NEPHAR407 Pharmaceutical Chemistry III Lab

Nuclear Magnetic Resonance (NMR) Spectroscopy

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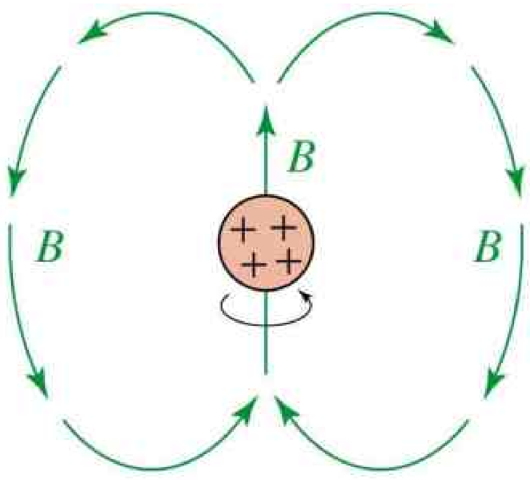
NEPHAR407 Pharmaceutical Chemistry III Laboratory Manual, 2010

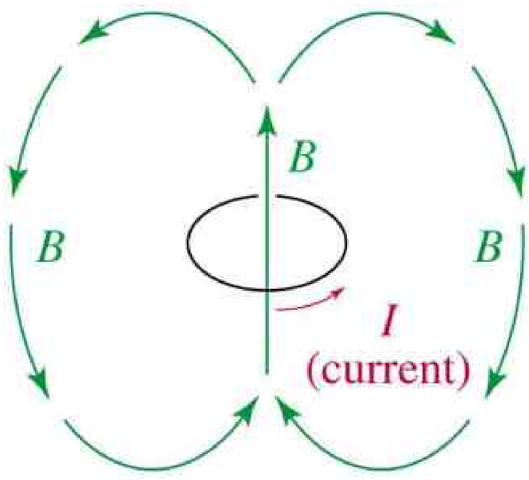
L. G. Wade, Jr. *Organic Chemistry*, 5th Edition

Nuclear Spin

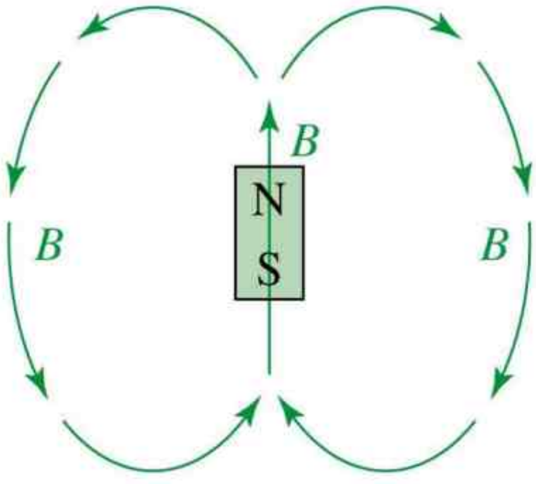
- A nucleus with an odd atomic number or an odd mass number has a nuclear spin.
- The spinning charged nucleus generates a magnetic field.



spinning proton



loop of current



bar magnet

=>

- The basis of the NMR experiment is to subject the nuclei to radiation which will result in a transition from the lower energy state to the higher one. In NMR, RF pulse is used to "flip" the alignment of nuclear spins from the low energy spin aligned state to the higher energy spin opposed state. The energy required for this transition depends on the strength of the applied magnetic field but is small.

- The energy required for the spin-flip depends on the magnetic field strength at the nucleus. With no applied field, there is no energy difference between the spin states, but as the field increases so does the separation of energies of the spin states and therefore so does the frequency required to cause the spin-flip, referred to as **resonance** or **precession (Larmor) frequency**

Active Nuclei in NMR

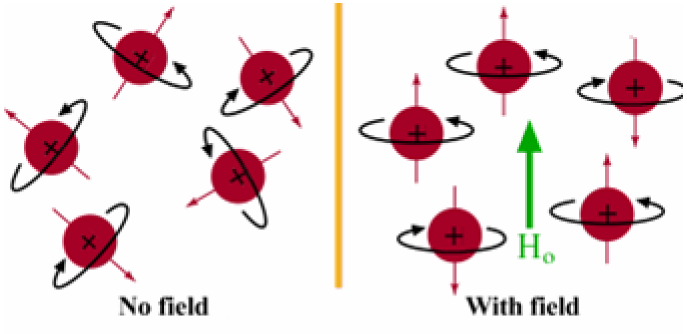
✓ Nuclei are composed of neutrons and protons.

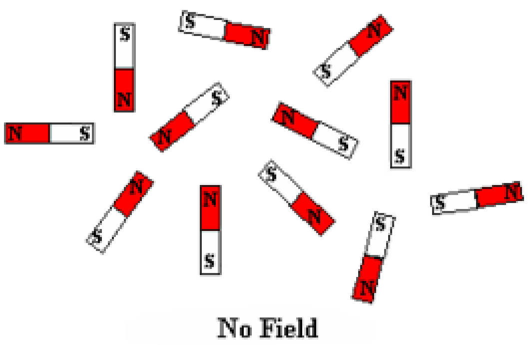
✓ To be active in NMR, the nuclei need to posses a property called the Spin (Spin is represented by the letter **I** in quantum mechanic).

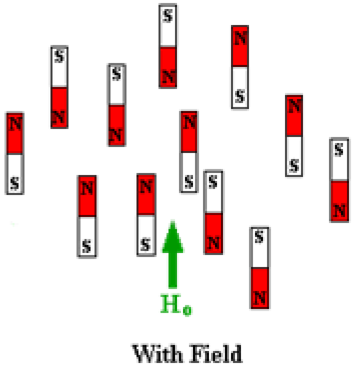
✓ When introduced in a magnetic field $\mathbf{B_0}$ the nuclei magnetic moment will orient in **(2nI+1)** orientations.

- The nuclei of many elemental isotopes have a characteristic spin (**I**).
- Some nuclei have integral spins (e.g. I = 1, 2, 3 ....),
- some have **fractional spins** (e.g. I = 1/2, 3/2, 5/2 ....),
- and a few have no spin, **I = 0** (e.g. ^{12}C, ^{16}O, ^{32}S, ...).
- Isotopes of particular interest and use are ^{1}H, ^{13}C, ^{19}F and ^{31}P, all of which have **I = 1/2.**

Isotope	**Natural Abundance (%)**
^{1}H	99.985
^{13}C	1.108
^{15}N	0.37
^{31}P	100







The frequency of radiation necessary to effect a transition between the two energy levels is given by the equation:

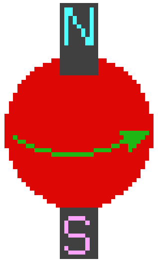
$$v = mB_o / hI$$

✓ where v is the frequency of radiation, m is the magnetic moment of the nucleus, B_o is the strength of the external magnetic field, h is Plank's constant, and I is the spin quantum number.

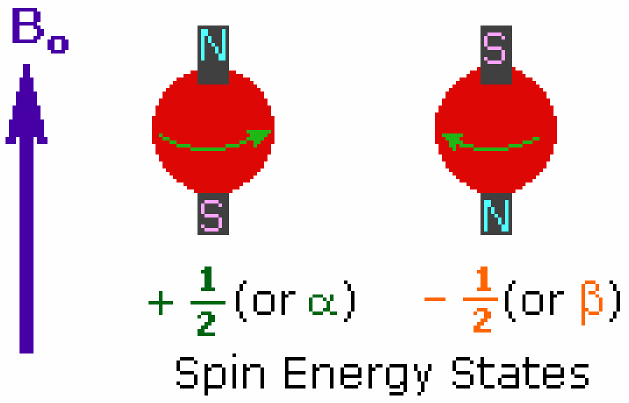
✓ The larger the applied magnetic field the greater energy difference between the two levels and the greater the frequency of radiation necessary to effect the transition between the two levels.

The NMR Phenomenon

1. A spinning charge generates a magnetic field. The resulting spin-magnet has a magnetic moment (**μ**) proportional to the spin.



2. In the presence of an external magnetic field (**B$_0$**), two spin states exist, **+1/2** and **-1/2**.The magnetic moment of the lower energy +1/2 state is aligned with the external field, but that of the higher energy -1/2 spin state is opposed to the external field.

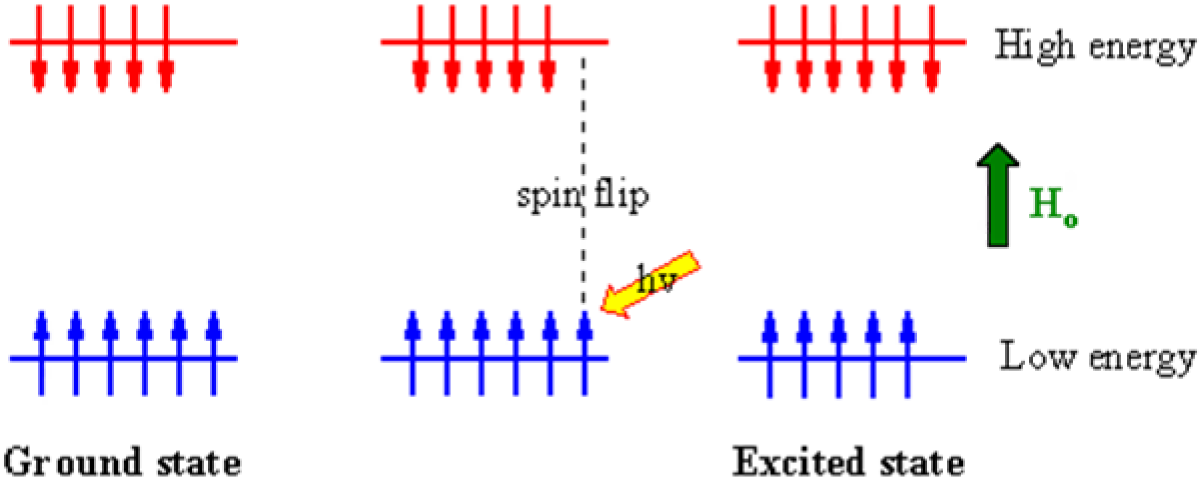


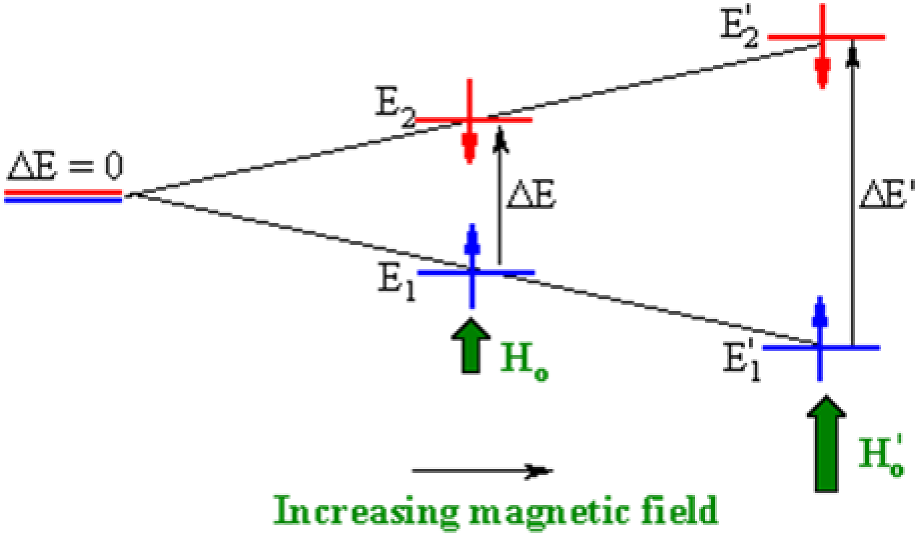
Examples of NMR Active Nuclei (I= ½)

^{1}H, ^{11}B, ^{13}C, ^{15}N, ^{31}P, ^{19}F

Examples of not NMR Active Nuclei (I= 0)

^{4}He, ^{12}C, ^{16}0

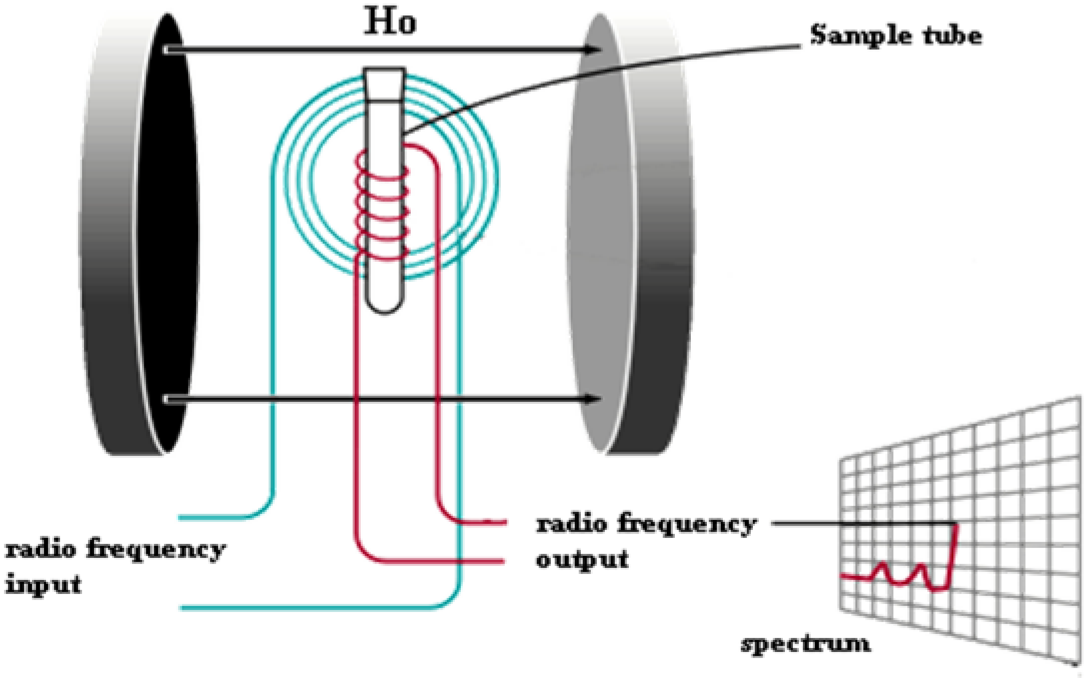

High energy
spin flip
hν
H_o
Low energy
Ground state
Excited state


E'_2
E_2
$\Delta E = 0$
ΔE
$\Delta E'$
E_1
H_o
E'_1
H'_o
Increasing magnetic field

The basic arrangement of an NMR spectrometer

The sample is positioned in the magnetic field and excited via pulsations in the radio frequency input circuit. The realigned magnetic fields induce a radio signal in the output circuit which is used to generate the output signal.

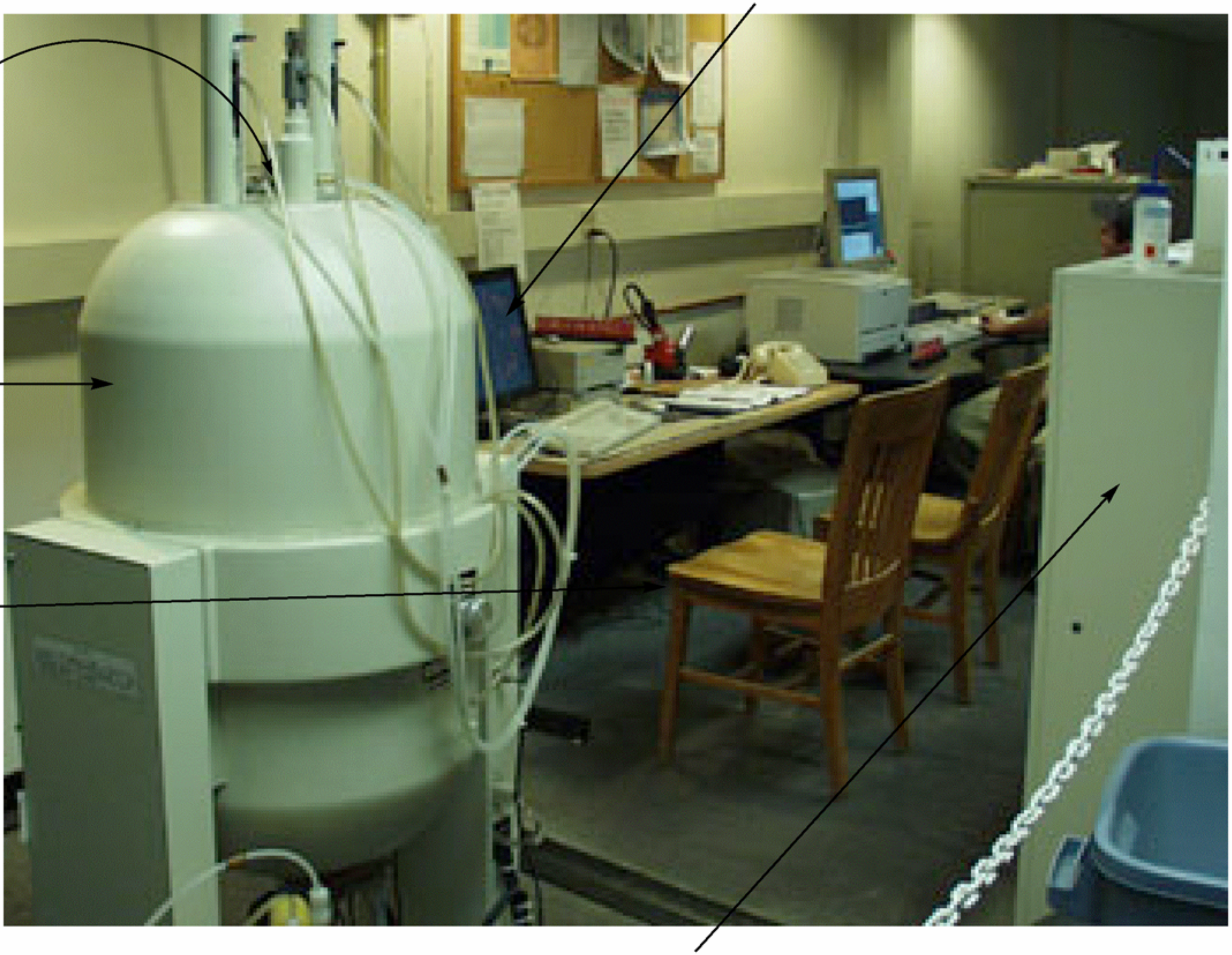
Fourier analysis of the complex output produces the actual spectrum (fourier transforms are used to transform FID from time domain to frequency domain). The pulse is repeated as many times as necessary to allow the signals to be identified from the background noise.



NMR Spectrometer Used by the Undergraduate Organic Labs

Spectrum observed on computer monitor here.

Sample is lowered into the magnetic field here (there's a hole in the center of the magnet).



Big superconducting magnet, cooled in liquid helium. Source of the magnetic field (B_o).

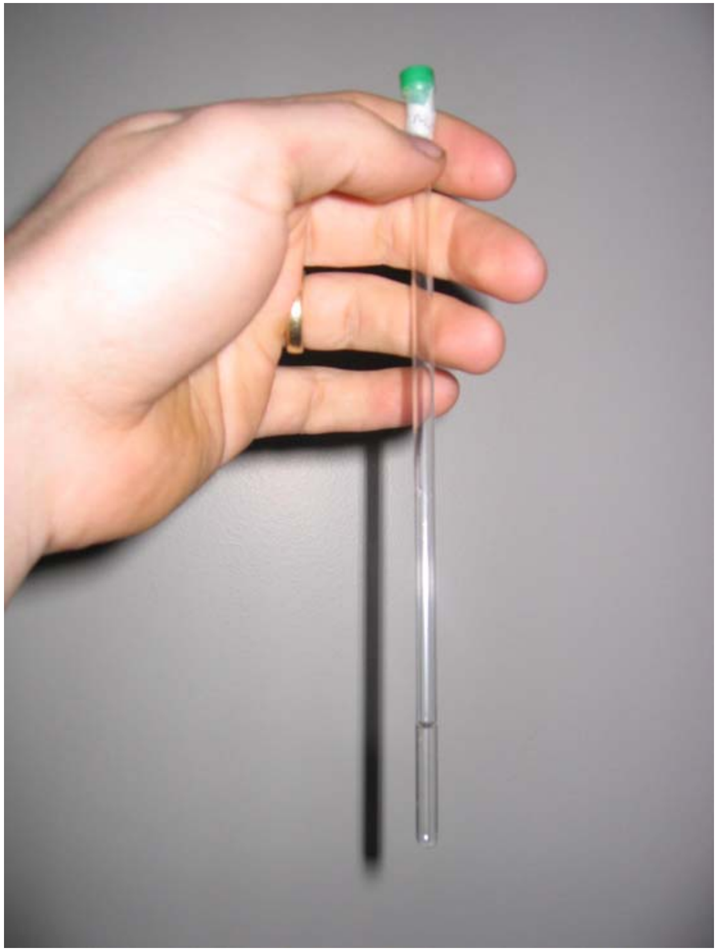
Wooden chair, no magnetic metals allowed near the magnet!

Radio transmiter and receiver tuned to 300 MHz

NMR Spectrometer- Console, Magnet, Computer

Varian NMR

NMR Sample



Application of NMR

✓ For organic structure determination, the two most important types of NMR spectra are the proton and carbon spectra.

✓ They give information about **the number of hydrogens and carbons** in a molecule and **how those hydrogens and carbons are connected together** as well as information about functional groups.

Chemical Shift

✓ An NMR spectrum is a plot of the ***radio frequency applied*** against ***absorption***.

✓ A signal in the spectrum is referred to as a ***resonance***.

✓ The frequency of a signal is known as its ***chemical shift***, **(δ).**

✓ Chemical shifts can be given in Hz, in this situation the applied frequency must be specified. The scale is made more manageable by expressing it in dimensionless units, parts per million (**ppm**), and is independent of the spectrometer frequency.

✓ Reference compound for both ^{13}C and ^{1}H spectra is tetramethysilane ($Si(CH_3)_4$ often just referred as TMS) which is defined to be at 0 ppm.

- ➢The NMR spectrum of the organic compound recorded as a **solution** (usually 5-10%)

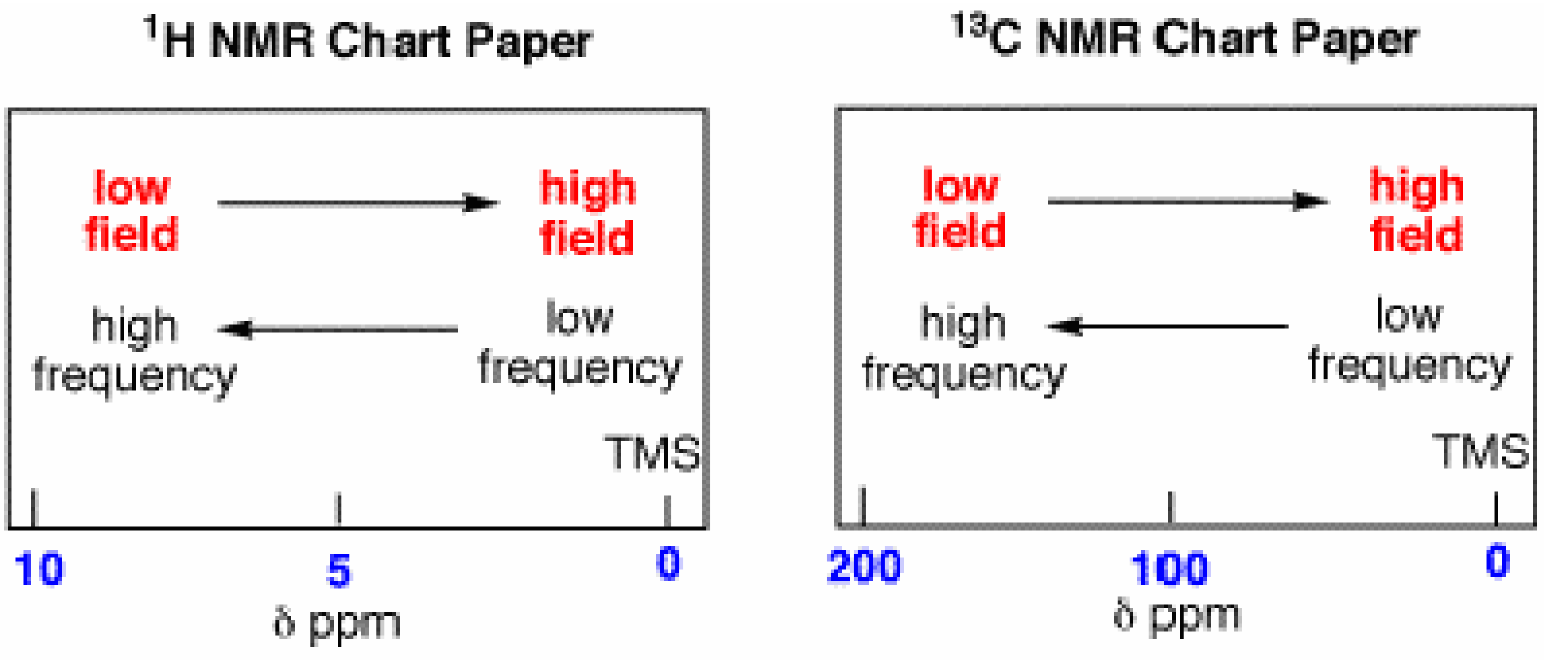
The solvents normally chosen do not contain hydrogen atom; e.g. **CCl_4, $CDCl_3$, $(CD_3)_2SO$, $(CD_3)_2CO$, CD_3OD, D_3C-CN, D_2O, C_6D_6, C_5D_5N.**

Usually, however, the **deuterated solvents** contain a small proportion of isotopically isomeric molecules containing hydrogen instead of deuterium, and this will giverise to an additional peak or peaks in the spectrum.

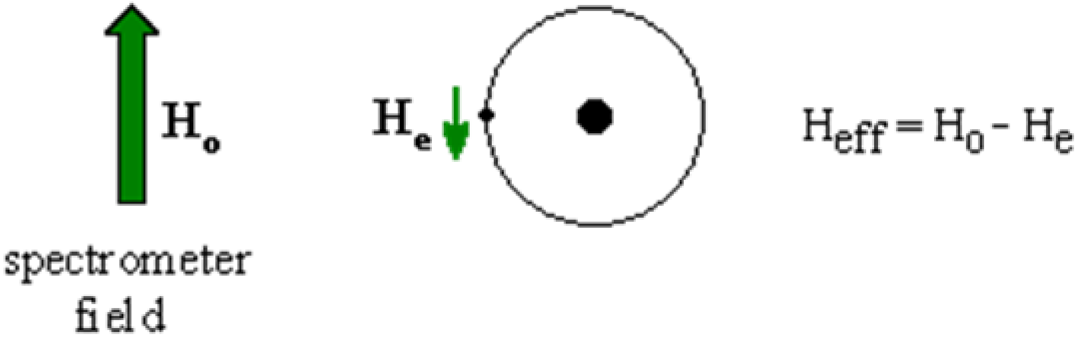
Solvent	**Formula**	**^{1}H NMR shift**	**^{13}C NMR shift**
Chloroform-d	$CDCl_3$	7.24	77.0
Acetone-d_6	CD_3COCD_3	2.04	29.8 206.3
Benzene-d_6	C_6D_6	7.2	128.0
Acetonitrile-d_3	CD_3CN	1.93	1.3 117.7
Dichloromethane-d_2	CD_2Cl_2	5.32	53.5
Dimethylsulfoxide-d_6	CD_3SOCD_3	2.49	39.7
Methanol-d_4	CD_3OD	3.35, 4.78	49.3
Tetrahydrofuran-d_8	C_4D_8O	1.73, 3.58	25.5 67.7
Toluene-d_8	C_7D_8	2.30, 7.19	
Pyridine-d_5	C_5D_5N	7.19, 7.55, 8.71	123.5 135.5 149.5
Water-d_2	D_2O	4.65	
Acetic acid-d_4	CD_3COOD	2.03, 11.53	20.0 178.4
Trifuoroacetic acid-d	CF_3COOD	11.5	116.5 164.4
Dioxane-d_8	$C_4D_8O_2$	3.58	66.5

NMR chart for ^{1}H and ^{13}C-NMR

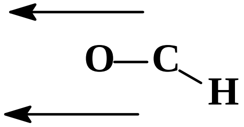
In the NMR spectra, externally applied magnetic field strength increases from left to right
A peak at a chemical shift, δ, of 10 ppm is said to be ***downfield*** or ***deshielded***
with respect to a peak at d 5 ppm, or if you prefer, the peak at 5 ppm is ***upfield*** or ***shielded***
with respect to the peak at d 10 ppm



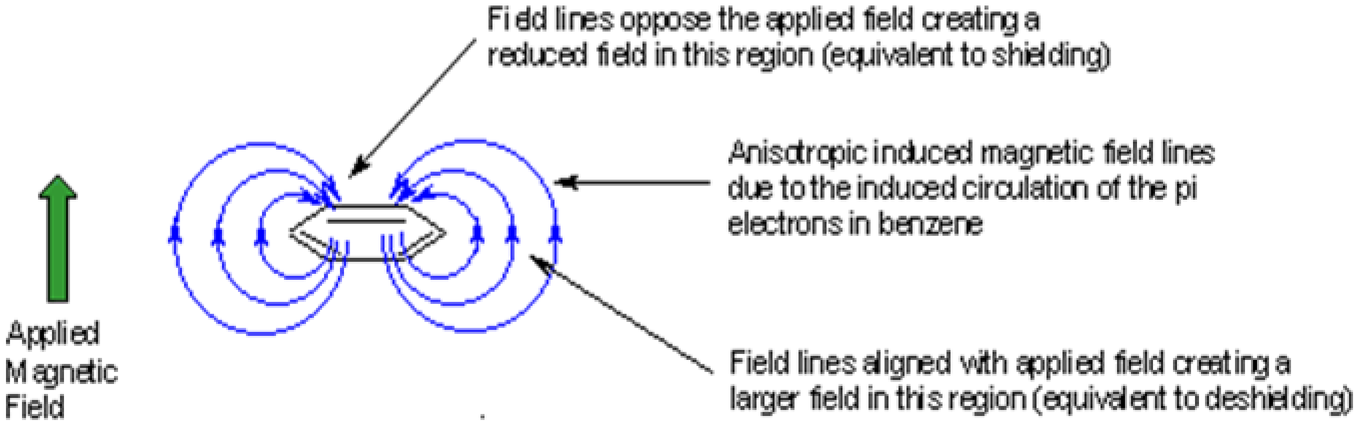
Shielding vs Deshielding



Electronegative elements such as oxygen pull electron density away from the hydrogen nucleus



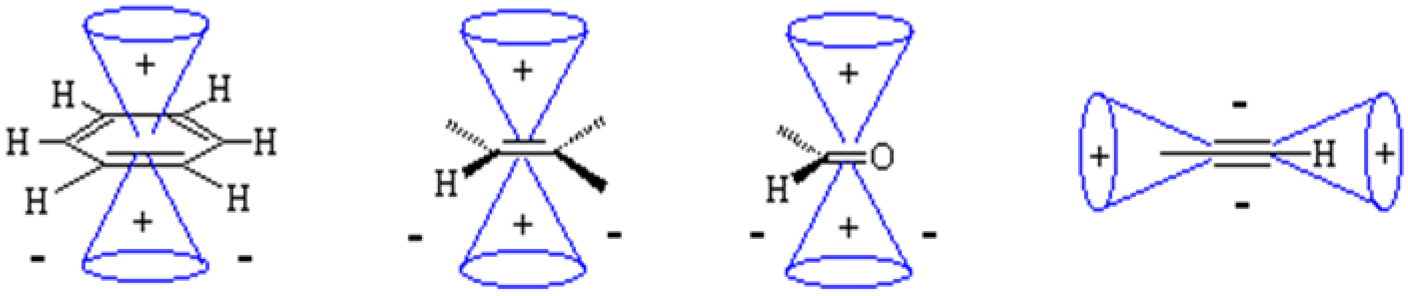
This decrases the magnitude of the shielding Be field and increases (Bo-Be)



Field lines oppose the applied field creating a reduced field in this region (equivalent to shielding)

Anisotropic induced magnetic field lines due to the induced circulation of the pi electrons in benzene

Field lines aligned with applied field creating a larger field in this region (equivalent to deshielding)



δ = 7-8 ppm δ = 5-7 ppm δ = 9-10 ppm δ = 2-3 ppm

Schematic diagram of shielding cones for common pi systems. The + denotes shielding areas and - denotes deshielding areas. Remember shielding lowers the chemical shift, δ and deshielding increases δ. Typical H δ values are also shown.

Deshielding groups:

1. electronegative atoms such as **O**, **N**, and **halogens**

2. functional groups containing **π-bonds**:

$C=O$

ketones
aldehydes
carboxylic acids
carboxylic esters
amides

$C=C$

alkenes

aromatic
compounds

Shielding from π-bonds

$R-C\equiv C-H$

terminal alkynes

NMR Signals

- The ***number*** of signals shows how many different kinds of protons are present.
- The ***location*** of the signals shows how shielded or deshielded the proton is.
- The ***intensity*** of the signal shows the number of protons of that type.
- Signal ***splitting*** shows the number of protons on adjacent atoms.

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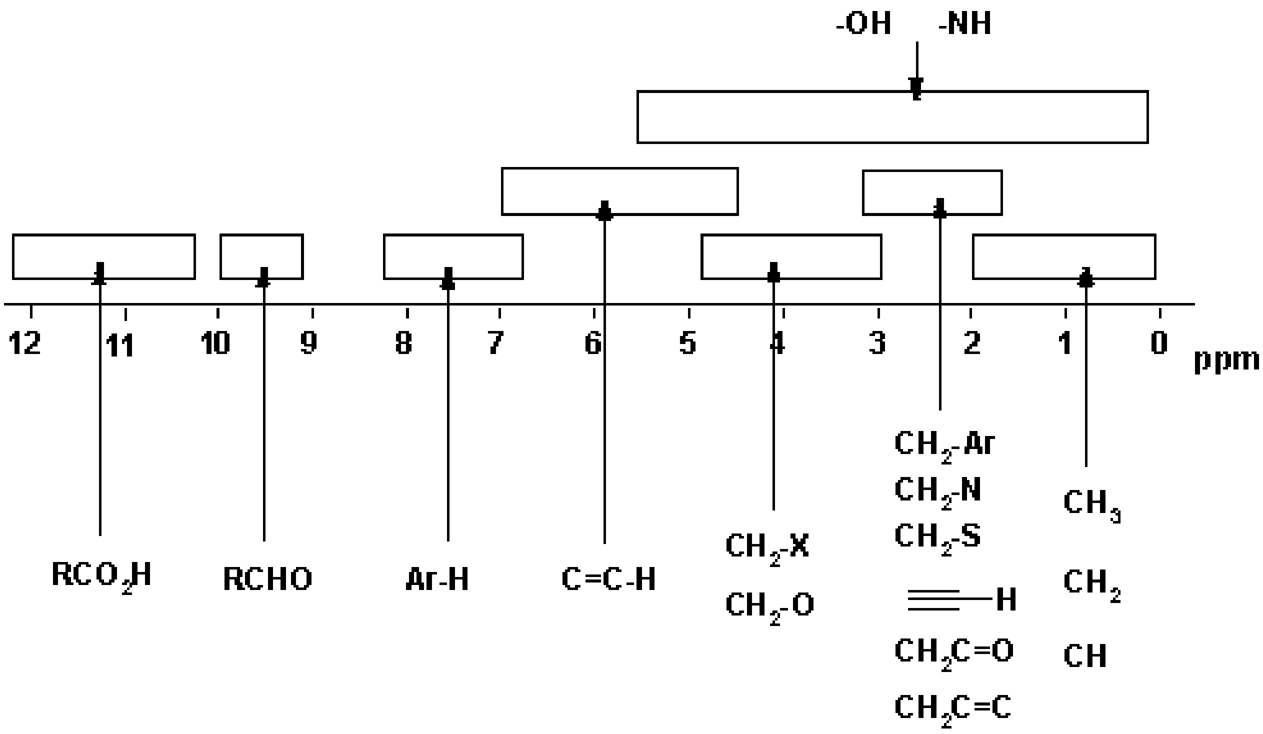
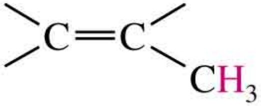
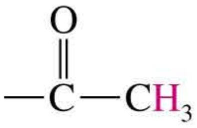
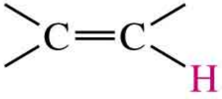


The typical chemical shifts for protons being influenced by a *single group*.
In cases where a proton is influenced by *more than one group*,
the effects are essentially cumulative.

Typical Values

Type of Proton	Approximate δ	Type of Proton	Approximate δ
alkane (—CH_3)	0.9		1.7
alkane (—CH_2—)	1.3	Ph—H	7.2
alkane  (—CH—)	1.4	Ph—CH_3	2.3
 $—\overset{\overset{\displaystyle O}{\|}}{C}—CH_3$	2.1	R—CHO	9–10
—C≡C—H	2.5	R—COOH	10–12
R—CH_2—X (X = halogen, O)	3–4	R—OH	variable, about 2–5
	5–6	Ar—OH	variable, about 4–7
		R—NH_2	variable, about 1.5–4

Note: These values are approximate, as all chemical shifts are affected by neighboring substituents. The numbers given here assume that alkyl groups are the only other substituents present. A more complete table of chemical shifts appears in Appendix 1.

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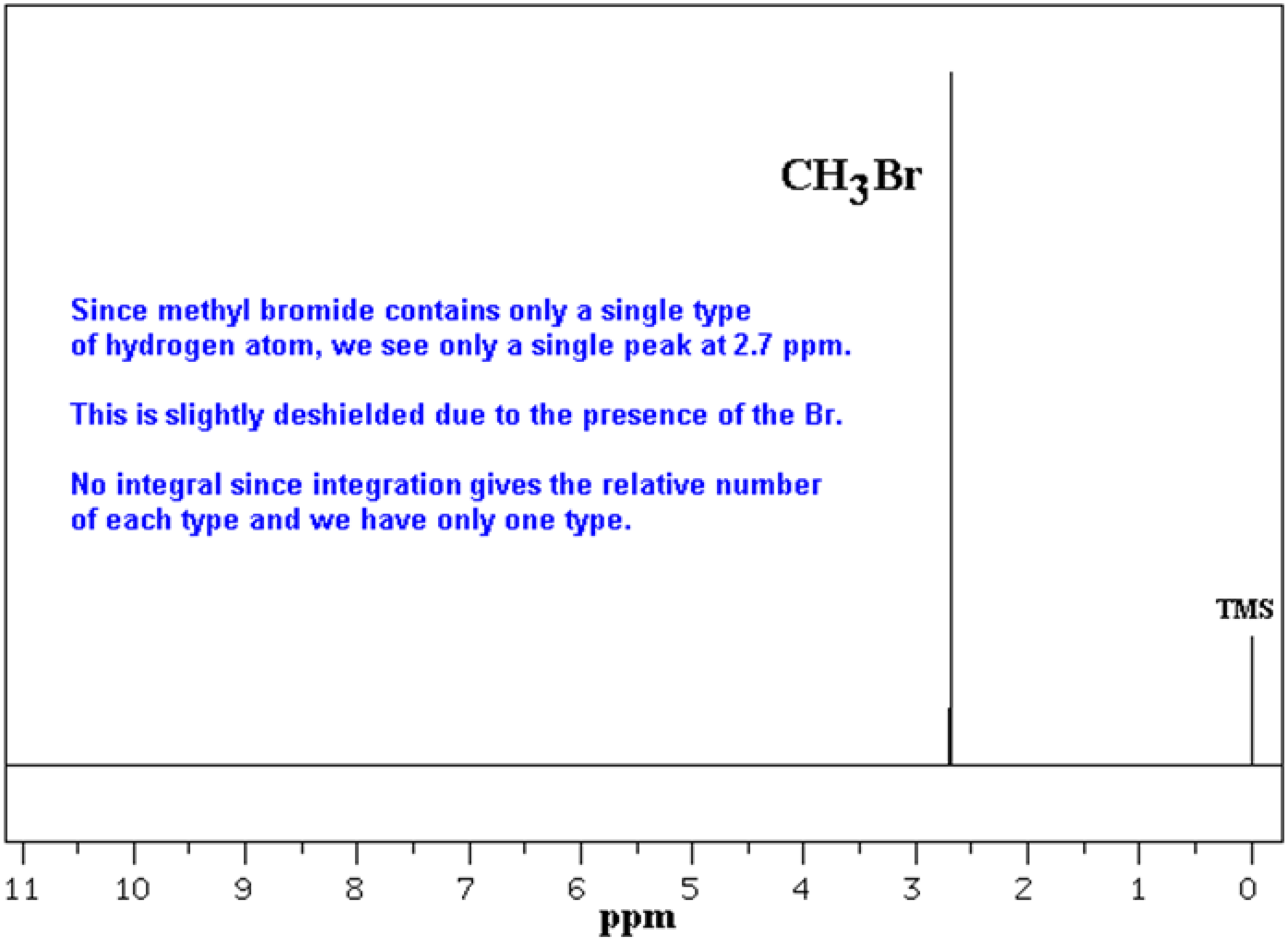
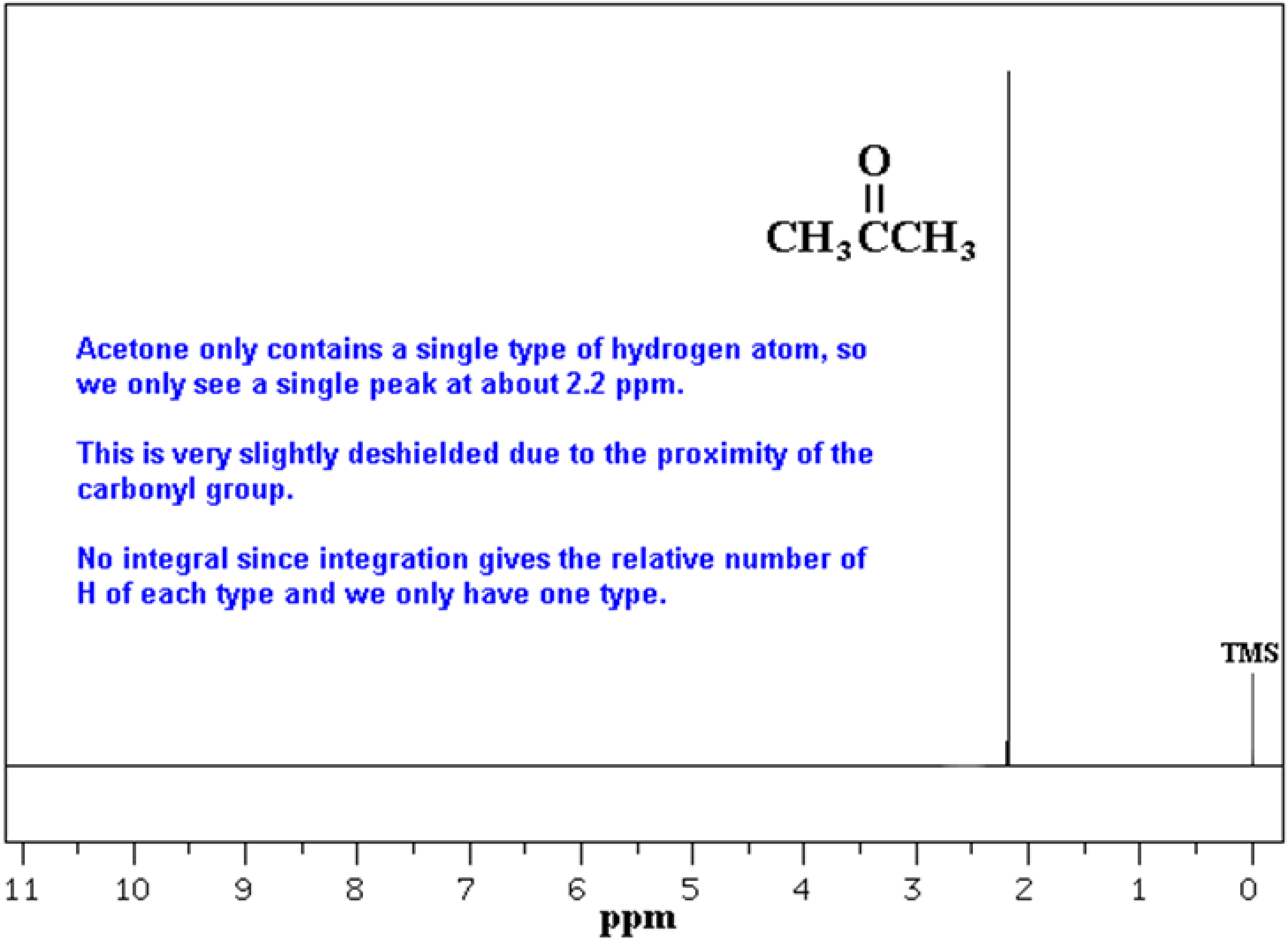


Since methyl bromide contains only a single type of hydrogen atom, we see only a single peak at 2.7 ppm.

This is slightly deshielded due to the presence of the Br.

No integral since integration gives the relative number of each type and we have only one type.

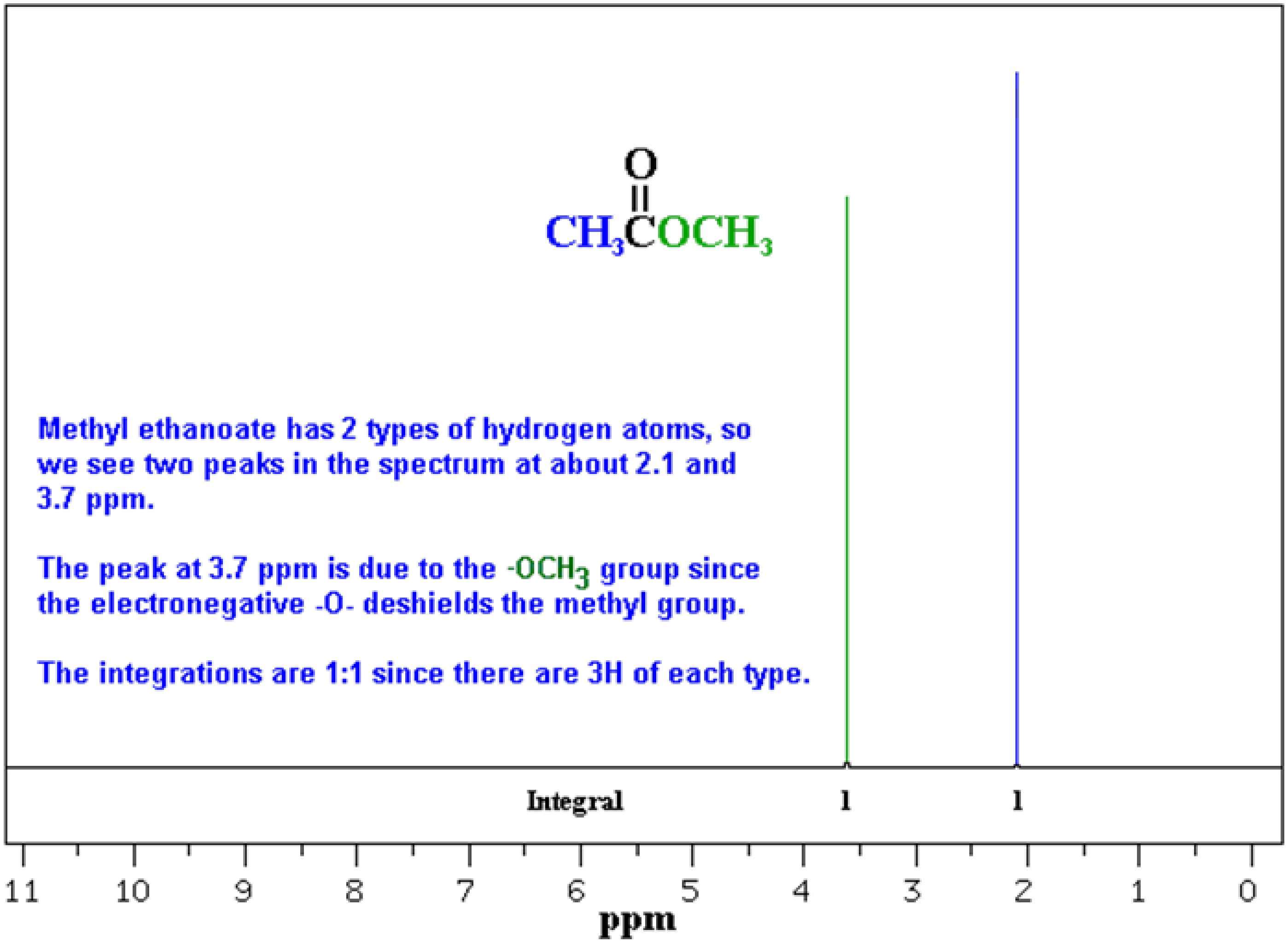


$$CH_3\overset{\overset{\displaystyle O}{\|}}{C}CH_3$$

Acetone only contains a single type of hydrogen atom, so we only see a single peak at about 2.2 ppm.

This is very slightly deshielded due to the proximity of the carbonyl group.

No integral since integration gives the relative number of H of each type and we only have one type.



$$CH_3\overset{\overset{\displaystyle O}{\|}}{C}OCH_3$$

Methyl ethanoate has 2 types of hydrogen atoms, so we see two peaks in the spectrum at about 2.1 and 3.7 ppm.

The peak at 3.7 ppm is due to the $-OCH_3$ group since the electronegative -O- deshields the methyl group.

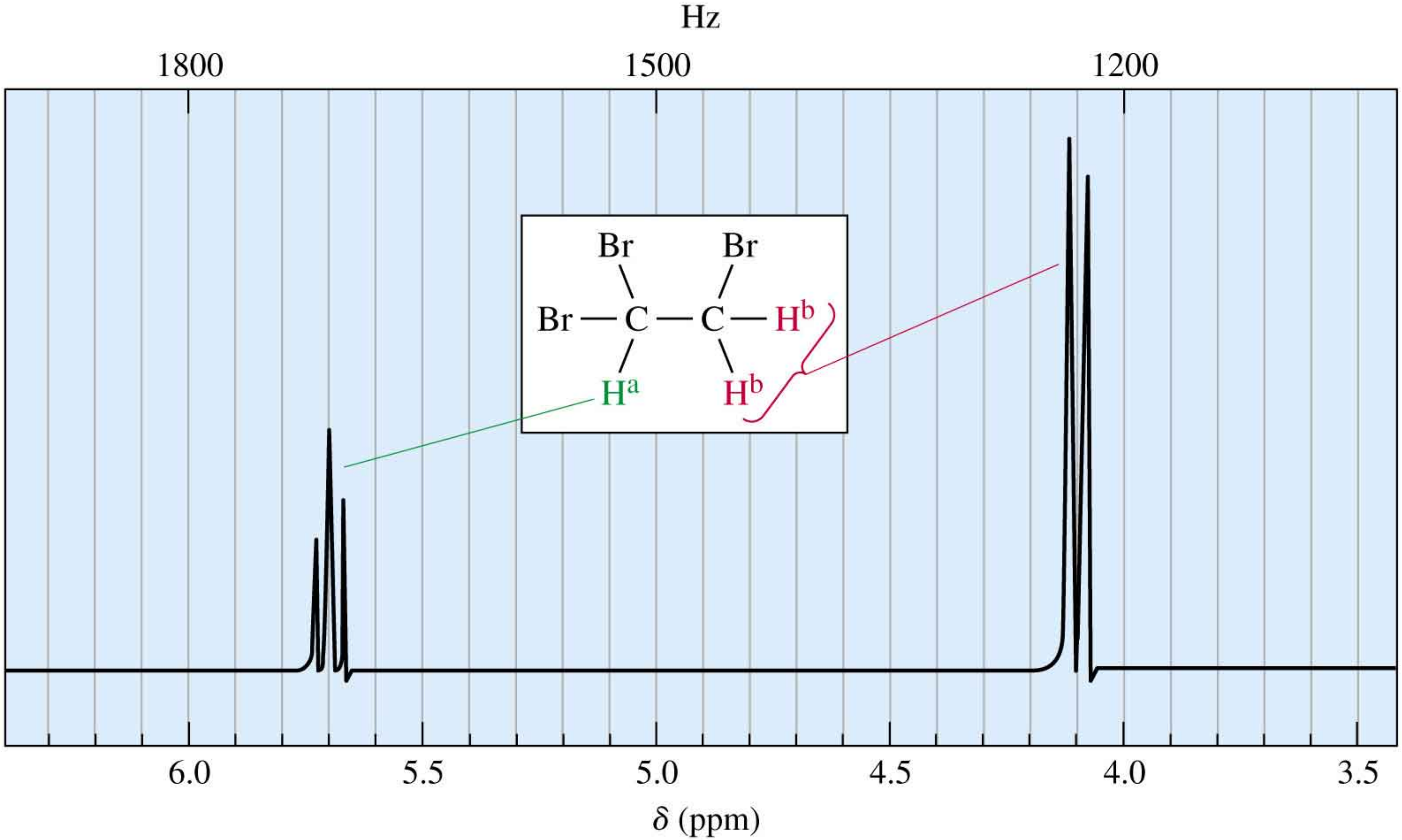
The integrations are 1:1 since there are 3H of each type.

Spin-Spin Splitting

- Nonequivalent protons on adjacent carbons have magnetic fields that may align with or oppose the external field.
- This magnetic coupling causes the proton to absorb slightly downfield when the external field is reinforced and slightly upfield when the external field is opposed.
- All possibilities exist, so signal is split. =>

1,1,2-Tribromoethane

Nonequivalent protons on adjacent carbons.



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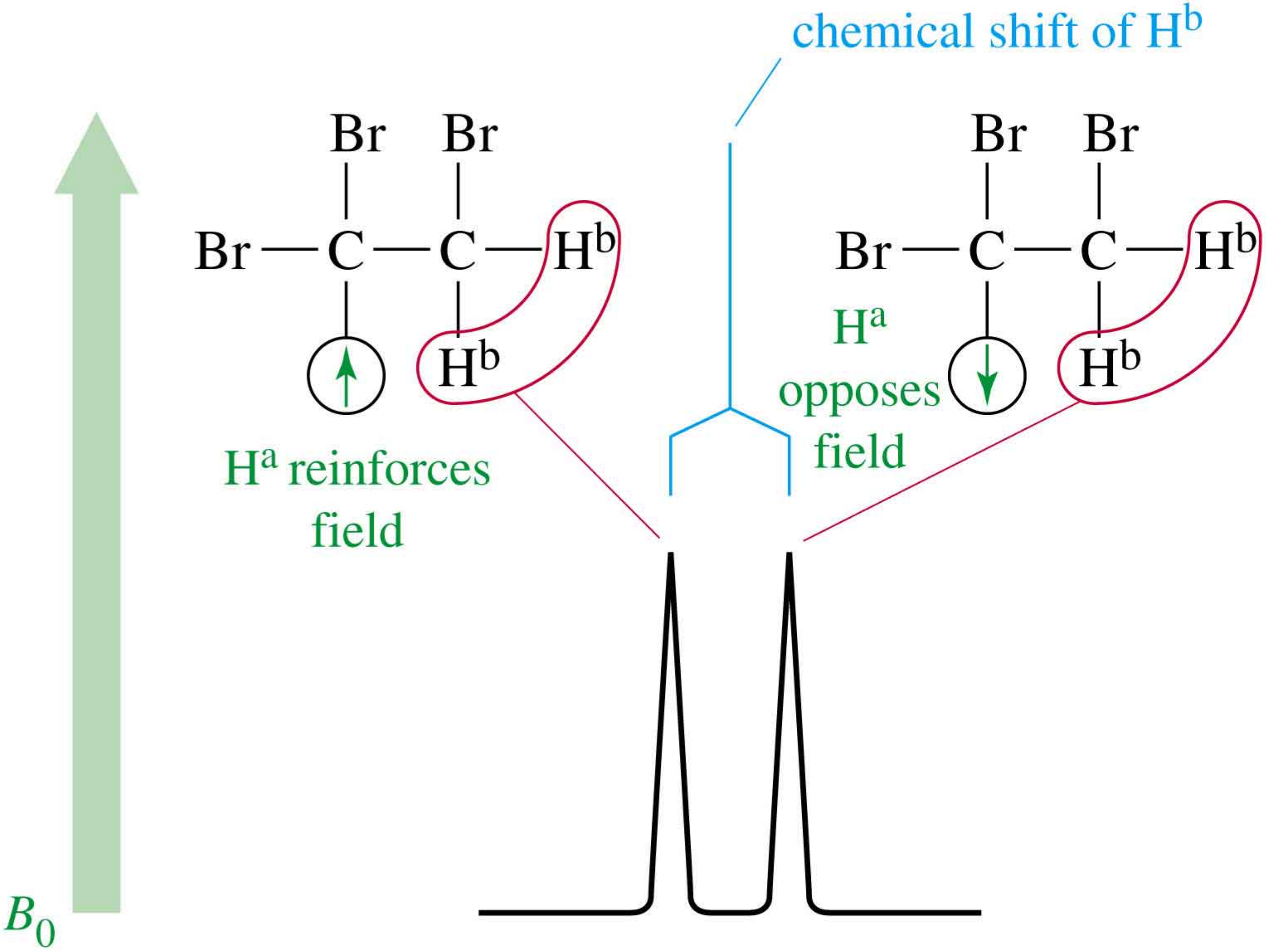
Doublet: 1 Adjacent Proton



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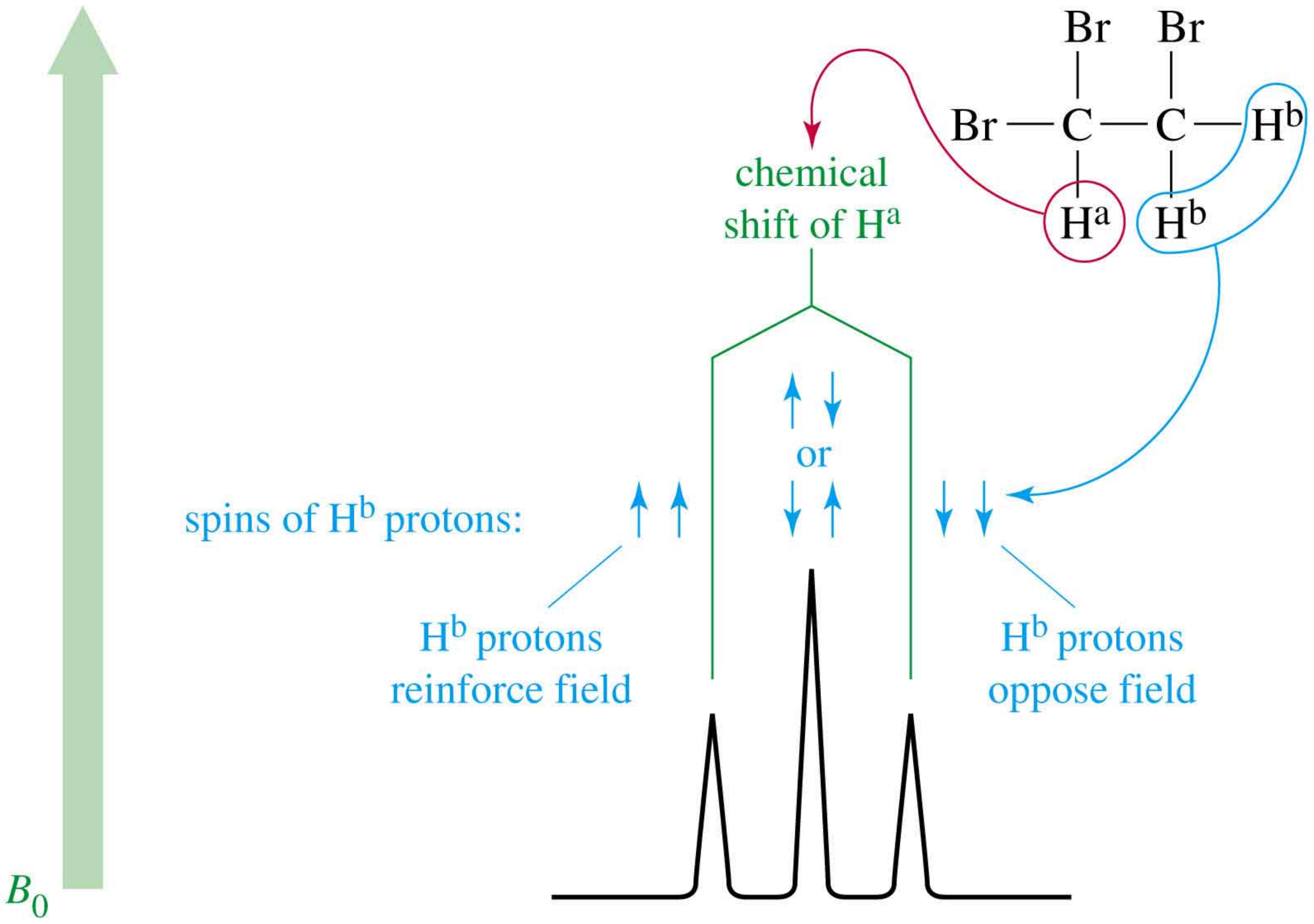
Triplet: 2 Adjacent Protons



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The *N* + 1 Rule

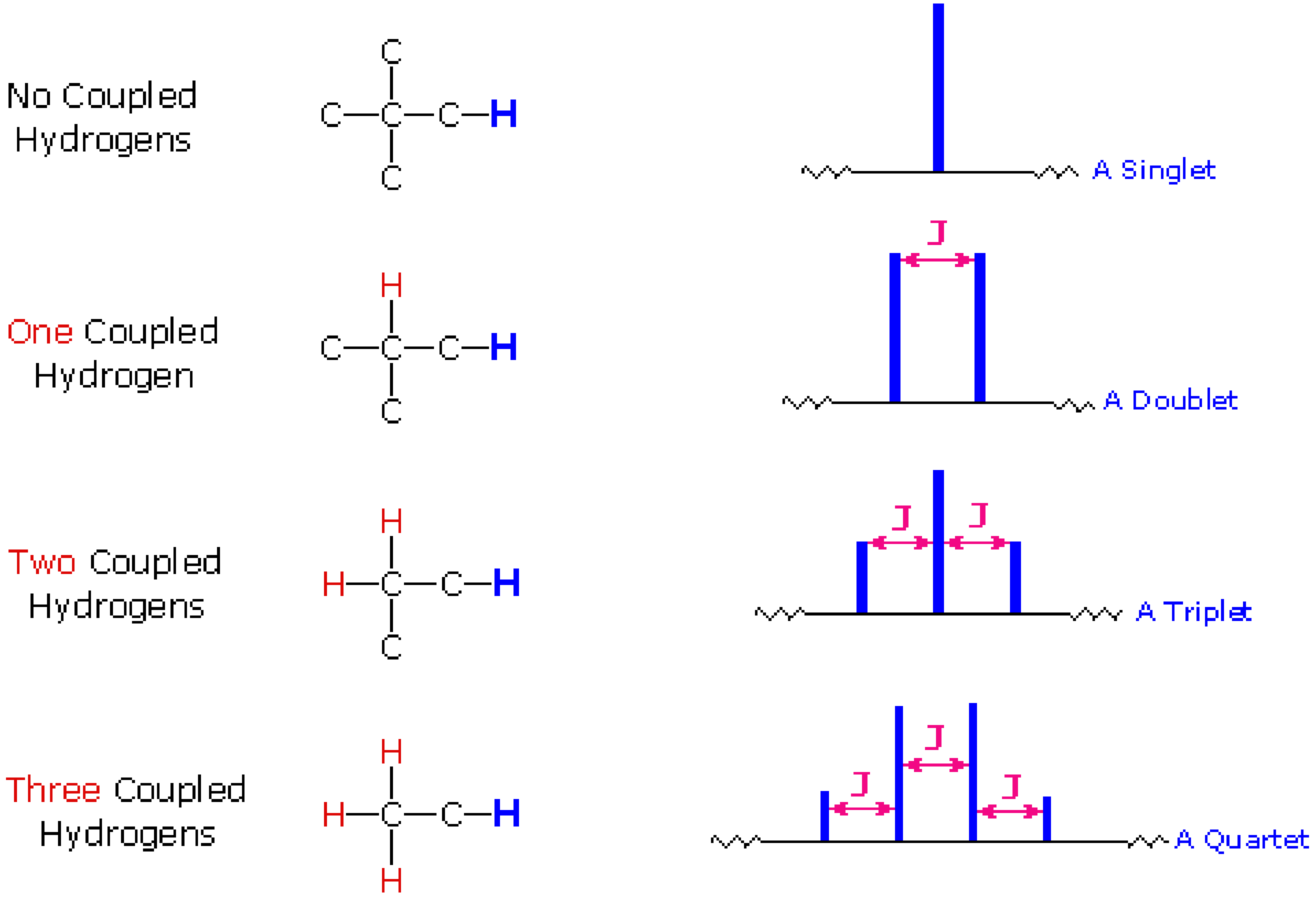
If a signal is split by *N* equivalent protons, it is split into *N* + 1 peaks.

Relative Peak Intensities of Symmetric Multiplets

Number of Equivalent Protons Causing Splitting	*Number of Peaks (multiplicity)*	*Area Ratios (Pascal's triangle)*
0	1 (singlet)	1
1	2(doublet)	1 1
2	3 (triplet)	1 2 1
3	4 (quartet)	1 3 3 1
4	5 (quintet)	1 4 6 4 1
5	6 (sextet)	1 5 10 10 5 1
6	7 (septet)	1 6 15 20 15 6 1

=>

Spin-Spin Splitting



No Coupled Hydrogens

One Coupled Hydrogen

Two Coupled Hydrogens

Three Coupled Hydrogens

Range of Magnetic Coupling

- Equivalent protons do not split each other.
- Protons bonded to the same carbon will split each other <u>only</u> if they are not equivalent.
- Protons on adjacent carbons normally will couple.
- Protons separated by four or more bonds will not couple.

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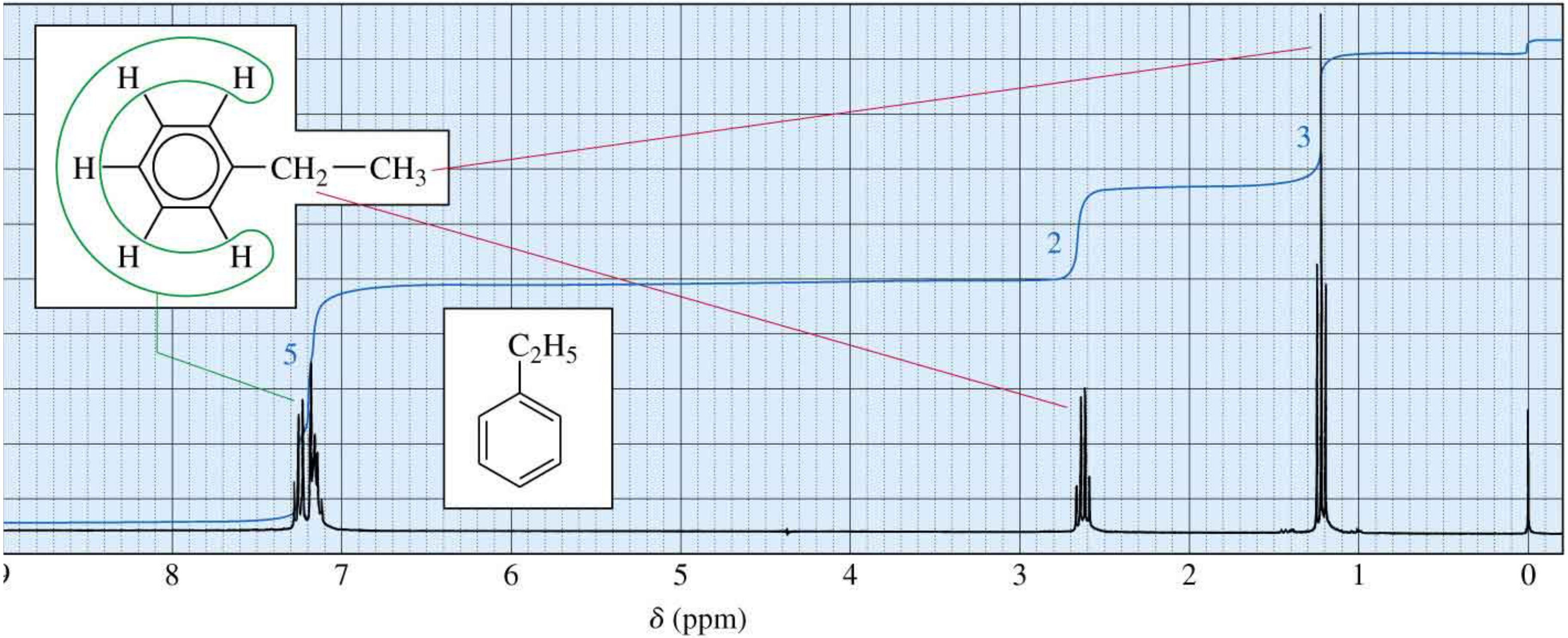
Splitting for Ethyl Groups



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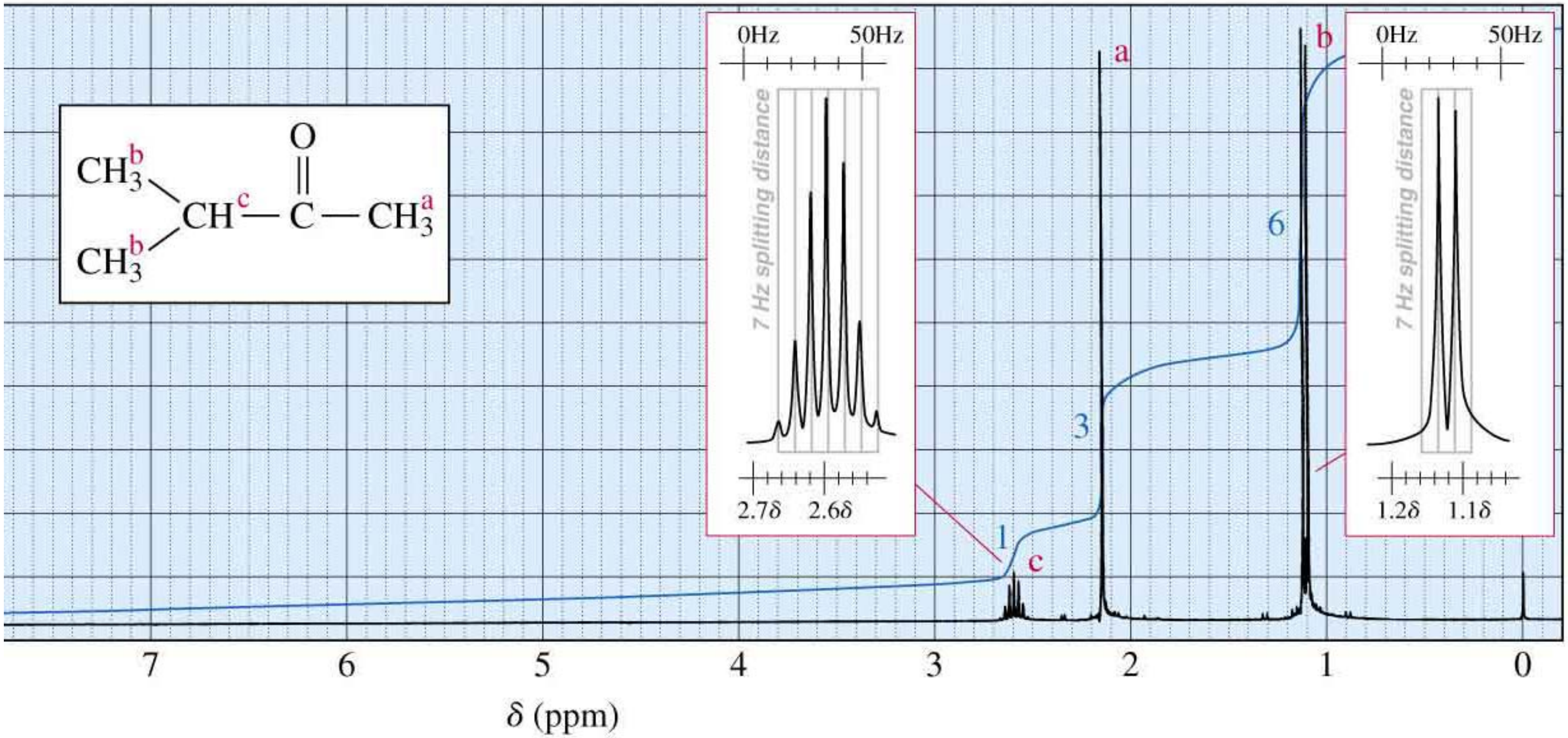
Splitting for Isopropyl Groups



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Coupling Constants

- Distance between the peaks of multiplet
- Measured in Hz
- Not dependent on strength of the external field
- Multiplets with the same coupling constants may come from adjacent groups of protons that split each other.

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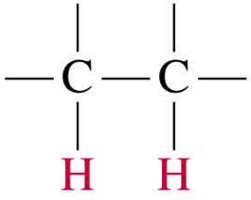
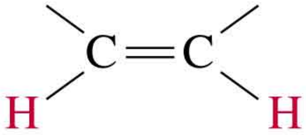
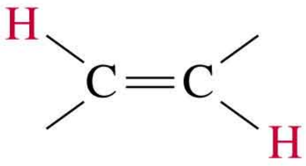
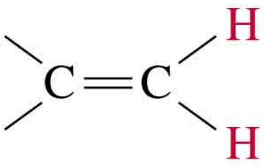
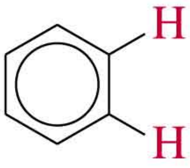
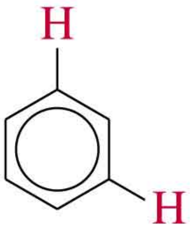
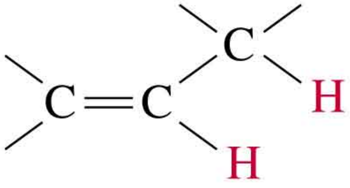
Values for Coupling Constants

		Approx. J
	(free rotation)	7 Hz[a]
	(cis)	10 Hz
	(trans)	15 Hz
	(geminal)	2 Hz

	Approx. J
 (ortho)	8 Hz
 (meta)	2 Hz
 (allylic)	6 Hz

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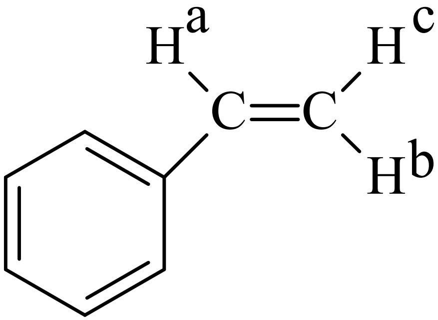
[a] The value of 7 Hz in an alkyl group is averaged for rapid rotation about the carbon–carbon bond. If rotation is hindered by a ring or bulky groups, other splitting constants may be observed.



Complex Splitting

- Signals may be split by adjacent protons, different from each other, with different coupling constants.
- Example: H^a of styrene which is split by an adjacent H *trans* to it (J = 17 Hz) and an adjacent H *cis* to it (J = 11 Hz).

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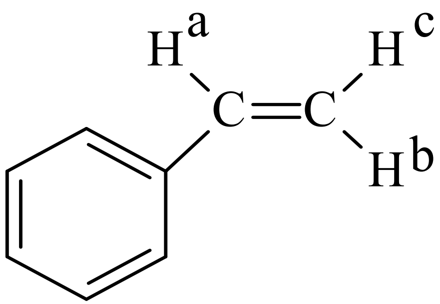
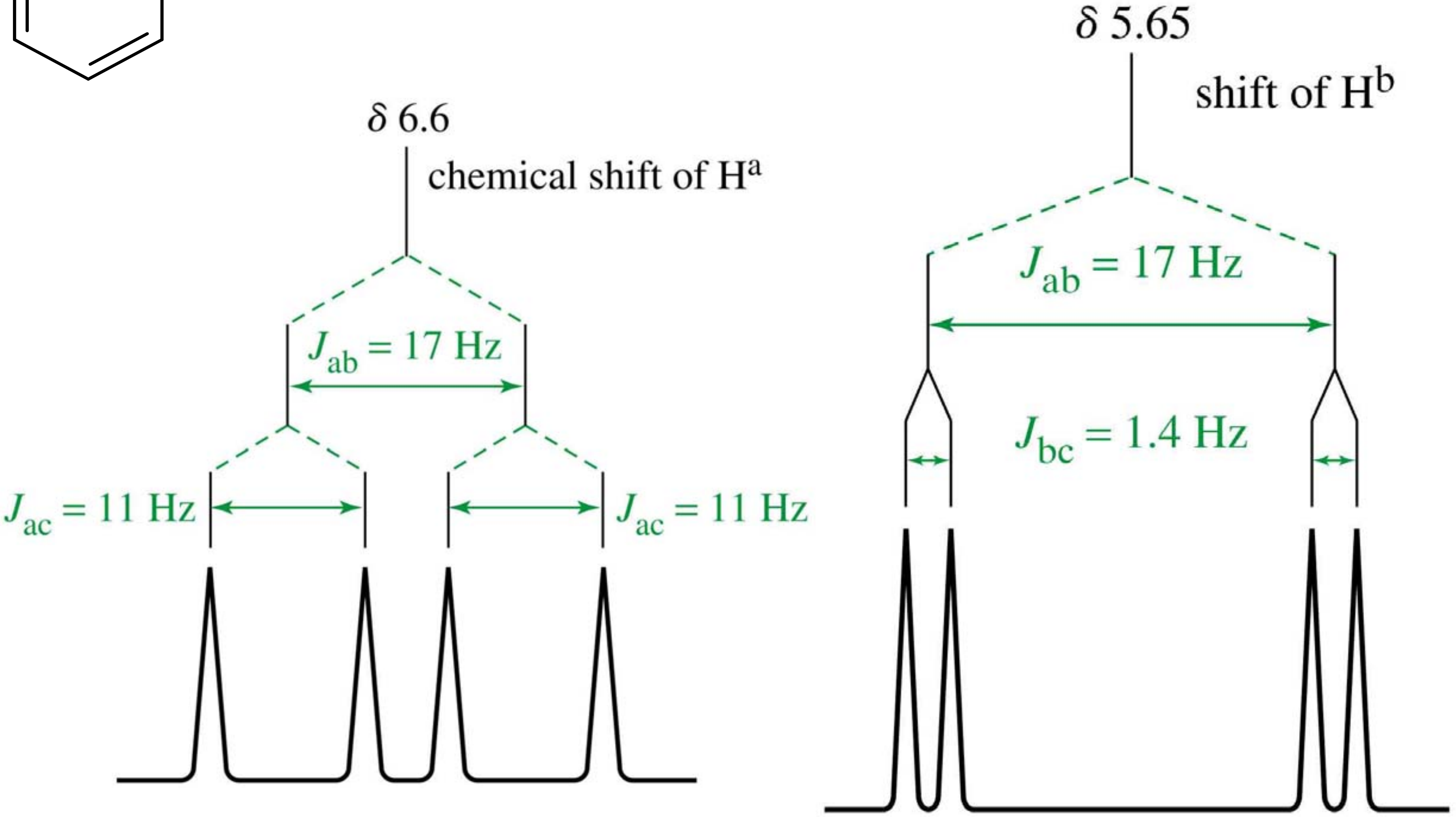
Splitting Tree





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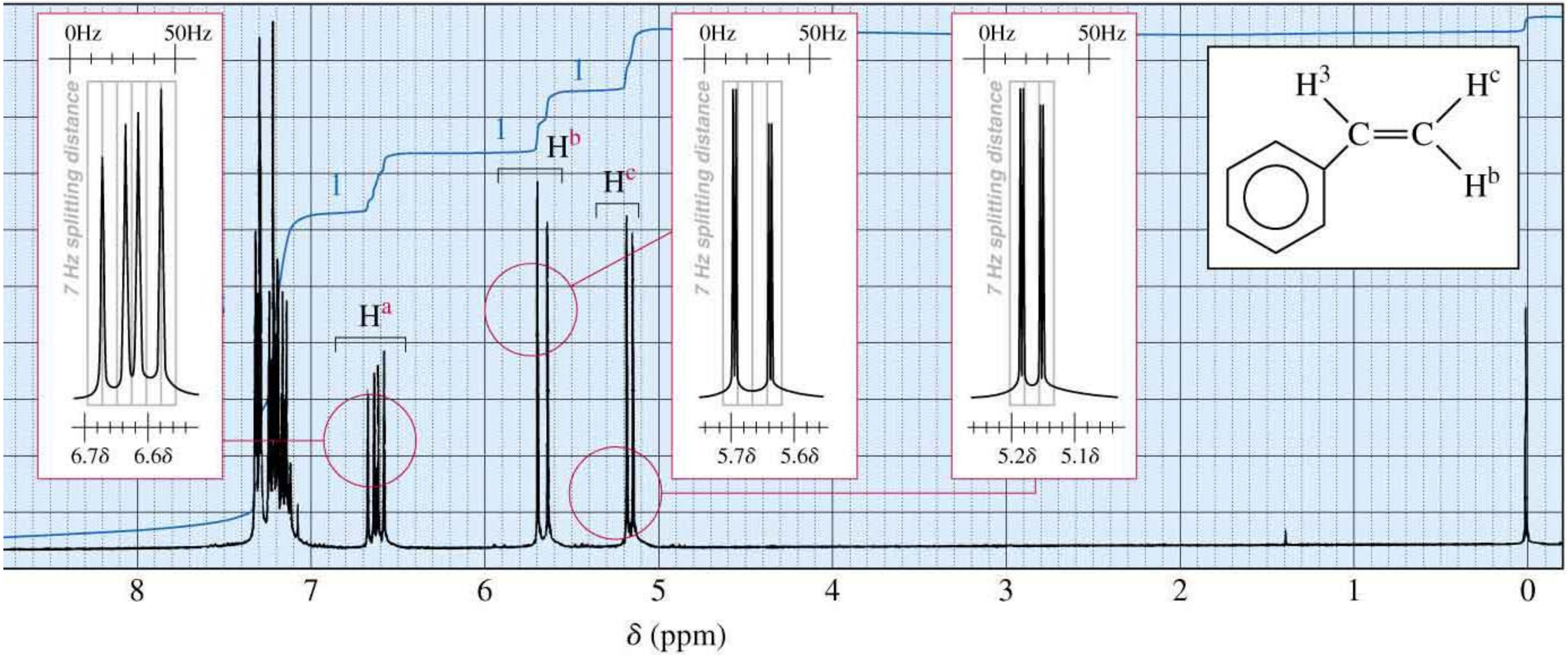
Spectrum for Styrene



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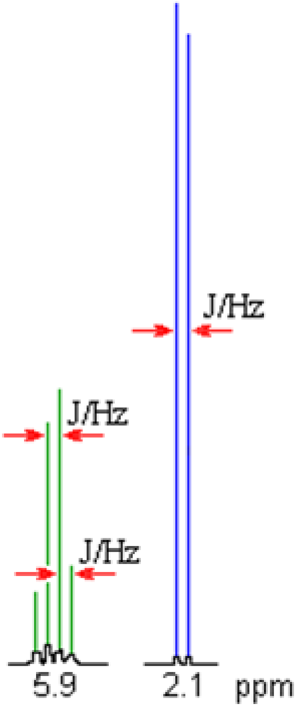
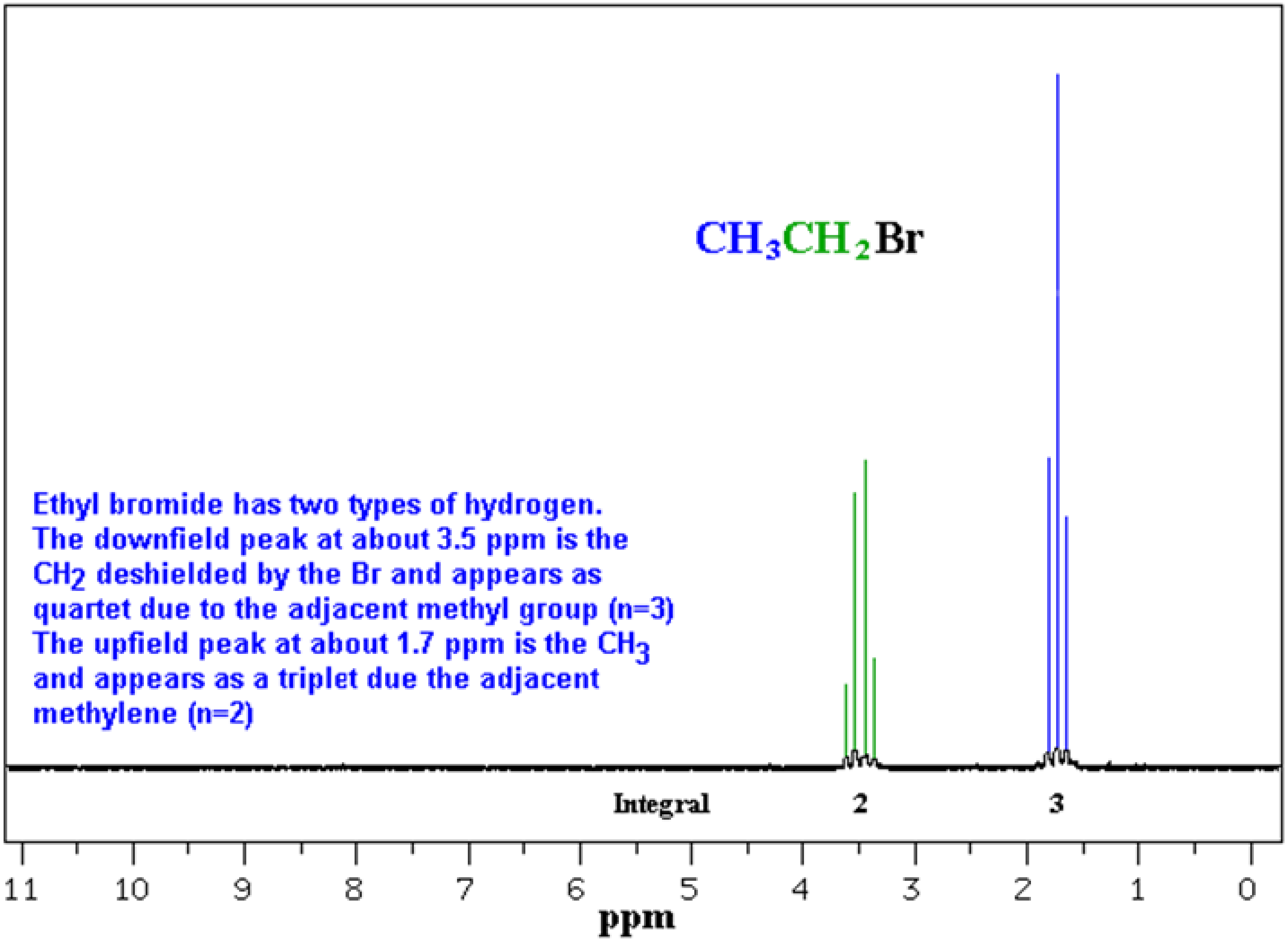
Coupling Constant, J

The coupling constant, *J* (usually in frequency units, Hz) is a measure of the interaction between a pair of protons. In a vicinal system of the general type, **H_a-C-C-H_b** then the coupling of **H_a** with **H_b**, J_{ab}, must be equal to the coupling of **H_b** with **Ha**, J_{ba}, therefore $J_{ab} = J_{ba}$.

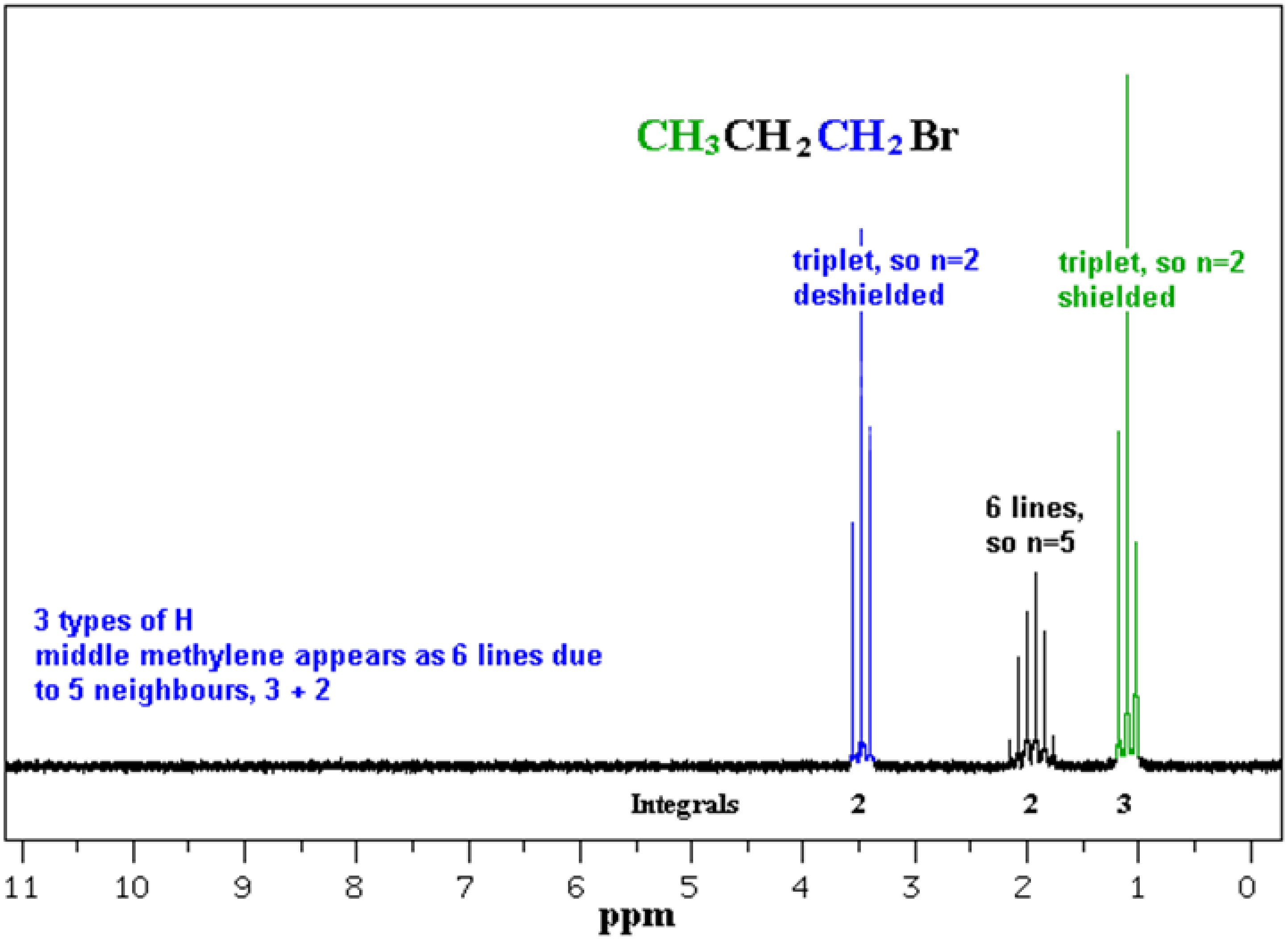
The coupling constant is independent of magnetic field strength because it is caused by the magnetic field of another nucleus, not the spectrometer magnet.

The implications are that the spacing between the lines in the coupling patterns are the same as can be seen in the coupling patterns from the ^{1}H-NMR spectra of 1,1-dichloroethane.





Ethyl bromide has two types of hydrogen. The downfield peak at about 3.5 ppm is the CH_2 deshielded by the Br and appears as quartet due to the adjacent methyl group (n=3) The upfield peak at about 1.7 ppm is the CH_3 and appears as a triplet due the adjacent methylene (n=2)



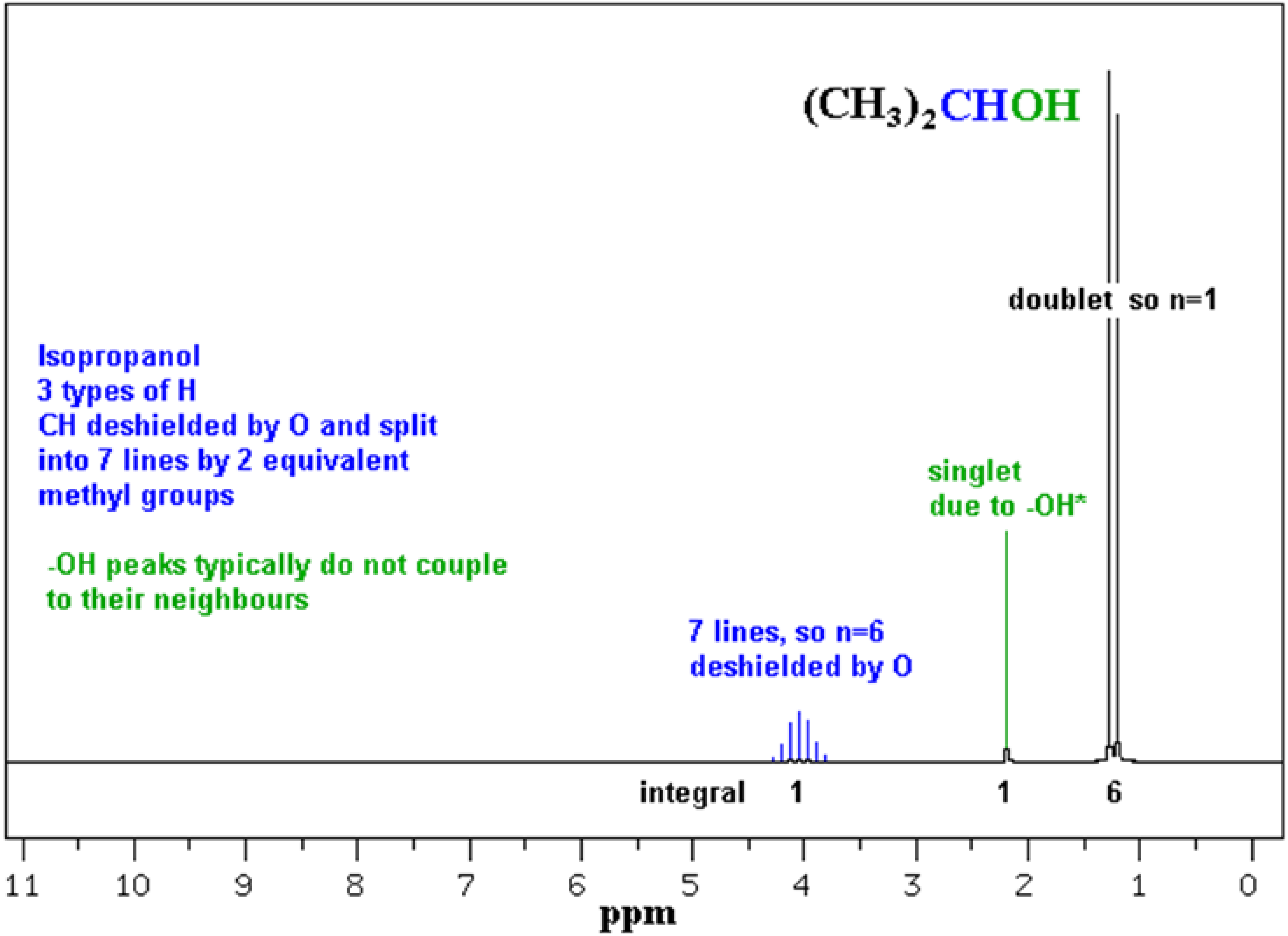
$CH_3CH_2CH_2Br$

3 types of H
middle methylene appears as 6 lines due
to 5 neighbours, 3 + 2

triplet, so n=2
deshielded

6 lines,
so n=5

triplet, so n=2
shielded



$(CH_3)_2CHOH$

Isopropanol
3 types of H
CH deshielded by O and split into 7 lines by 2 equivalent methyl groups

-OH peaks typically do not couple to their neighbours

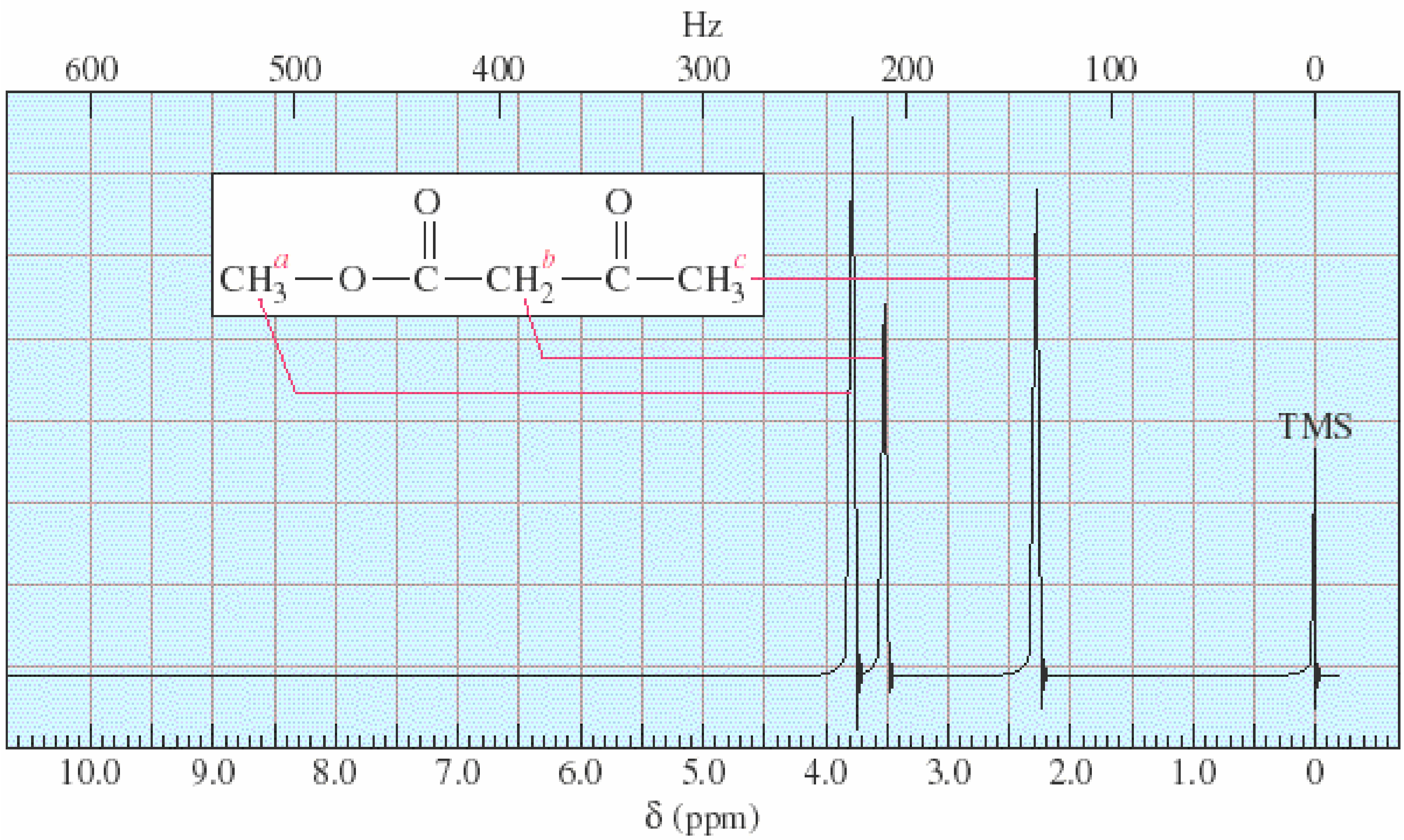
7 lines, so n=6
deshielded by O

singlet
due to -OH*

doublet so n=1

Number of Signals

Equivalent hydrogens have the same chemical shift.



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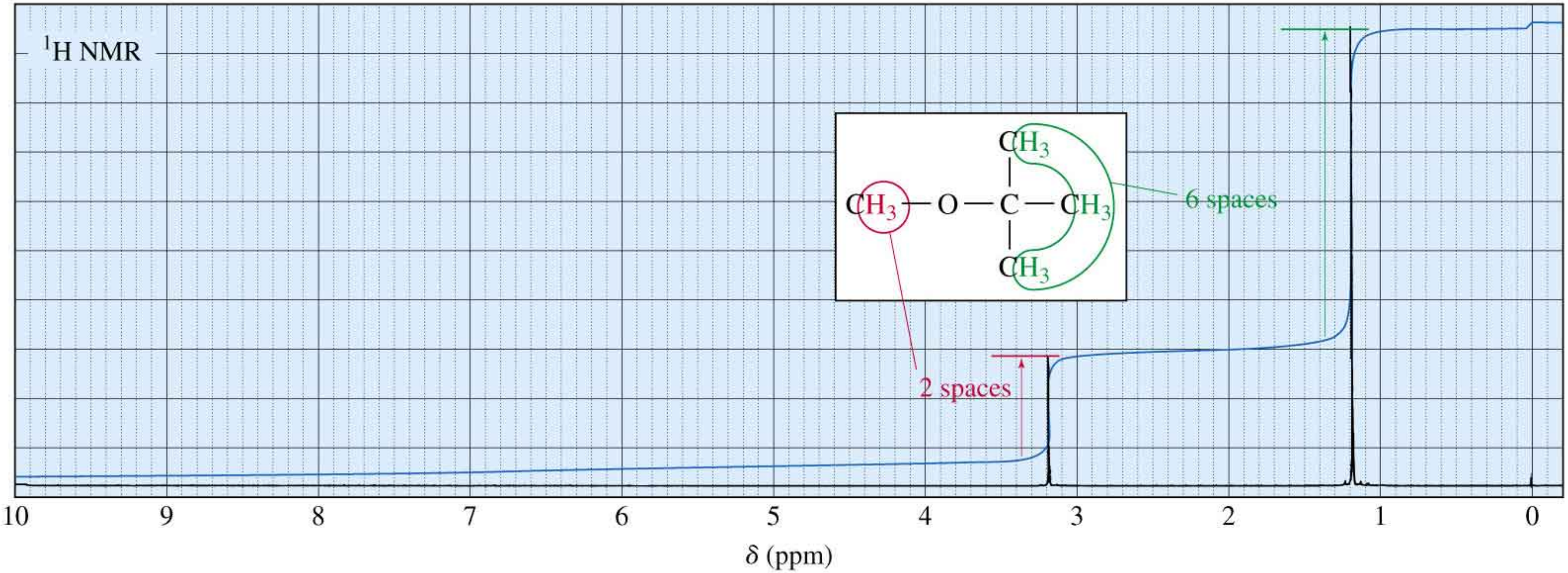
Intensity of Signals

- The area under each peak is proportional to the number of protons.
- Shown by integral trace.



=>

O-H and N-H Signals

- Chemical shift depends on concentration.
- Hydrogen bonding in concentrated solutions deshield the protons, so signal is around $\delta 3.5$ for N-H and $\delta 4.5$ for O-H.
- Proton exchanges between the molecules broaden the peak.

=>

Identifying the O-H or N-H Peak

- Chemical shift will depend on concentration and solvent.
- To verify that a particular peak is due to O-H or N-H, shake the sample with D_2O
- Deuterium will exchange with the O-H or N-H protons.
- On a second NMR spectrum the peak will be absent, or much less intense.

=>

Interpretting ^{1}H NMR Spectra

How many types of H?

Indicated by how many groups of signals there are in the spectra

What types of H?

Indicated by the *chemical shift* of each group

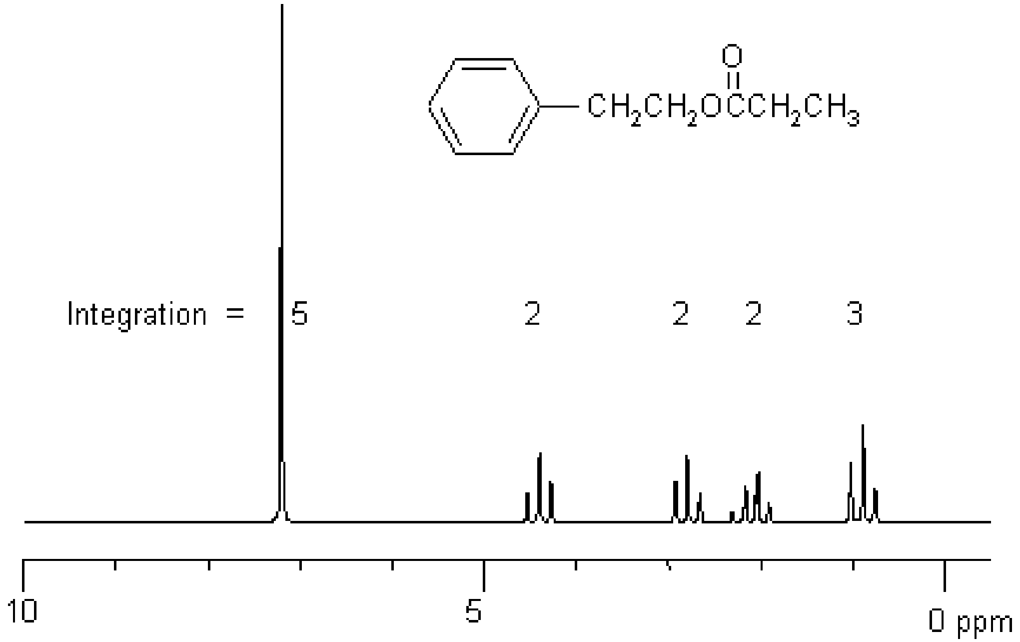
How many H of each type are there?

Indicated by the *integration* (relative area) of the signal for each group.

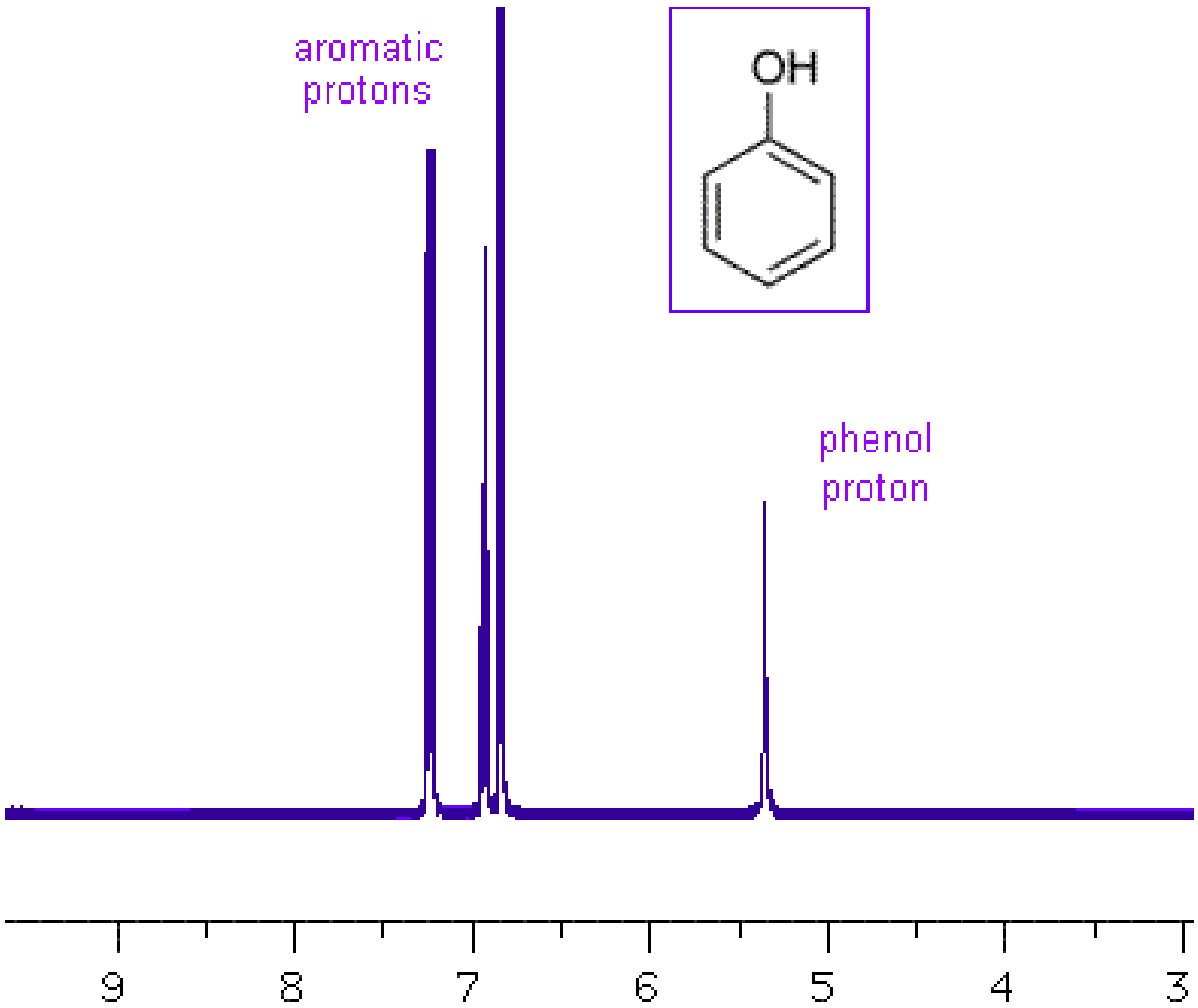
What is the connectivity?

Look at the *coupling patterns*. This tells you what is *next to* each group.

An example of an ^{1}H-NMR is shown below:



Based on the outline given above the four sets of information we get are:
Five basic types of H present in the ratio of 5:2:2:2:3. These are seen as a 5H "singlet"(ArH), two 2H triplets, a 2H quartet and a 3H triplet. Each triplet tells us that there are 2H in the adjacent position, and a quartet tells us that there are 3H in adjacent position (think of it as the lines you see, L = n + 1, where n = number of equivalent adjacent H).
This tells us that the peaks at 4.4 and 2.8 ppm must be connected as a CH_2CH_2 unit. The peaks at 2.1 and 0.9 ppm as a CH_2CH_3 unit. Using the chemical shift charts, the H can be assigned to the peaks:
7.2 ppm (5H)= Ar**H** ; 4.4 ppm (2H)= C$\mathbf{H_2}$O; 2.8 ppm (2H)= Ar-C$\mathbf{H_2}$;
2.1 ppm (2H)= O=CC$\mathbf{H_2}$$CH_3$ and 0.9 ppm (3H)= CH_2C$\mathbf{H_3}$.


aromatic
protons
OH
phenol
proton
9
8
7
6
5
4
3

^{13}C NMR SPECTROSCOPY

- ^{13}C has only about 1.1% natural abundance (of carbon atoms), ^{12}C does not exhibit NMR behaviour (nuclear spin, $I = 0$). As a result, C is about 400 times less sensitive than H nucleus to the NMR phenomena.

- Due to low abundance, there is no carbon-carbon coupling that causes splitting of signals into multiple peaks.

- Chemical shift range is normally 0 to 220 ppm.

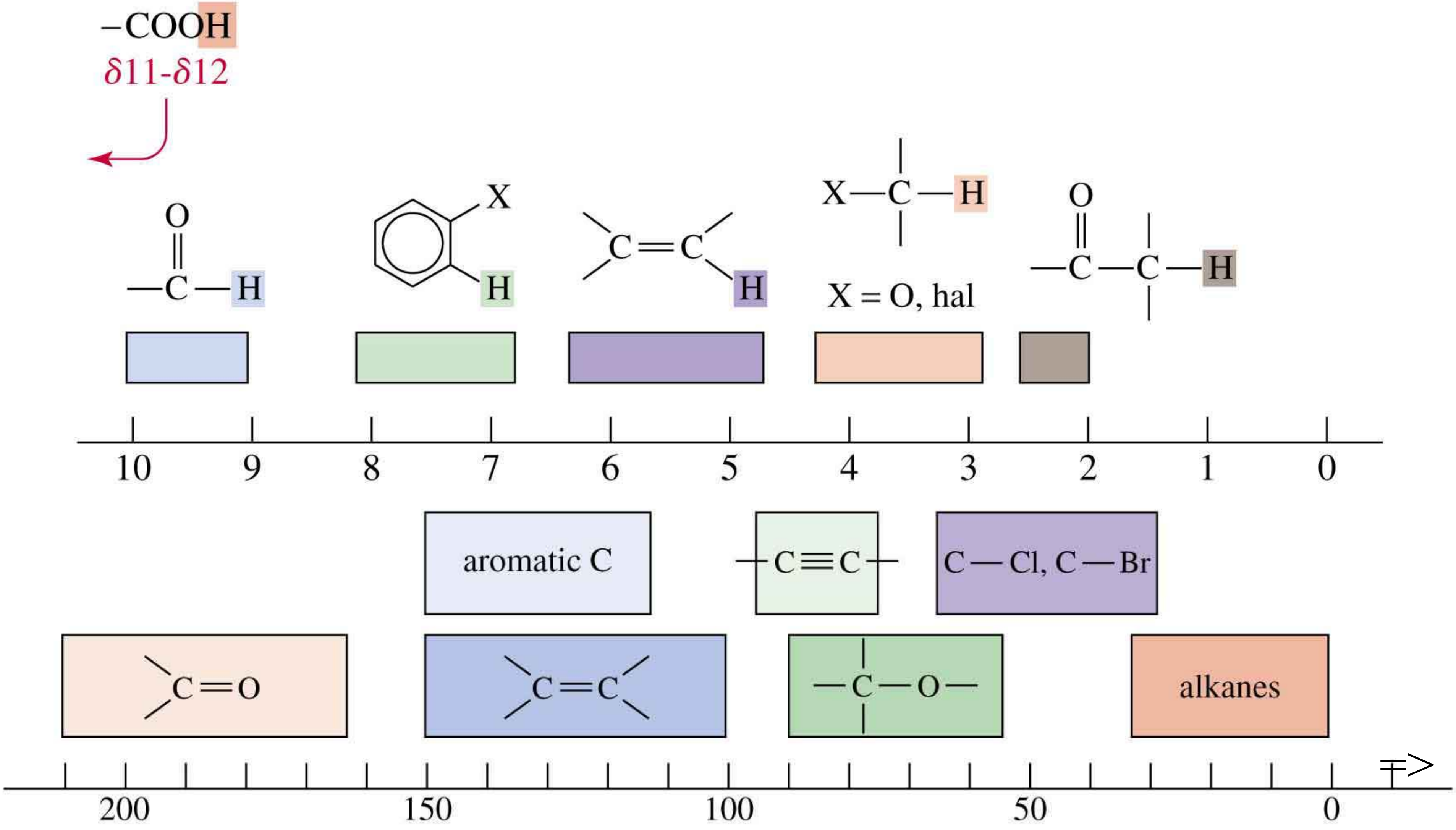
- Chemical shifts measured with respect to tetramethylsilane, $(CH_3)_4Si$ (*i.e.* TMS).

- Similar factors affect the chemical shifts in ^{13}C as seen for ^{1}H NMR.

- In further contrast to ^{1}H NMR, the intensities of the signals are not normally proportional to the number of equivalent ^{13}C atoms and are instead strongly dependent on the number of surrounding spins (typically ^{1}H). *(The carbon not attached to any protons is called 'quaternary' carbon. It usually give weaker signals than other carbons)*

- Number of peaks indicates the number of types of C.

Hydrogen and Carbon Chemical Shifts



Differences in ^{13}C Technique

- Resonance frequency is ~ one-fourth, 15.1 MHz instead of 60 MHz.
- Peak areas are not proportional to number of carbons.
- Carbon atoms with more hydrogens absorb more strongly.

=>

Spin-Spin Splitting

- It is unlikely that a ^{13}C would be adjacent to another ^{13}C, so splitting by carbon is negligible.
- ^{13}C will magnetically couple with attached protons and adjacent protons.
- These complex splitting patterns are difficult to interpret.

=>

Proton Spin Decoupling

- To simplify the spectrum, protons are continuously irradiated with “noise,” so they are rapidly flipping.
- The carbon nuclei see an average of all the possible proton spin states.
- Thus, each different kind of carbon gives a single, unsplit peak.

=>

Proton Spin Decoupling

- To simplify the spectrum, protons are continuously irradiated with “noise,” so they are rapidly flipping.
- The carbon nuclei see an average of all the possible proton spin states.
- Thus, each different kind of carbon gives a single, unsplit peak.

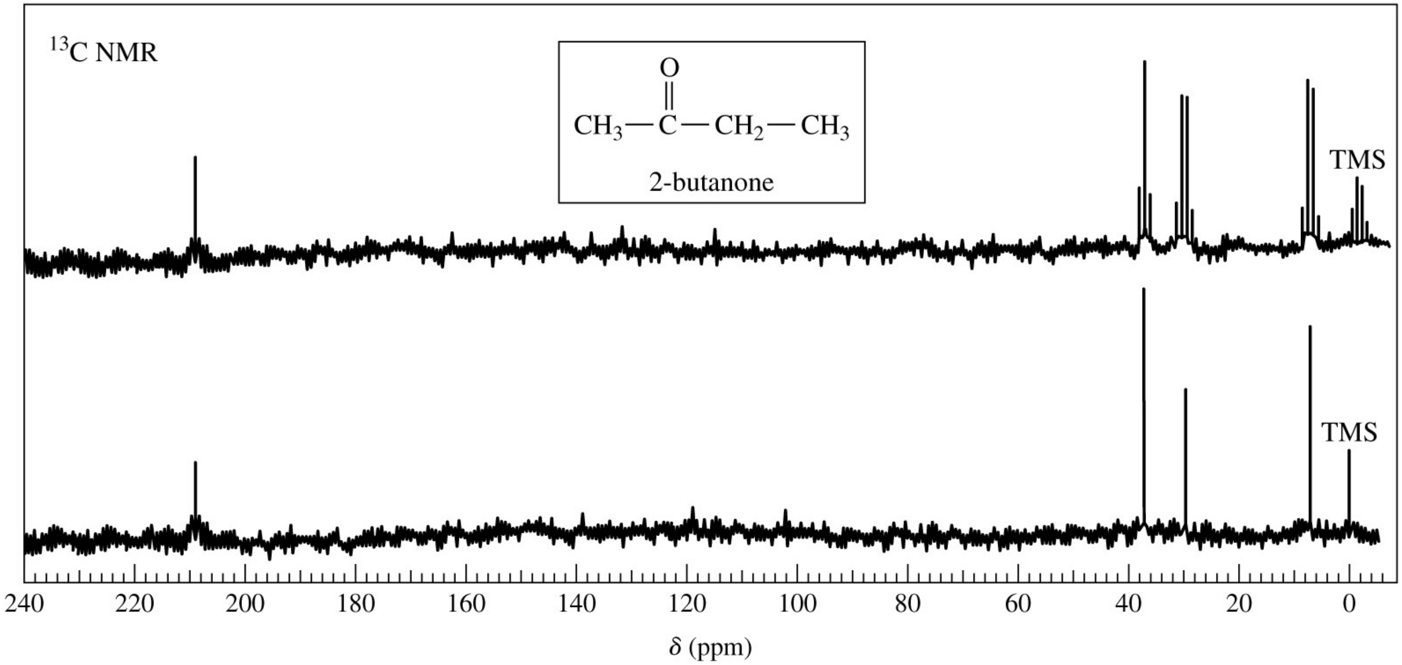
=>

Off-Resonance Decoupling

- ^{13}C nuclei are split only by the protons attached directly to them.
- The *N* + 1 rule applies: a carbon with *N* number of protons gives a signal with *N* + 1 peaks.

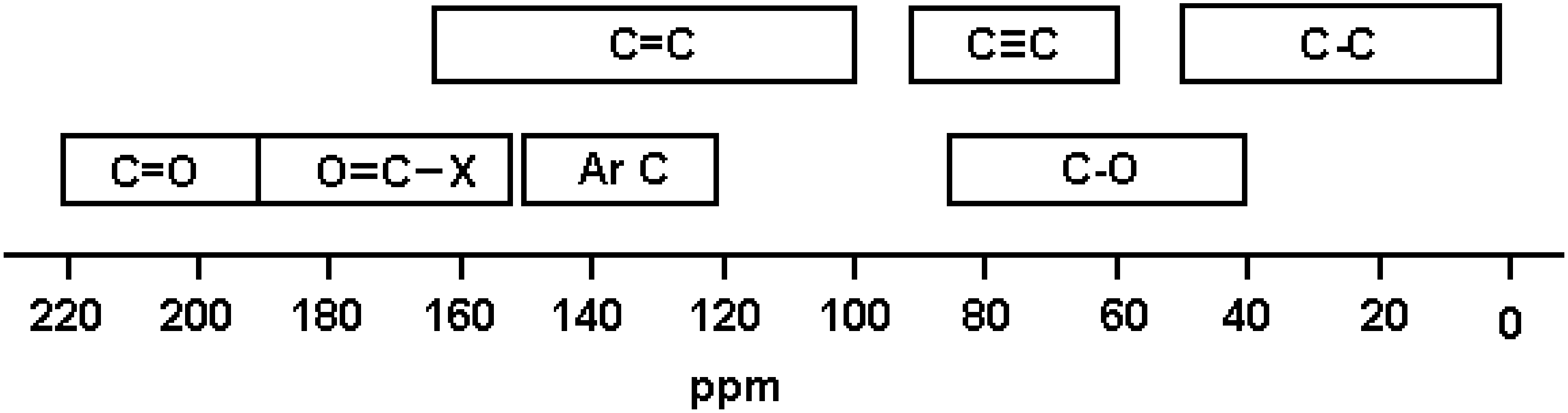
=>

Off-Resonance-decoupled and broadband decoupled ^{13}C-NMR spectrum of 2-butanone.



Interpreting ^{13}C NMR

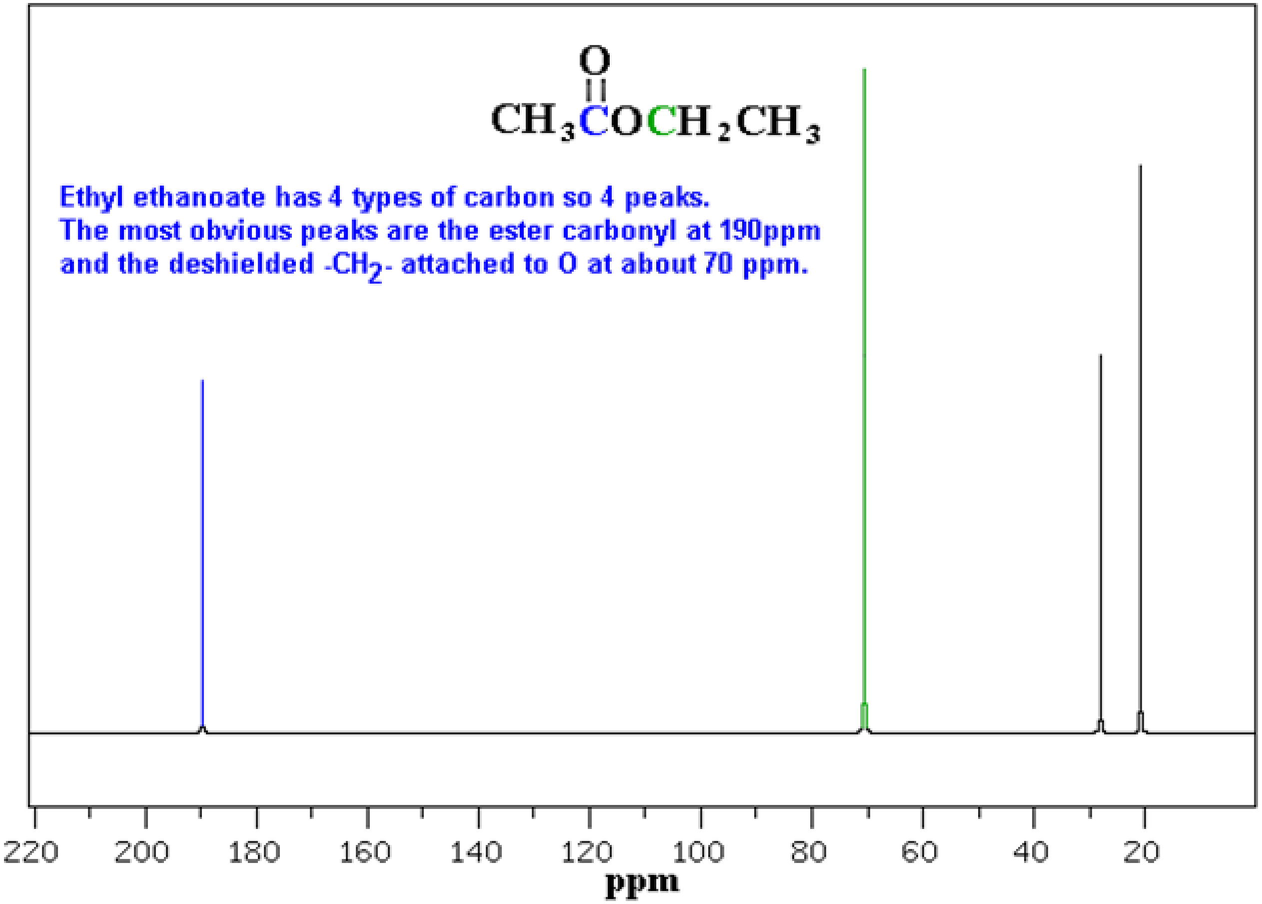
- The number of different signals indicates the number of different kinds of carbon.
- The location (chemical shift) indicates the type of functional group.
- The peak area indicates the numbers of carbons (if integrated).
- The splitting pattern of off-resonance decoupled spectrum indicates the number of protons attached to the carbon. =>



The simple correlation table of ^{13}C chemical shifts:

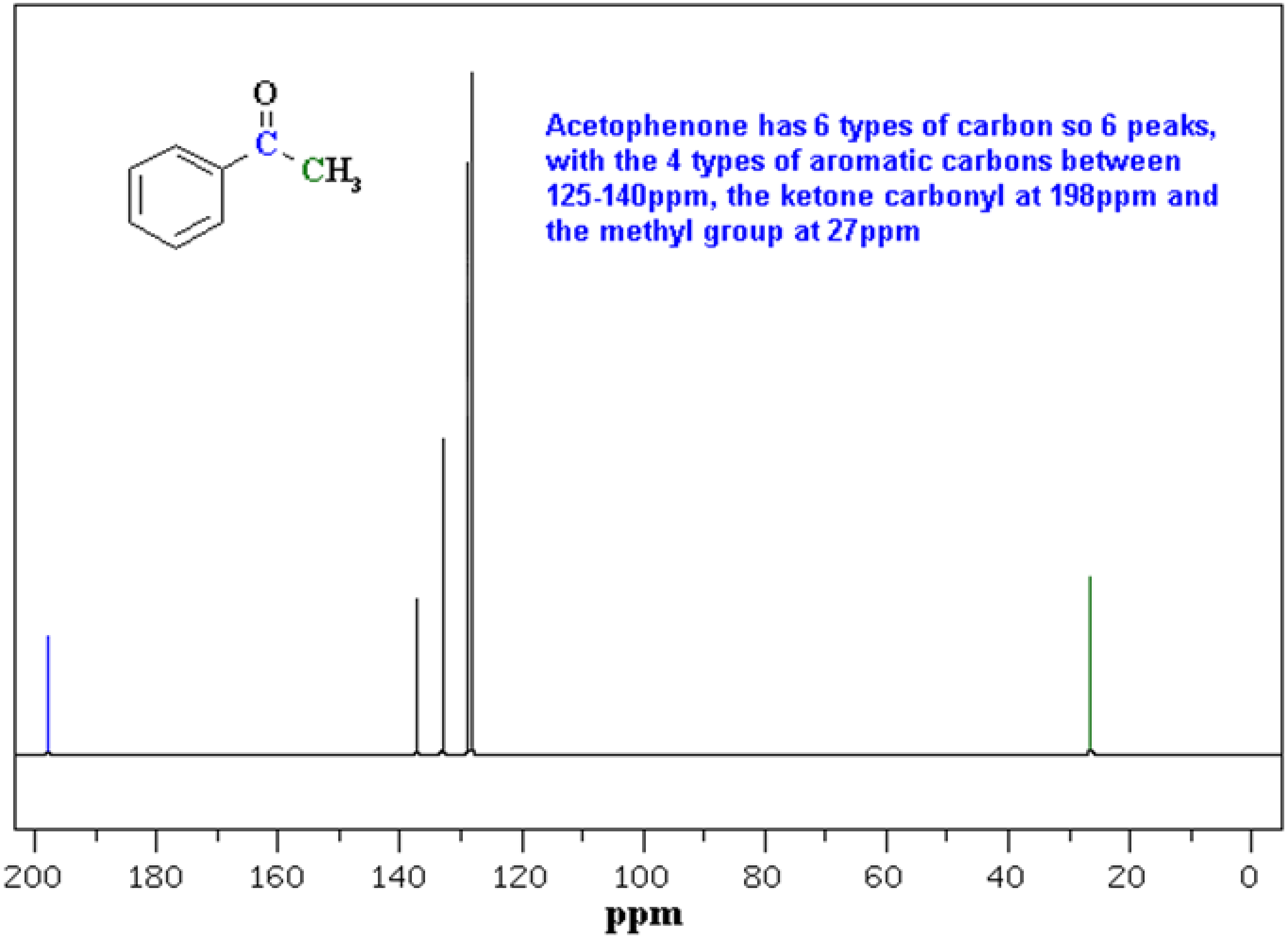
C=O indicates aldehydes and ketones;

O=C-X indicates carboxylic acids and derivatives such as esters and amides.



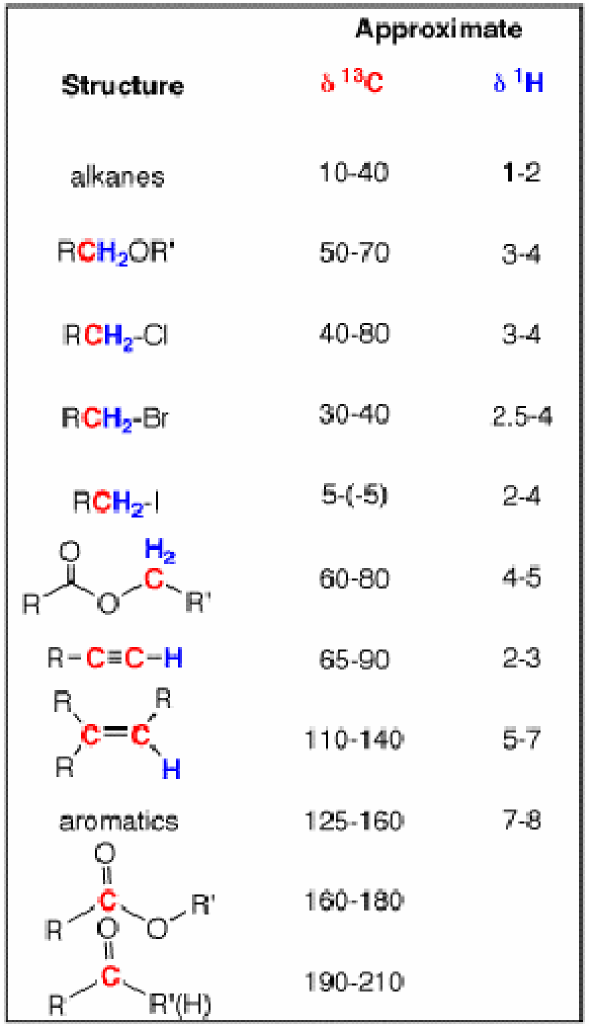
$$CH_3\overset{\overset{\displaystyle O}{\|}}{C}OCH_2CH_3$$

Ethyl ethanoate has 4 types of carbon so 4 peaks.
The most obvious peaks are the ester carbonyl at 190ppm
and the deshielded $-CH_2-$ attached to O at about 70 ppm.

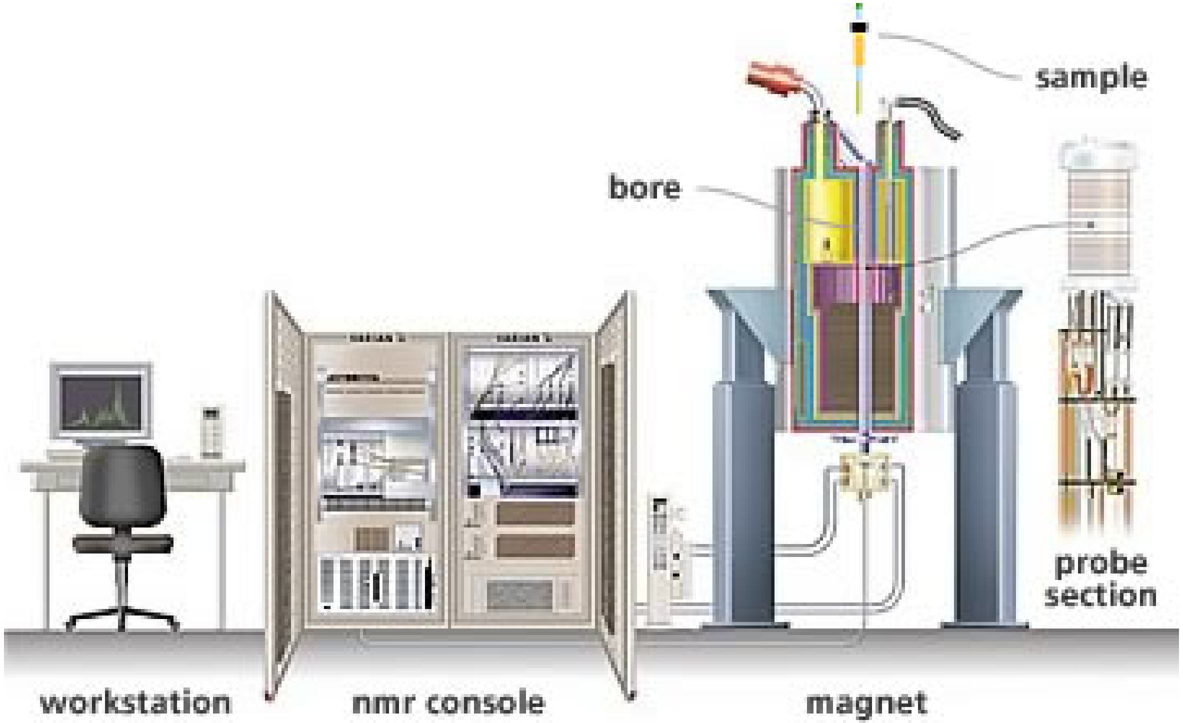


Acetophenone has 6 types of carbon so 6 peaks, with the 4 types of aromatic carbons between 125-140ppm, the ketone carbonyl at 198ppm and the methyl group at 27ppm

Summary of the approximate ^{13}C chemical shifts versus ^{1}H chemical shifts as a function of structure



	Approximate	
Structure	δ ^{13}C	δ ^{1}H
alkanes	10-40	1-2
RCH_2OR'	50-70	3-4
RCH_2-Cl	40-80	3-4
RCH_2-Br	30-40	2.5-4
RCH_2-I	5-(-5)	2-4
$RC(=O)OCH_2R'$	60-80	4-5
$R-C\equiv C-H$	65-90	2-3
$R_2C=CRH$	110-140	5-7
aromatics	125-160	7-8
$RC(=O)OR'$	160-180	
$RC(=O)R'(H)$	190-210	


sample
bore
probe section
workstation
nmr console
magnet

Degree of Unsaturation

For molecules containing only carbon, hydrogen, monovalent halogens, nitrogen, and oxygen, the formula

$$C + 1 - 0.5 * (H + X - N)$$

where C = number of carbons, H = number of hydrogens, X= number of halogens and N = number of nitrogens, gives an equivalent result.

Oxygen and other divalent atoms do not contribute to the degree of unsaturation, as (2-2) = 0.

The degree of unsaturation is used to calculate the number of rings and pi bonds, where

- Rings count as one degree of unsaturation
- Double bonds count as one degree of unsaturation
- Triple bonds count as two degrees of unsaturation.

Example 1:

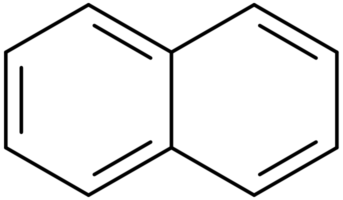
What is the degree of unsaturation of a compound that has the formula $C_{10}H_8$.

Apply $C + 1 - 0.5 * (H + X - N)$

$= 10 + 1 - 0.5 \times (8)$

$= 10 + 1 - 4 =$ **7** (sum of the number of rings and double bonds)

The formula of $C_{10}H_8$ belongs to naphthalene ring.



- ➢*When we look at the chemical formula of naphalene, it is seen that it contains 5 double bonds and 2 rings.*

Example 2: C8H8NOBr.

Apply $C + 1 - 0.5 * (H + X - N)$

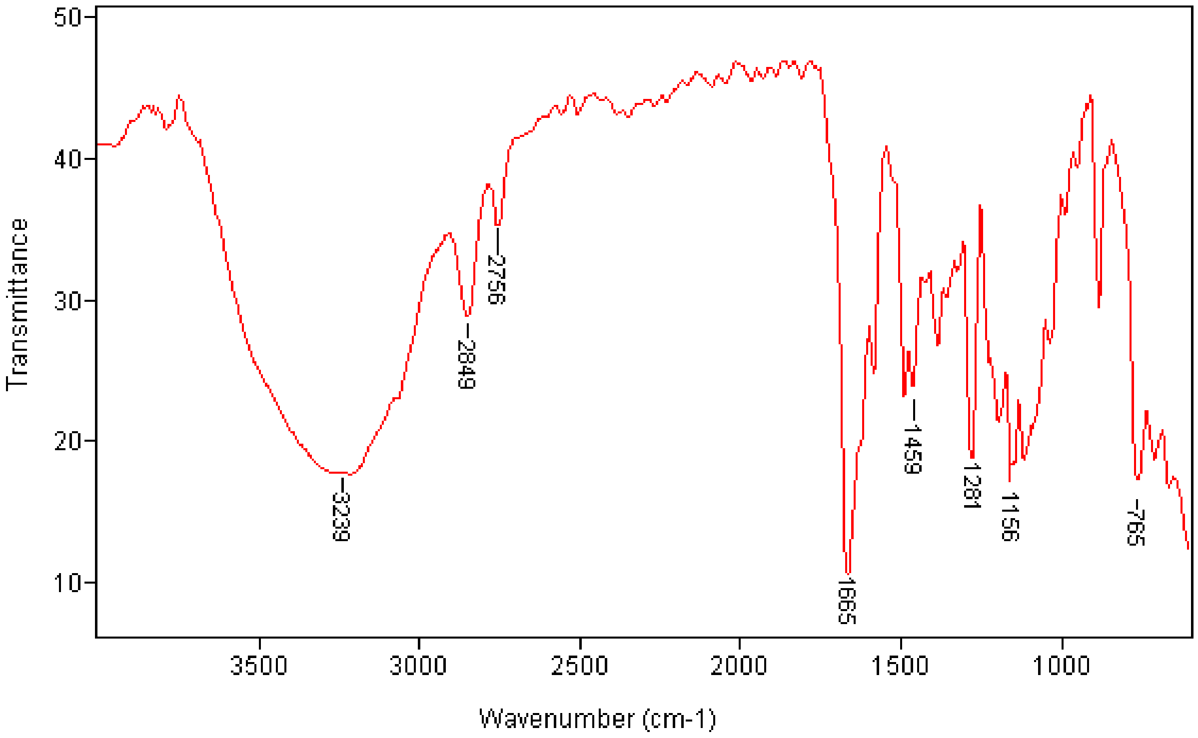
$= 8 + 1 - 0.5 \times (8 + 1 - 1)$

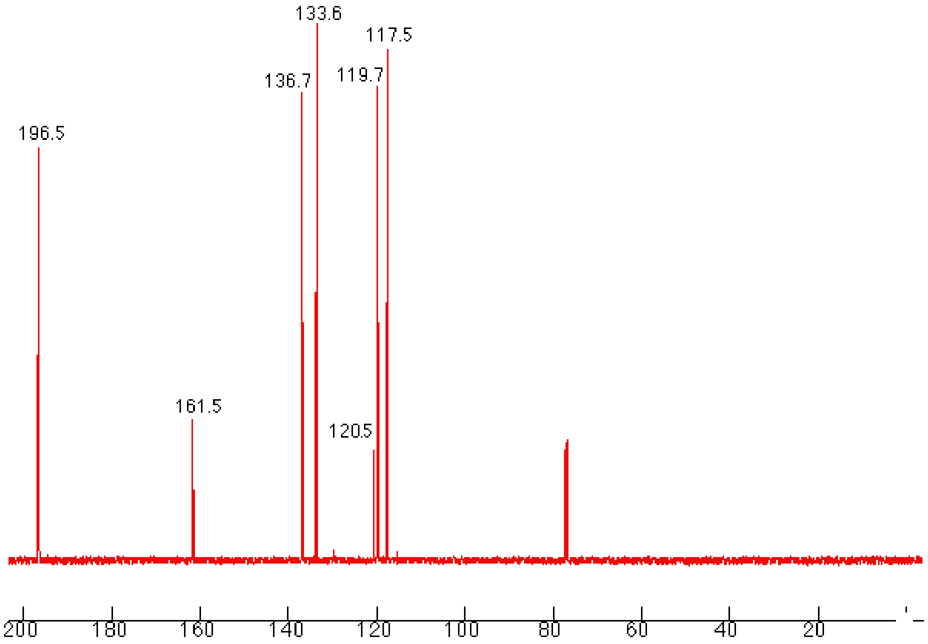
$= 8 + 1 - 4 =$ **5** (sum of the number of rings and double bonds)

** omit oxygens and substract nitrogens

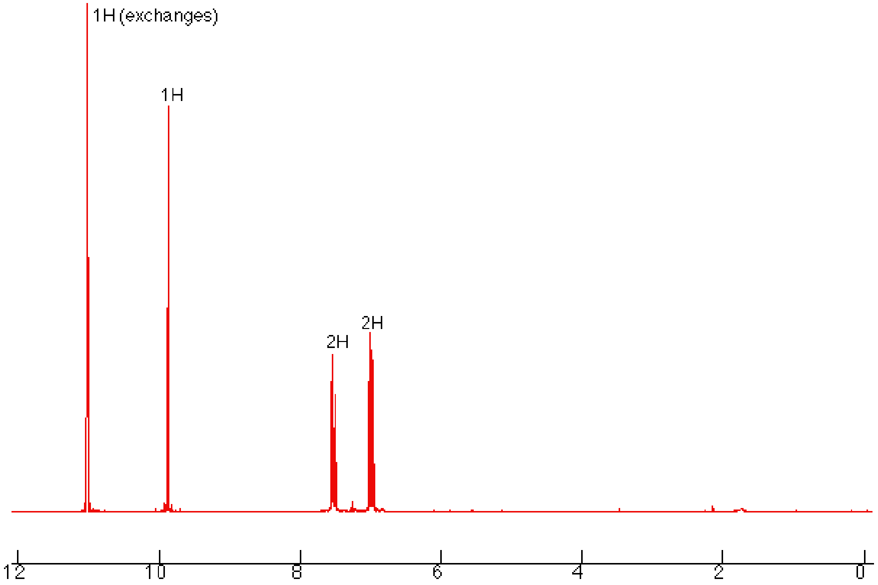
- ➢ A degree of unsaturation greater than or equal to 4 doesn’t demand but should suggest the possibility of an aromatic (benzene) ring.

- Find the structure by comparing IR, ^{1}H and ^{13}C NMR of an unknown compound with a formula of C7H6O2





196.5
161.5
136.7
133.6
120.5
119.7
117.5
200
180
160
140
120
100
80
60
40
20



1H (exchanges)
1H
2H
2H
12
10
8
6
4
2
0