Although computer programs exist for simulation of such complex coupling patterns, the best remedies are to try different solvents (e.g., d_6-acetone or d_6-benzene) using a spectrometer at a high enough MHz to remove the effects of strong coupling.

AA'XX' Second Order Patterns. First, consider the molecule cyclopropene. The two protons on the double bond (H_A) are chemically equivalent by symmetry, and the two CH_2 protons (H_X) are chemically equivalent by symmetry. Because the molecule has a high degree of symmetry, another fact is true: the geometric relationship of a single H_A proton to each of the H_X protons is identical. When a molecule has the two possible J_{AX} values equal, each pair of protons is called *magnetically equivalent*. and the spectrum will be *first order*. In this case, it is designated $\mathbf{A_2X_2}$. Note also that no effect of coupling between the two H_A protons is observed.



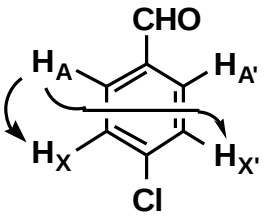
$\mathbf{A_2X_2}$

7.01 ppm, t, 2H, J_{AX} =1.8 Hz

0.92 ppm, t, 2H, J_{AX} =1.8 Hz

Most molecules of this type do not have sufficient symmetry to make the two possible J_{AX} values equal. Consider the two aromatic compounds shown below. For all *p*-disubstituted aromatic compounds (and therefore all monosubstituted benzene derivatives) the *ortho* protons are rendered chemically equivalent by free rotation of the ring. However, the proton H_A is *ortho* to one of the H_X protons and *para* to the other. Because the coupling constant J_{AX} ? $J_{AX'}$, the more complicated **AA'XX'** pattern results. It is important to recognize on sight that this pattern is *not* first order. Although it is tempting to blur your eyes and call each pattern a doublet, the four smaller peaks of each resonance are too large to be overlooked. Alternatively, analysis of each pattern as a doublet of triplets would also be erroneous because the four smaller peaks are too small to be consistent with the expected 1:2:1:1:2:1 ratio of intensities (and such an analysis would clearly not be consistent with the known structure!).

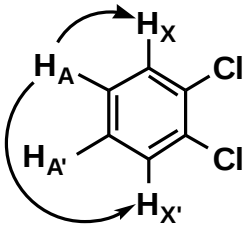
In the case of *ortho*- substitution with equal substituents (*o*-dichlorobenzene), the proton H_A is *ortho* to one of the H_X protons and *meta* to the other. Again two complex **AA'XX'** patterns result which should not be mistaken for first order dd patterns.



AA'XX'

7.22 ppm,m, 2H

6.98 ppm,m, 2H



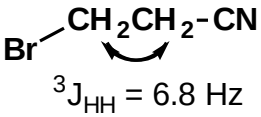
AA'XX'

7.22 ppm,m, 2H

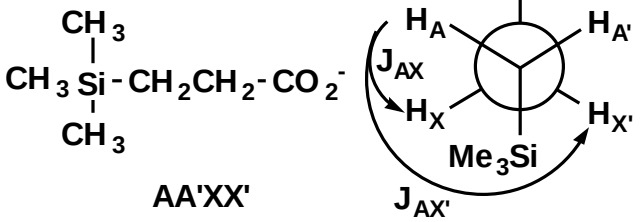
6.98 ppm,m, 2H

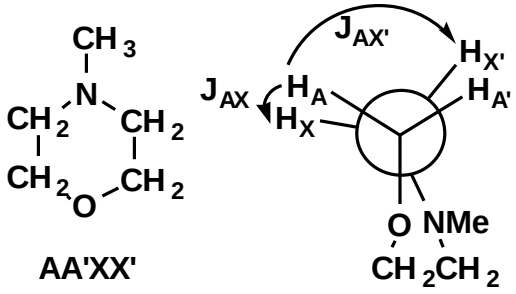
The appearance of AA'XX' patterns depends on the exact values of $J_{AA'}$ and $J_{XX'}$ as well as J_{AX} and $J_{AX'}$. Although computer simulation can be used to help figure out the exact values, the most important skill to acquire is learn to recognize typical **AA'XX'** patterns which show up for certain types of organic functional groups.

Consider a second series of examples, the **XCH_2CH_2Y** molecules. For many molecules of this type ($BrCH_2CH_2CN$ is a good example), two triplets are observed which leads one to proceed with a first order analysis. However, in a rigorous sense, the XCH_2CH_2Y subunit is a *magnetically inequivalent* (AA'XX') spin system. In the *anti* and eclipsed conformations shown below, it can be seen that J_{AX} ? $J_{AX'}$. However, for 3-bromoacetonitrile, free rotation averages the coupling constants J_{AX} and $J_{AX'}$ over all conformations and this leads to a very nearly first order spectrum (J_{AX} ~ $J_{AX'}$).



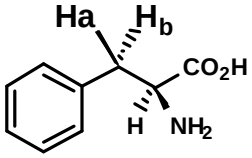
In the case of 3-trimethylsilylpropionic acid, rotation is severely restricted due to the size of the trimethylsilyl substituent. The anti- conformation predominates in which it is apparent that J_{AX} ? $J_{AX'}$. Alternatively, *N*-methylmorpholine is locked in a cyclic conformation for which J_{AX} ? $J_{AX'}$.



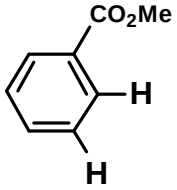


TABLES OF NMR COUPLING CONSTANTS AND CHEMICAL SHIFTS

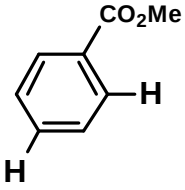
Once the multiplets of a ^{1}H NMR spectrum are correctly analyzed, the coupling constants are very powerful for determining the structure of the organic compound. Spin-spin coupling constants are **constant in Hz** and the mechanism of spin-spin coupling involves entirely a **through bond** effect. So the magnitude of coupling constants depends on the **number of bonds** separating the atoms and the **geometry of the bonds** relative to each other. The first can be observed in the following series:



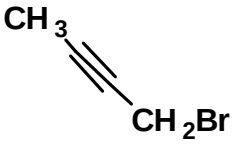
$^2J_{HaHb}$ (~ 14 Hz)
Geminal Coupling



$^3J_{HH}$ (= 6 Hz)
Vicinal Coupling

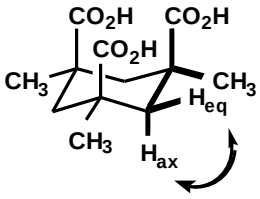


$^4J_{HH}$ (~ 1.5 Hz)

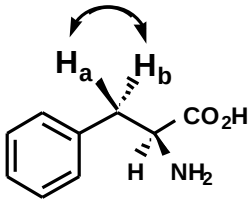


$^5J_{HH}$ (~ 1 Hz)
Long-Range Coupling

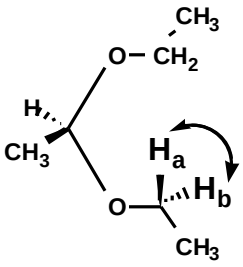
GEMINAL COUPLING is **only** observed for **diastereotopic CH_2** groups. (If the protons of a CH_2 group are equivalent, then no coupling will be observed between them). For phenylalanine, we learned that the property of chirality renders the Ha and Hb protons inequivalent and so they couple to each other. Geminal coupling constants are often quite large, and the exact value of the geminal coupling constant can tell a lot about the groups attached. Reviewing the three compounds discussed in the previous section, the geminal coupling constant of 12.4 Hz for Kemp's Triacid is representative for a CH_2 group flanked by two sp^3 carbons. The phenyl group of phenylalanine is electron-rich and facilitates more efficient coupling (J = 14.2 Hz) between the hydrogens of an attached CH_2 group. Alternatively, an oxygen atom is electronegative and leads to a smaller coupling constant (J = 9.4 Hz) between the hydrogens of an attached CH_2 group.



J_{gem} = 12.4 Hz

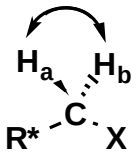


J_{gem} = 14.2 Hz

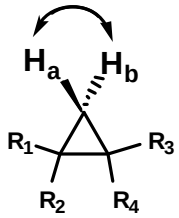


J_{gem} = 9.4 Hz

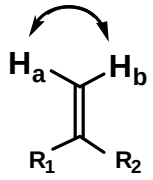
Some other values for geminal coupling constants are listed below. In each case, **R*** is an alkyl substiituent containing a chiral carbon atom. For small rings, the coupling geminal coupling constant is small as is the case for terminal alkenes.



X = CH_3	J ˜ 12.5 Hz
X = OR	J ˜ 10.9 Hz
X = Cl	J ˜ 11 Hz
X = Ph or C=C	J ˜ 14.2 Hz
X = $C(O)CH_3$	J ˜ 15 Hz
X = C≡CH	J ˜ 17 Hz

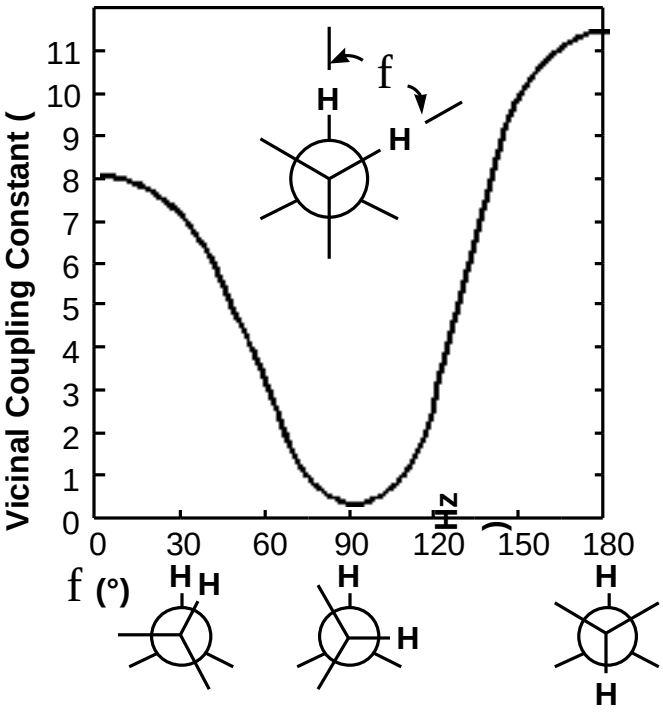


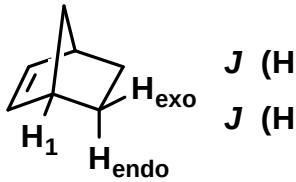
***J* = 4-8 Hz**



***J* = 0-2 Hz**

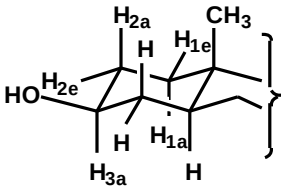
VICINAL COUPLING is sensitive to the **dihedral angle** between the two adjacent **C-H** bonds (shown by the Karplus curve at right). The magnitude of vicinal coupling constants can be **zero** if the dihedral angle ~ 90 ° or as large as 13 Hz for ϕ = 180 °. This is frequently the case for bicyclic systems. For cyclohexane rings which adopt a rigid chair conformation, the coupling constants for axial and equatorial protons are easily understood. For freely rotating systems, the coupling constant is averaged over all angles during rotation.





J **(H_1-H_{endo}) = 0 Hz**

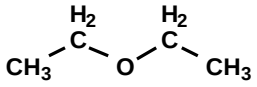
J **(H_1-H_{exo}) = 1-2 Hz**



J **(axial-axial) = 10-13 Hz**

J **(axial-equatorial) = 4-7 Hz**

J **(equatorial-equatorial) = 2-4 Hz**

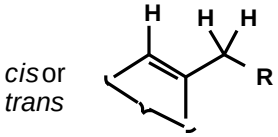


J **(CH_2-CH_3) = 6.7-7.4 Hz**

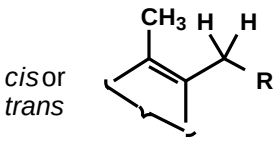
LONG-RANGE COUPLING

Coupling of protons separated by four or five bonds will generally be no larger than 1-2 Hz and will often be 0 Hz. Two factors which facilitate long-range coupling are (a) intervening p bonds and (b) a "W" arrangement of bonds.

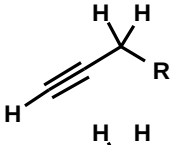
Allylic Coupling



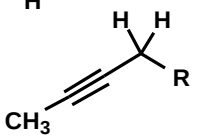
^4J = 0.5-2 Hz



^5J = 0-1 Hz

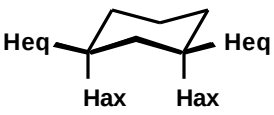


^4J = 2-4 Hz



^5J = 1-3 Hz

W Coupling

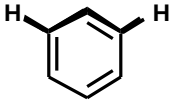


^4J (eq-eq) = 1-2 Hz

^4J (ax-ax) = 0 Hz

^4J = 1.5 Hz

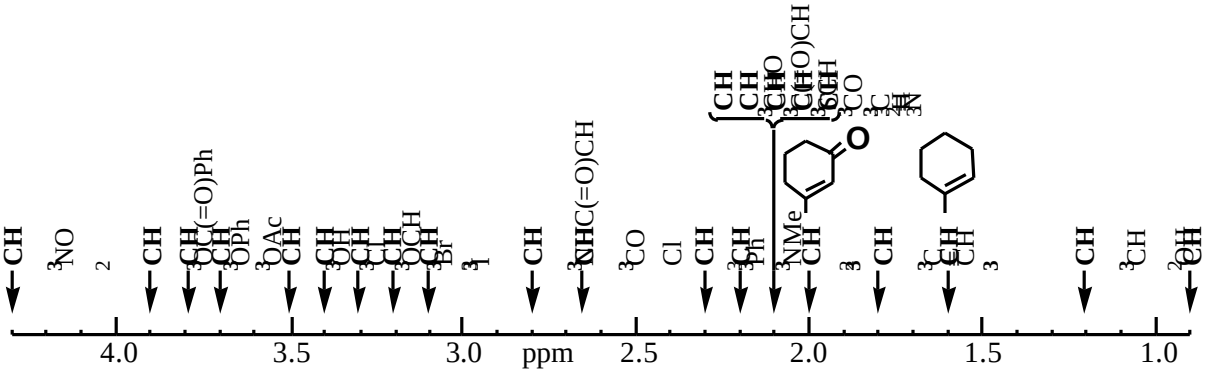
^4J = 7 Hz



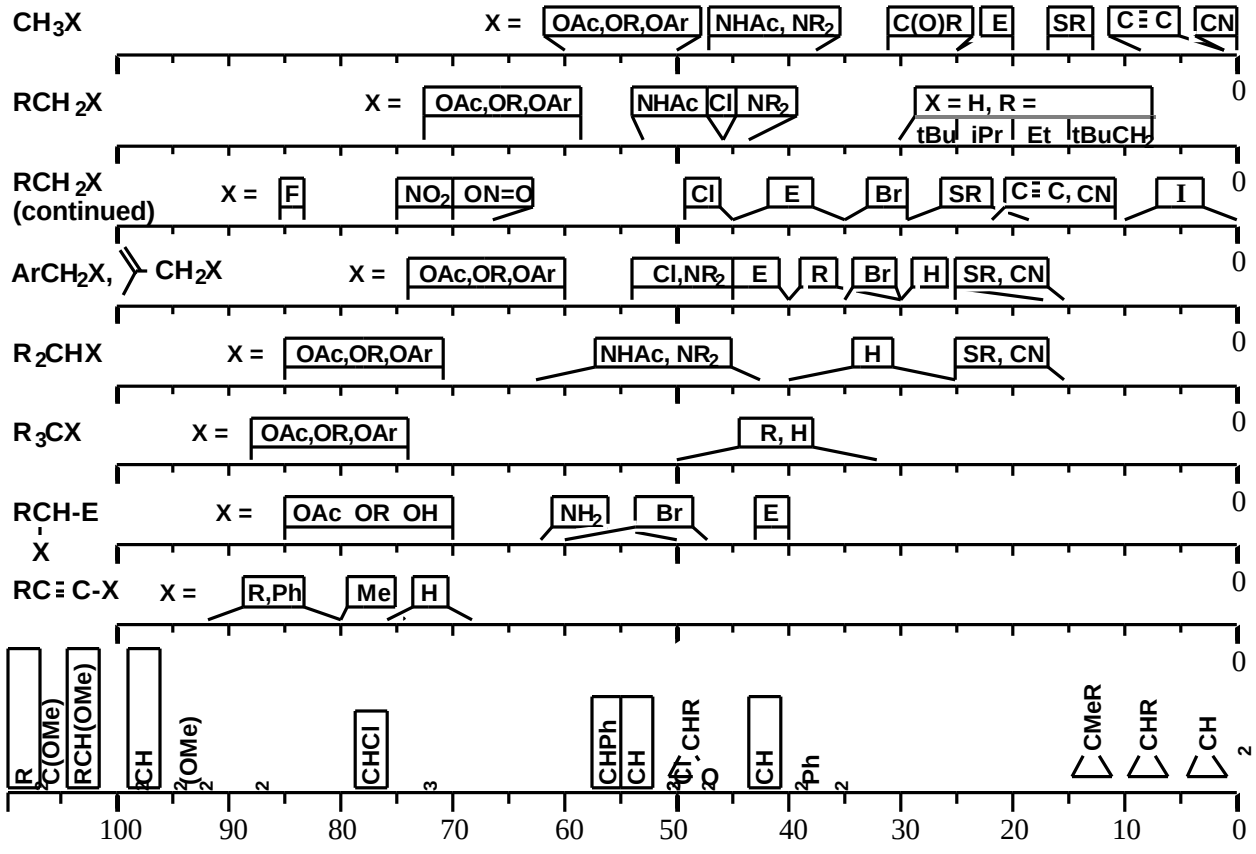
^4J = 2 Hz

^{1}H NMR CHEMICAL SHIFTS for CH_3X COMPOUNDS ($CDCl_3$)

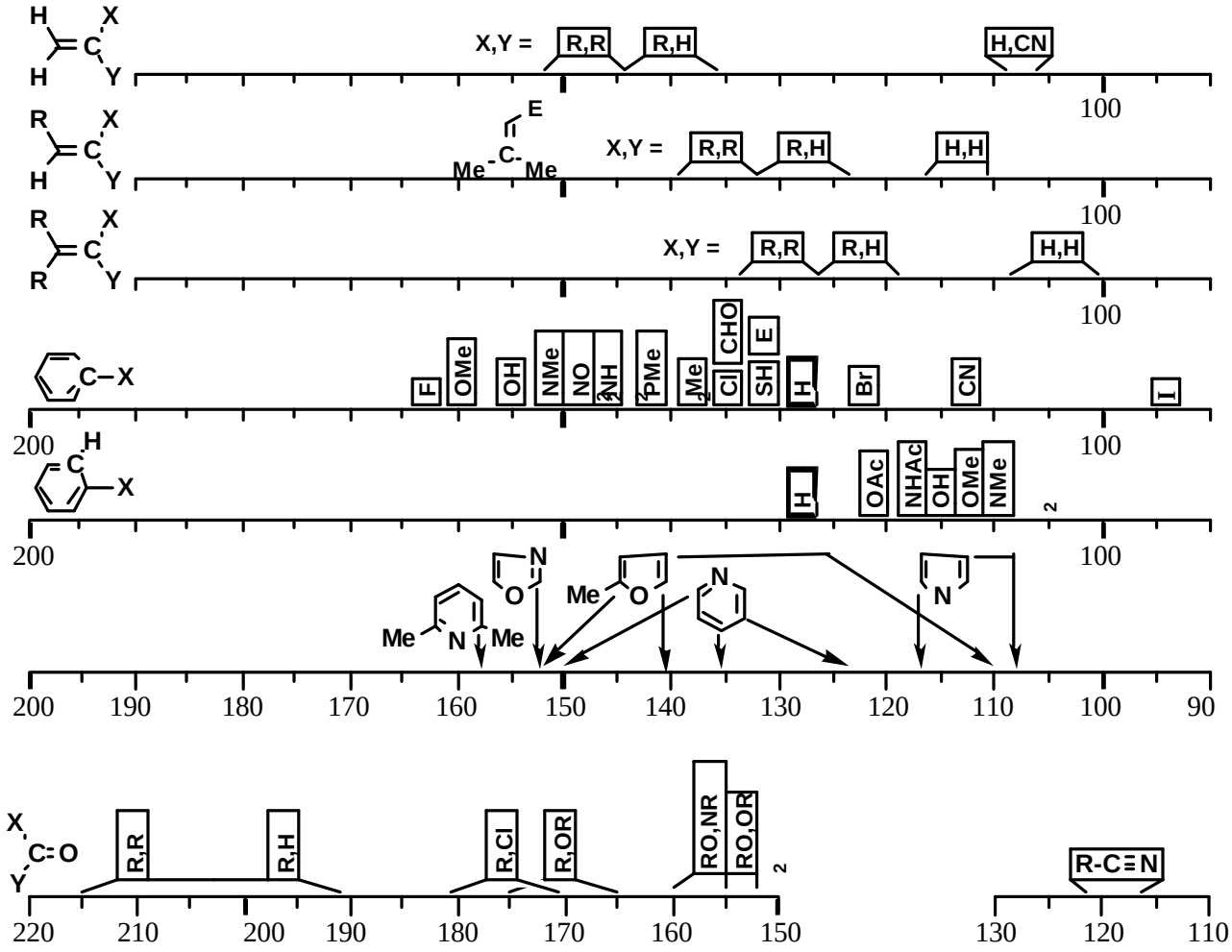
(add 0.2 to 0.4 ppm for **CH_3CH_2-X**)



^{13}C NMR CHEMICAL SHIFTS for SP and SP^3 CARBONS



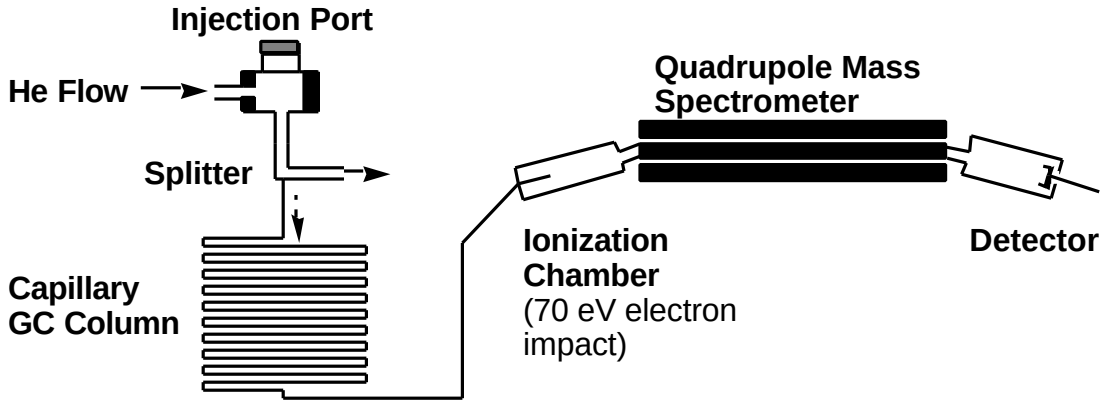
^{13}C NMR CHEMICAL SHIFTS for SP^2 CARBONS



HEWLETT PACKARD GC/MASS SPECTROMETER

The GC-Mass Spectrometer has become the most sensitive and most powerful instrument for the identification of organic compounds. Analysis of trace quantities of organic compounds - especially organochlorine compounds - has advanced to a stage that 1 part in 10^{15} can be identified unambiguously as a contaminant in food or water sources. For this purpose, thousands of spectra of insecticides and industrial pollutants have been compiled in databases.

A solution of the organic compound containing 1-5 milligrams in 0.5 mL is prepared and a special syringe is used to inject 0.05 microliters of this solution into the heated injector port of the GCMS. The volatilized compound is carried in the helium flow to a splitter where only 1% of the sample is diverted to the GC column (the rest goes out the window). The GC column used in this instrument is 10-30 meters long and only 0.4 mm in diameter! (called a capillary column). The conditions above assure that no more than 10^{-9} gram of sample are applied to this column.



As the organic compounds elute from the GC, they are introduced immediately into a Quadrupole Mass Spectrometer and a series of mass spectra are repeatedly recorded for each two-second interval. In the ionization chamber, the molecules are bombarded by electrons travelling across a 70 eV potential. When a molecule is struck by one of these high energy electrons, it loses one of its own electrons and become a radical cation $\mathbf{M}^{\bullet+}$. Many of the molecular ions will be formed with enough excess energy to undergo subsequent reactions in the ionization chamber. From the ionization chamber, the positively charged ions are attracted through the magnetic field of a quadrupole ion chamber maintained under high vacuum. For a given strength of the magnetic field, only those ions having the correct charge-mass ration (m/e) will be deflected through the magnetic field and reach the detector. The instrument varies the magnetic field continuously to produce a spectrum of m/e peaks.

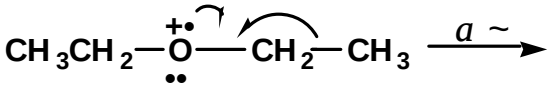
In the next section are detailed mechanisms for common fragmentation and ionization reactions. It is much more difficult to solve the structure of an organic compound from the mass spectrum alone than it is to match the spectrum with that of the known compound. But with the help of NMR and IR spectroscopy, mass spectrometry can play an important role in structure determination.

REACTIONS OBSERVED IN MASS SPECTROSCOPY

A FRAGMENTATION REACTION involves the cleavage of one bond of the radical cation $\mathbf{M}^{\bullet+}$ to form a cation $\mathbf{A}^{+}$ and a radical $\mathbf{B}^{\bullet}$. The reaction is especially facilitated by substituents which stabilize the cation $\mathbf{A}^{+}$.

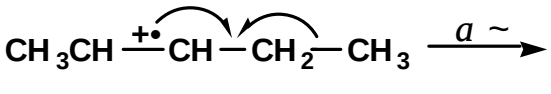
$$\mathbf{M}^{\bullet+} \rightarrow \mathbf{A}^{+} + \mathbf{B}^{\bullet}$$

α-Cleavage Reaction. The α-cleavage reaction is driven by the formation of a stable carbocation as shown for the three examples below. In the first case, ethers readily undergo the α-cleavage reaction forming a stable oxonium ion (also common for similar **N**, **S** compounds). In the second example, alkenes undergo α-cleavage reaction to form a stable allylic cation (also common for aryl compounds and alkynes). In the third example, ketones readily undergo α-cleavage to produce stable acylium ions.



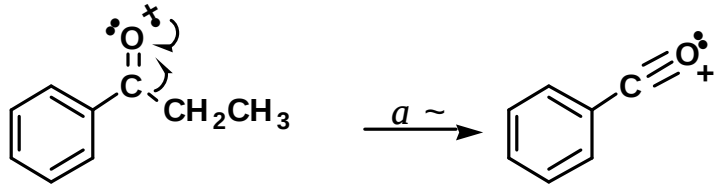
$\mathbf{M}^{\bullet+} = 74$ $\quad \mathbf{A}^{+} = 59$ $\quad \mathbf{B}^{\bullet}$

Formation of Stable Oxonium ion (Also common for **N**,**S**)



2-Pentene → $\mathbf{M}^{\bullet+} = 70$ $\quad \mathbf{A}^{+} = 55$ $\quad \mathbf{B}^{\bullet}$

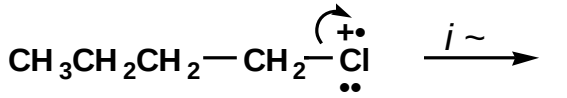
Formation of Stable Allylic and Benzylic Cations



Propiophenone → $\mathbf{M}^{\bullet+} = 134$ $\quad \mathbf{A}^{+} = 105$ $\quad \mathbf{B}^{\bullet}$

Formation of Stable Acylium Ion

Inductive Cleavage Reaction. Alkyl halides often undergo the inductive cleavage reaction in which the halogen atom (e.g. **Cl•**) simply breaks off the initial radical cation. This is most prevalent for bromides and iodides and for compounds which can produce a stable carbocation.



$\mathbf{M}^{\bullet+} = 92$ $\quad \mathbf{A}^{+} = 57$ $\quad \mathbf{B}^{\bullet}$

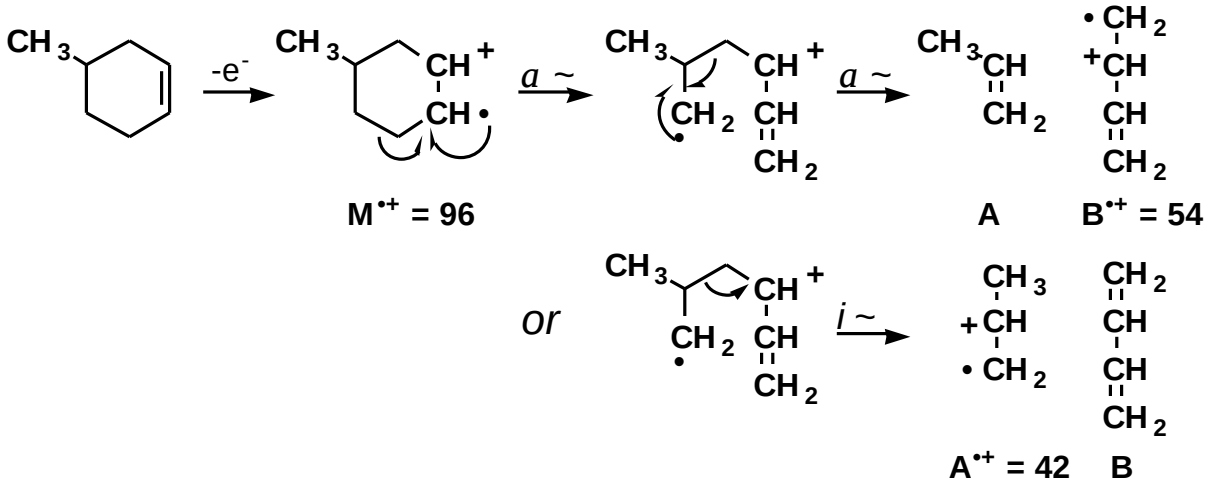
Halogens do this easily because they afford stable **X•**

In each case, fragmentation reactions above produce both a radical and a cation from the initial radical cation $\mathbf{M}^{\bullet+}$. Also, a consequence of the nitrogen rule: For molecules that do not contain nitrogen, fragmentation reactions of the **even-massed** radical cations will produce **odd-massed** $\mathbf{A}^{+}$ ions. This can be a useful method for finding which ions are produced by one of the above reactions.

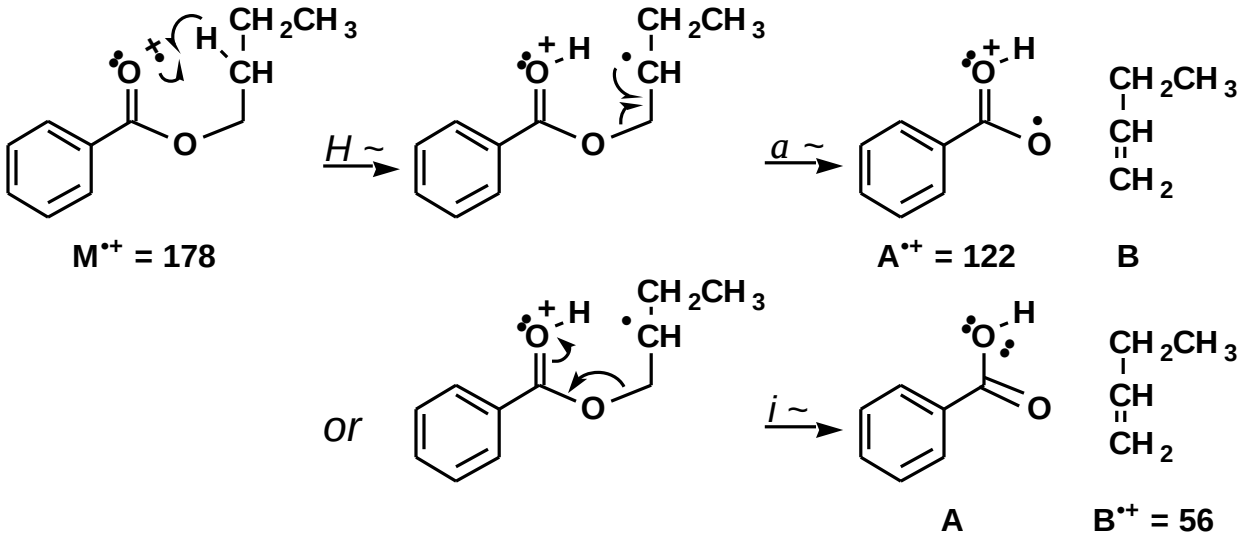
A REARRANGEMENT REACTION involves the cleavage of more than one bond of the radical cation $\mathbf{M}^{\bullet+}$ to ultimately form a new radical cation $\mathbf{A}^{\bullet+}$ and a neutral compound **B**. Often, rearrangement reactions may proceed by complementary pathways so that the mass numbers for both parts of the molecule can be found in the mass spectrum.

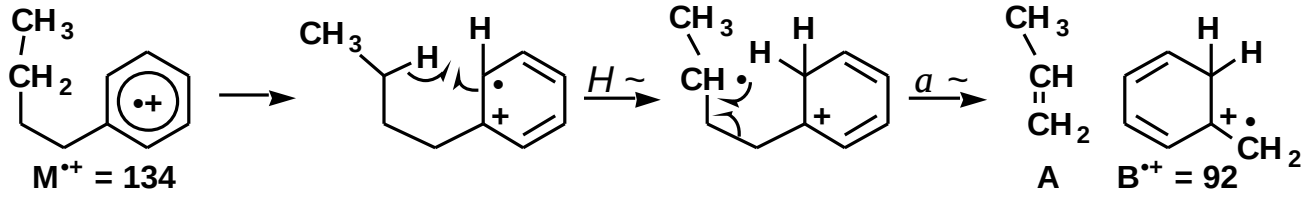
$$\mathbf{M}^{\bullet+} \text{ Æ } \mathbf{A}^{\bullet+} + \mathbf{B} \textit{ or } \mathbf{A} + \mathbf{B}^{\bullet+}$$

Rearrangement of Cyclic Structures. When a cyclohexene derivative is ionized in the mass spectrum, a particularly facile cleavage of the ring is reminiscent of a retro-Diels-Alder reaction.



Radical site rearrangements (McLafferty Rearrangement). Carbonyl derivatives readily undergo a rearrangement reaction in which $\mathbf{M}^{\bullet+}$ undergoes intramolecular H atom transfer to the carbonyl oxygen atom. The best geometry involves a six-membered transition state and subsequent α-cleavage or inductive cleavage leads to the fragment ions of each piece.





For each example of rearrangement reactions, the observed ions are radical cations. For a molecule which does not contain nitrogen, the McLafferty rearrangement of the **even-massed $M^{\bullet+}$** ion produces an **even-massed $A^{\bullet+}$** ion and so are easily distinguished from the odd-massed ions from a simple fragmentation reaction. Also, many molecules display a peak in the mass spectrum arising from a McLafferty + 1 rearrangement. For the ester above, the mechanism involves a second H atom transfer.