Pharmaceutical analysis 838802

Nuclear Magnetic resonance spectroscopy

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NMR SPECTROSCOPY

- Nuclear magnetic resonance spectroscopy has become a very powerful tool for structure elucidation to an organic chemist.
- This technique is based on transitions between nuclear spin states by absorption of electromagnetic radiations in the radiofrequency region of roughly 4 to 900 MHz by certain organic molecules when they are placed in a strong magnetic field.
- **E. Bloch and F. Purcell** (1952) were awarded nobel prize for demonstrating the NMR effect in 1946.

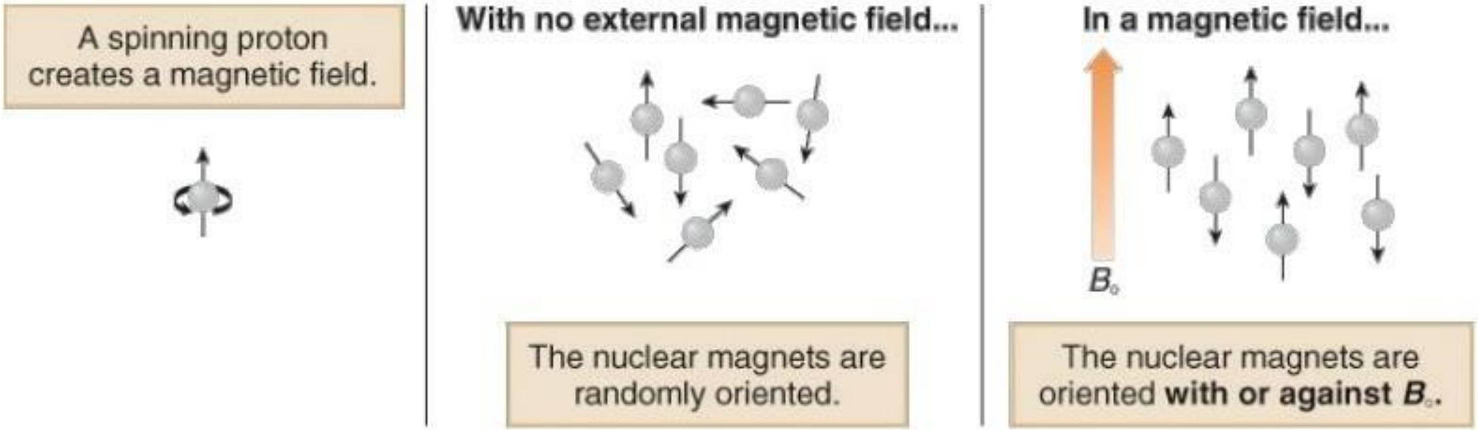
Nuclear Spin States

- **An electron has a spin quantum number of 1/2 with allowed values of +1/2 and -1/2.**
  - This spinning charge has an associated magnetic field.
  - In effect, an electron behaves as if it is a tiny bar magnet and has what is called a magnetic moment.
- **The same effect holds for certain atomic nuclei.**
  - Any atomic nucleus that has an odd mass number, an odd atomic number, or both, also has a spin and a resulting nuclear magnetic moment.
  - The allowed nuclear spin states are determined by the spin quantum number, *I*, of the nucleus given by the formula **2I+1**

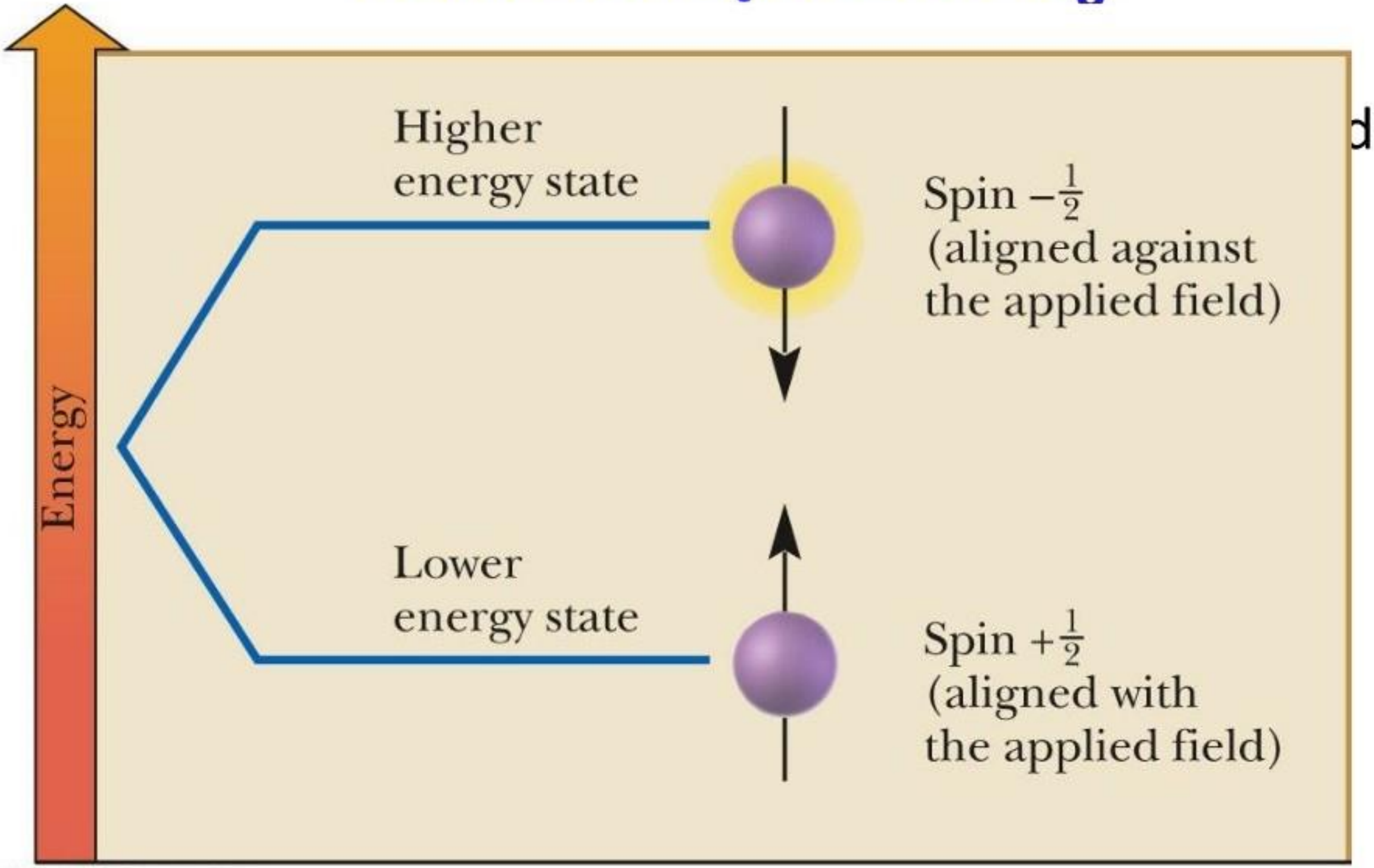
No. of protons	No. of Neutrons	Spin Quantum No.(I)	Examples
Even	Even	0	^{12}C, ^{16}O, ^{32}S
Odd	Even	1/2	^{1}H, ^{19}F, ^{31}P
		3/2	^{11}B, ^{35}Cl, ^{79}Br, ^{127}I
Even	Odd	1/2	^{13}C
		5/2	^{17}O
Odd	Odd	1	^{2}H, ^{14}N

Introduction to NMR Spectroscopy

- When a charged particle such as a proton spins on its axis, it creates a magnetic field. Thus, the nucleus can be considered to be a tiny bar magnet.
- Normally, these tiny bar magnets are randomly oriented in space. However, in the presence of a magnetic field B_0, they are oriented with or against this applied field. More nuclei are oriented with the applied field because this arrangement is lower in energy.
- The energy difference between these two states is very small (<0.1 cal).



Nuclear Spins in B_0



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Nuclear Magnetic Resonance

- **Resonance:** In NMR spectroscopy, resonance is the absorption of energy by a precessing nucleus and the resulting "flip" of its nuclear spin from a lower energy state to a higher energy state.
- The precessing spins induce an oscillating magnetic field that is recorded as a signal by the instrument.
  - **Signal:** A recording in an NMR spectrum of a nuclear magnetic resonance.

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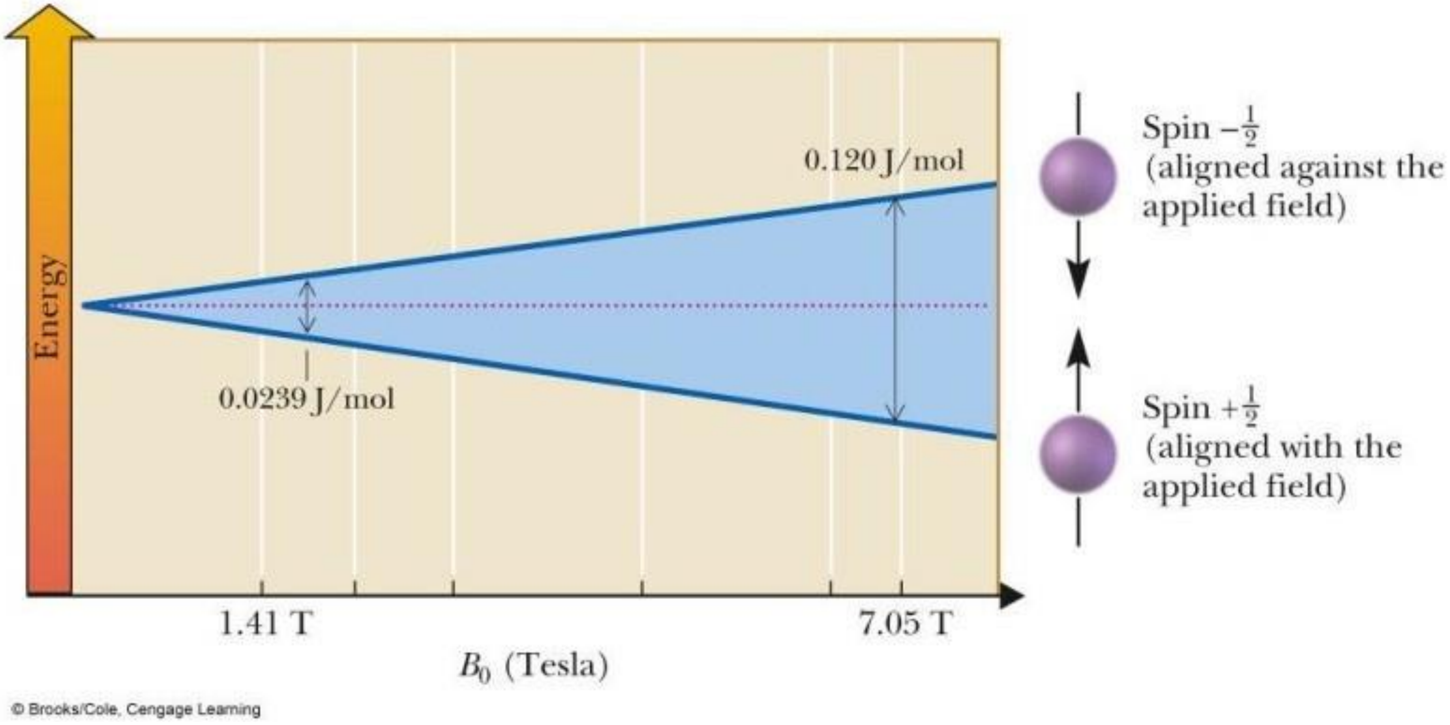
Nuclear Spin in B_0

- The energy difference between allowed spin states increases linearly with applied field strength.
- Values shown here are for ^1H nuclei.



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ΔE and Magnet Strength

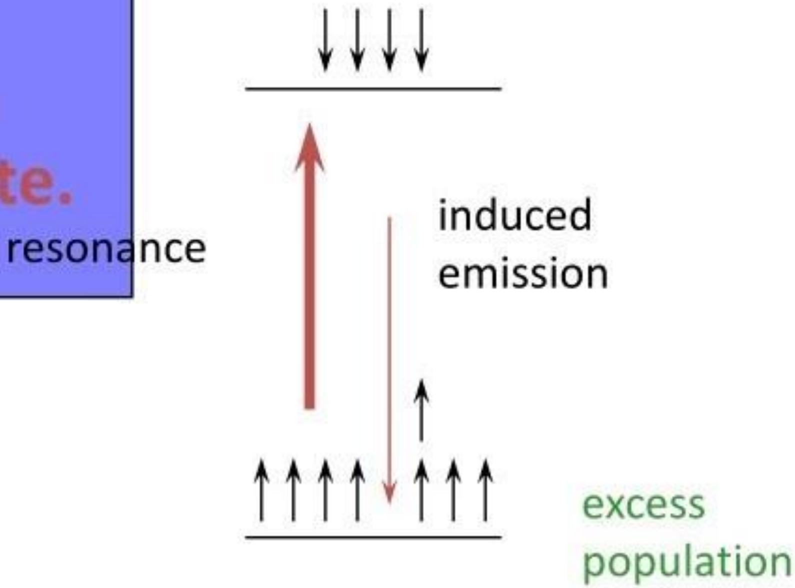
- Energy difference is proportional to the magnetic field strength.
- $\Delta E = h\nu = \frac{\gamma h}{2\pi} B_0$ **The Larmor Equation**
- Gyromagnetic ratio, γ, is a constant for each nucleus (26,753 $s^{-1}gauss^{-1}$ for H).

NMR Field Strength	^1H Operating Frequency
1.41 T	60 Mhz
2.35 T	100 MHz
7.05 T	300 MHz

POPULATION AND SIGNAL STRENGTH

The strength of the NMR signal depends on the <u>Population Difference</u> of the two spin states

For a net positive signal there must be an excess of spins in the lower state.



Saturation = equal populations = no signal

Relaxation processes

- In actual case saturation stage is never reached because higher energy nuclei are constantly returning to the lower energy state by two radiationless processes called
- Spin-spin relaxation
- Spin-lattice relaxation

SPIN-LATTICE Relaxation

T_1 relaxation is sometimes called spin-lattice relaxation.

In this type of relaxation the energy lost as the nucleus returns to the lower

energy state is transferred to the molecule in the form of heat. This means that

the total number of nuclei in the excited state decreases.

SPIN-SPIN Relaxation

T_2 relaxation is commonly referred as spin-spin relaxation.

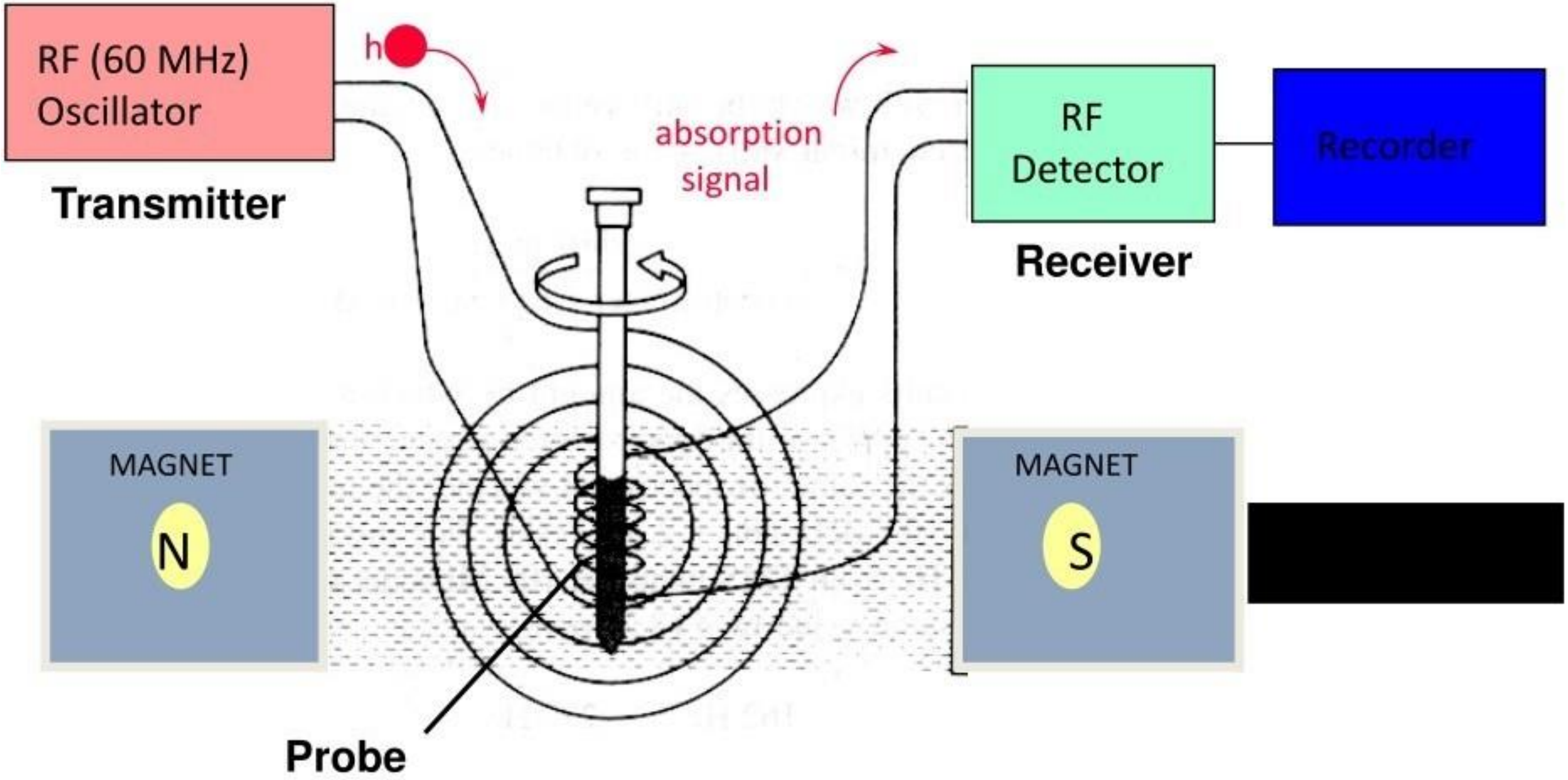
In this type of relaxation the energy released when a nucleus makes the transition from high to low energy state is absorbed by another nucleus. This allows the other nucleus to move from low energy to high. In this case the total number of nuclei in the excited state doesn’t change.

Short relaxation times result in broad signals. This is a result of the Heisenberg uncertainty principle. The shorter the time frame for observation the more uncertainty exists in the frequency. Longer relaxation times then produce narrower signals.

NMR Spectrometer

- Essentials of an NMR spectrometer are a powerful magnet, a radio-frequency generator, and a radio-frequency detector.
- The sample is dissolved in a solvent, most commonly $CDCl_3$ or D_2O, and placed in a sample tube which is then suspended in the magnetic field and set spinning.
- Using a Fourier transform NMR (FT-NMR) spectrometer, a spectrum can be recorded in about 2 seconds.

A Simplified 60 MHz NMR Spectrometer



RF (60 MHz) Oscillator

Transmitter

h

absorption signal

RF Detector

Recorder

Receiver

MAGNET

N

MAGNET

S

Probe

CONTINUOUS WAVE (CW) METHOD

THE OLDER, CLASSICAL METHOD

The magnetic field is “scanned” from a low field strength to a higher field strength while a constant beam of radiofrequency (continuous wave) is supplied at a fixed frequency (say 100 MHz).

Using this method, it requires several minutes to plot an NMR spectrum.

SLOW, HIGH NOISE LEVEL

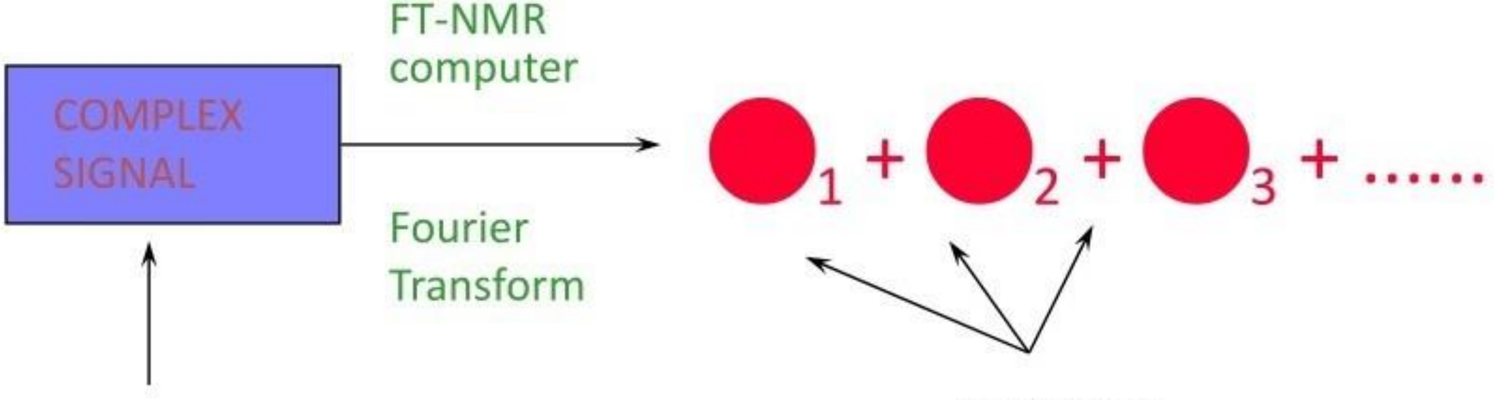
FOURIER TRANSFORM

A mathematical technique that resolves a complex FID signal into the individual frequencies that add together to make it. (Details not given here.)

TIME DOMAIN → converted to → FREQUENCY DOMAIN

FID — NMR SPECTRUM

DOMAINS ARE MATHEMATICAL TERMS



a mixture of frequencies decaying (with time)

individual frequencies

converted to a spectrum

PULSED FOURIER TRANSFORM (FT) METHOD

THE NEWER COMPUTER-BASED METHOD

FAST
LOW NOISE

Most protons relax (decay) from their excited states very quickly (within a second).

The excitation pulse, the data collection (FID), and the computer-driven Fourier Transform (FT) take only a few seconds.

The pulse and data collection cycles may be repeated every few seconds.

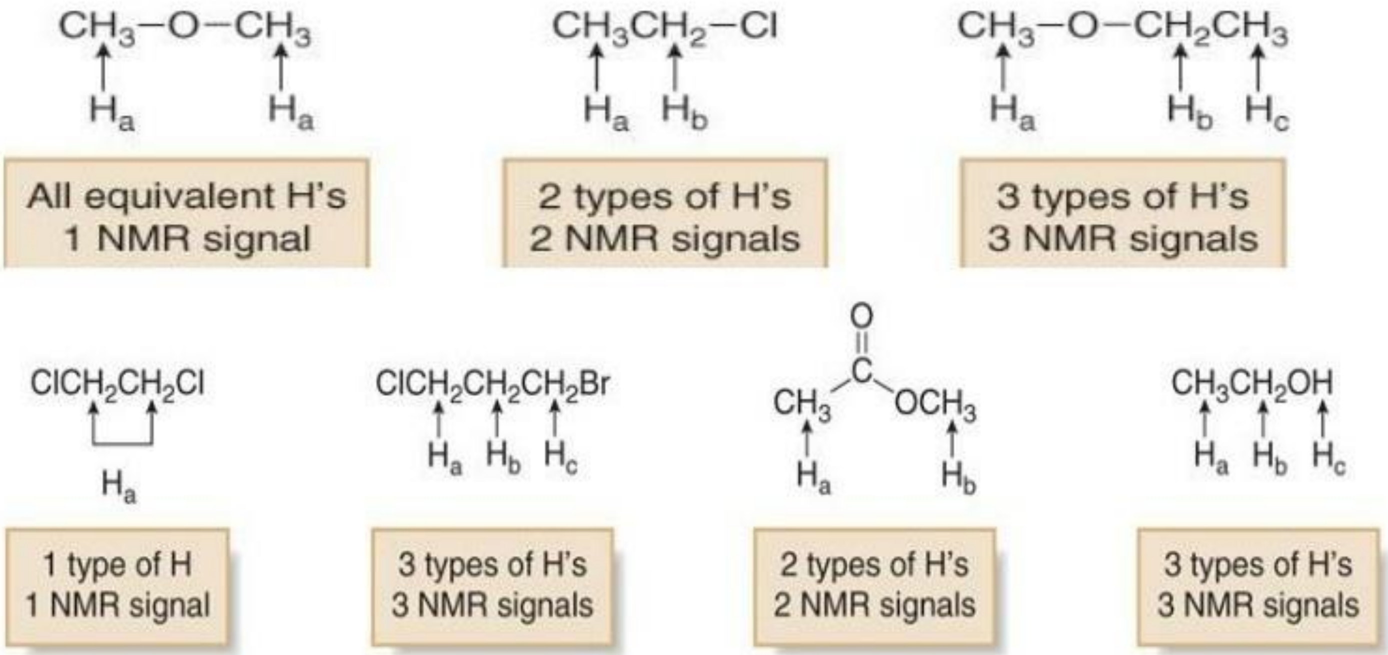
Many repetitions can be performed in a very short time, leading to improved signal .....

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. =>

^1H NMR : Number of Signals

- **Equivalent hydrogens:** Hydrogens that have the same chemical environment The number of NMR signals ~ the number of different types of ^1Hs.
- Equivalent ^1Hs give the same NMR signal.



All equivalent H's
1 NMR signal

2 types of H's
2 NMR signals

3 types of H's
3 NMR signals

1 type of H
1 NMR signal

3 types of H's
3 NMR signals

2 types of H's
2 NMR signals

3 types of H's
3 NMR signals

Classification of Protons

- **If replacement of one hydrogen at a time in separate models creates <u>enantiomers</u>, the hydrogens are enantiotopic.**

$CH_3CH_2CH_2CH_3$:

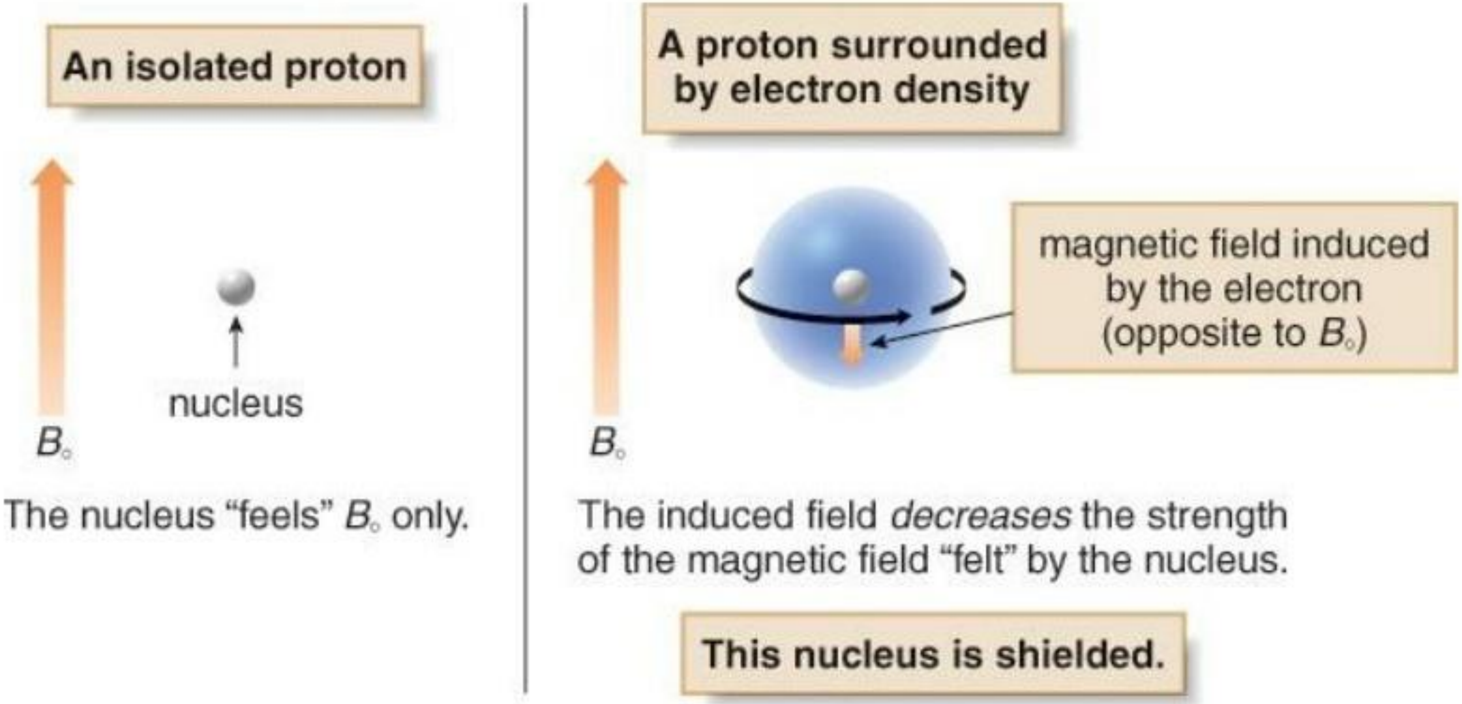
Enantiotopic protons have **the <u>same</u> chemical shifts.**

If replacement of hydrogens in separate models creates <u>diastereomers</u>, the hydrogens are diastereotopic

Diastereotopic protons have **<u>different</u> chemical shifts**

^{1}H NMR: Position of Signals (Chemical shift)

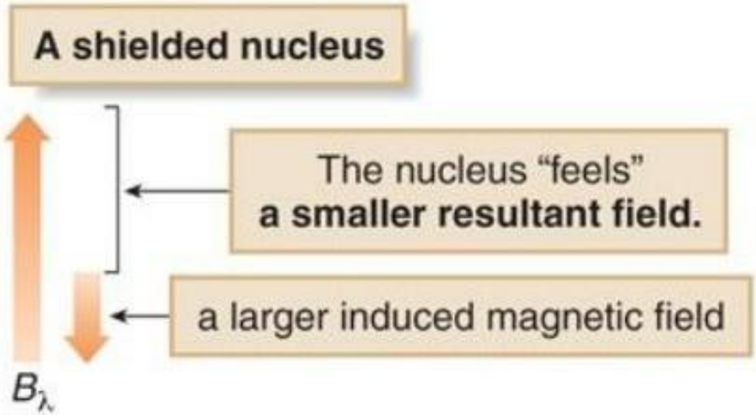
- In the vicinity of the nucleus, the magnetic field generated by the circulating electron decreases the external magnetic field that the proton "feels".
- Since the electron experiences a lower magnetic field strength, it needs a lower frequency to achieve resonance. Lower frequency is to the right in an NMR spectrum, toward a lower chemical shift, so shielding shifts the absorption upfield.



The nucleus "feels" B_0 only.

The induced field *decreases* the strength of the magnetic field "felt" by the nucleus.

This nucleus is shielded.

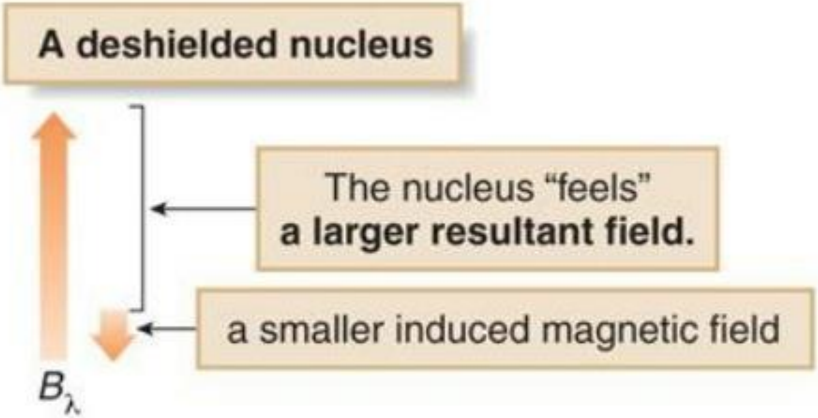


A shielded nucleus

The nucleus "feels"
a smaller resultant field.

a larger induced magnetic field

- As the electron density around the nucleus increases, the nucleus feels a smaller resultant magnetic field, so a lower frequency is needed to achieve resonance.
- **The absorption shifts upfield.**

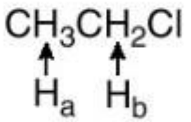


A deshielded nucleus

The nucleus "feels"
a larger resultant field.

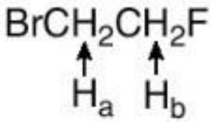
a smaller induced magnetic field

- As the electron density around the nucleus decreases, the nucleus feels a larger resultant magnetic field, so a higher frequency is needed to achieve resonance.
- **The absorption shifts downfield.**



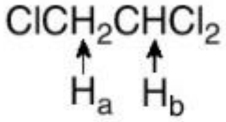
$\underset{H_a}{\underset{\uparrow}{CH_3}}\underset{H_b}{\underset{\uparrow}{CH_2}}Cl$

- The H_b protons are **deshielded** because they are closer to the electronegative Cl atom, so they absorb **downfield** from H_a.



$Br\underset{H_a}{\underset{\uparrow}{CH_2}}\underset{H_b}{\underset{\uparrow}{CH_2}}F$

- Because F is more electronegative than Br, the H_b protons are more **deshielded** than the H_a protons and absorb farther **downfield.**

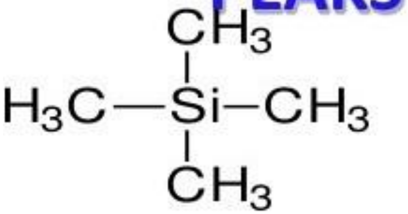


$Cl\underset{H_a}{\underset{\uparrow}{CH_2}}\underset{H_b}{\underset{\uparrow}{CH}}Cl_2$

- The larger number of electronegative Cl atoms (two versus one) **deshields** H_b more than H_a, so it absorbs **downfield** from H_a.

Tetramethylsilane:

PEAKS ARE MEASURED RELATIVE TO TMS



$H_3C-\underset{CH_3}{\overset{CH_3}{Si}}-CH_3$

- TMS has following advantages as the reference compound:

- 1. It is chemically inert and non-toxic.
- 2. It is volatile (b.pt 270c) and soluble in most organic solvents.
- 3. It gives a single sharp peak as it has 12equivalent hydrogens.
- 4. Since silicon is less electronegative than carbon, TMS protons are highly shielded. Signal defined as zero.
- 5. Organic protons absorb downfield (to the left) of the TMS signal.

=>

CHEMICAL SHIFT

- NMR absorptions generally appear as sharp peaks.
- Increasing chemical shift is plotted from left to right.
- Most protons absorb between 0-10 ppm.
- The terms “upfield” and “downfield” describe the relative location of peaks. Upfield means to the right. Downfield means to the left.
- NMR absorptions are measured relative to the position of a reference peak at 0 ppm on the d scale due to tetramethylsilane (TMS). TMS is a volatile inert compound that gives a single peak upfield from typical NMR absorptions.

THE CHEMICAL SHIFT

The shifts from TMS in Hz are bigger in higher field instruments (300 MHz, 500 MHz) than they are in the lower field instruments (100 MHz, 60 MHz).

We can adjust the shift to a field-independent value, the “chemical shift” in the following way:

parts per million

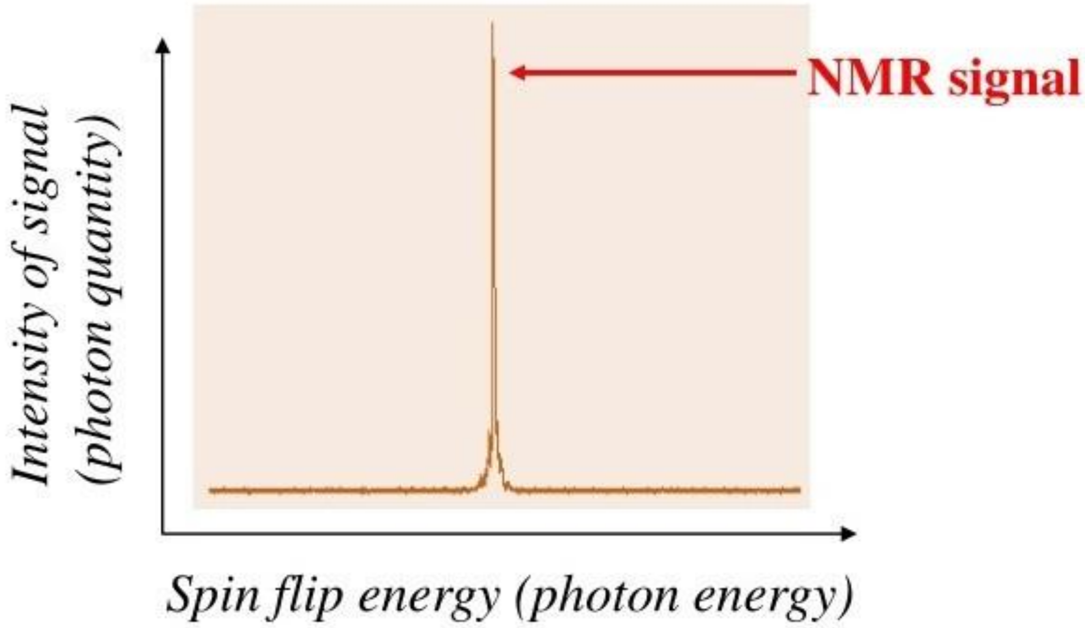
$$\text{chemical shift} = \mathbf{d} = \frac{\text{shift in Hz}}{\text{spectrometer frequency in MHz}} = \text{ppm}$$

This division gives a number independent of the instrument used.

A particular proton in a given molecule will always come <u>at the same chemical shift</u> (constant value).

The NMR Spectrum

- Spectrum = plot of photon energy versus photon quantity



Deshielded (downfield)
Low magnetic field strength

Shielded (upfield)
High magnetic field strength

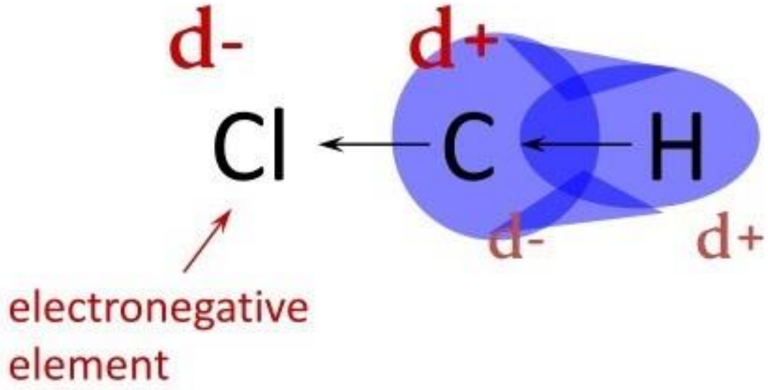
Factors affecting Chemical Shift

Three major factors account for the resonance positions (on the ppm scale) of most protons.

1. Deshielding by electronegative elements.
2. Anisotropic fields usually due to pi-bonded electrons in the molecule.
3. Deshielding due to hydrogen bonding.

We will discuss these factors in the sections that follow.

1. DESHIELDING BY AN ELECTRONEGATIVE ELEMENT



Chlorine "deshields" the proton, that is, it takes valence electron density away from carbon, which in turn takes more density from hydrogen deshielding the proton.

NMR CHART

"deshielded"
protons appear
at low field

highly shielded
protons appear
at high field

←

deshielding moves proton
resonance to lower field

Electronegativity Dependence of Chemical Shift

Dependence of the Chemical Shift of CH_3X on the Element X

Compound CH_3X	CH_3F	CH_3OH	CH_3Cl	CH_3Br	CH_3I	CH_4	$(CH_3)_4Si$
Element X	F	O	Cl	Br	I	H	Si
Electronegativity of X	4.0	3.5	3.1	2.8	2.5	2.1	1.8
Chemical shift d	4.26	3.40	3.05	2.68	2.16	0.23	0

most deshielded

TMS

deshielding increases with the electronegativity of atom X

2. ANISOTROPIC EFFECTS

DUE TO THE PRESENCE OF PI BONDS

Anisotropy refers to the dissimilar electron density in all directions.The presence of a nearby pi bond or pi system greatly affects the chemical shift.

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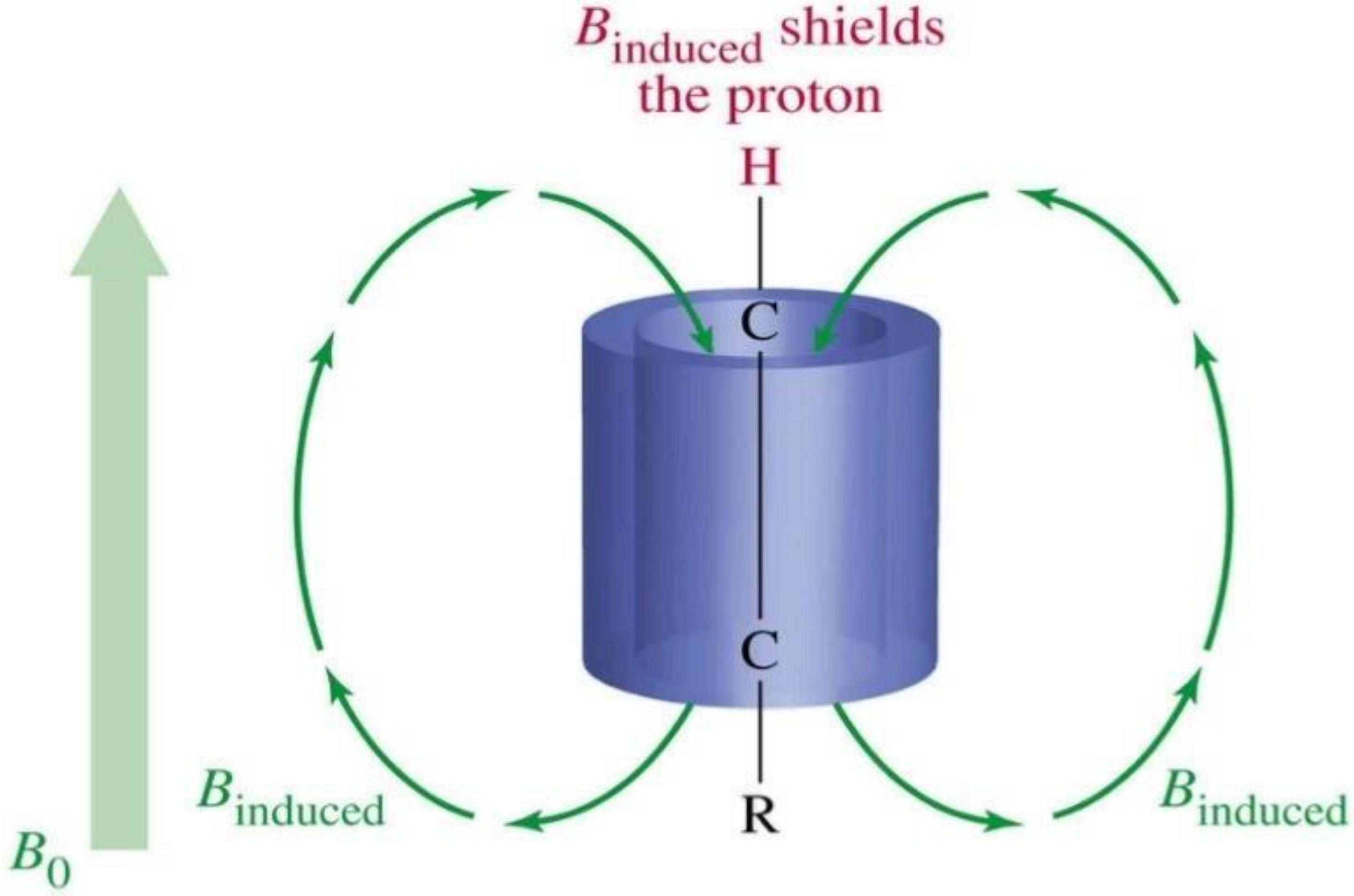
Acetylenic Protons, δ2.5

In a magnetic field, the π electrons of a carbon-carbon triple bond are induced to circulate, but in this case the induced magnetic field opposes the applied magnetic field (B_0).

Thus, the proton feels a weaker magnetic field, so a lower frequency is needed for resonance. The nucleus is shielded and the absorption is upfield.



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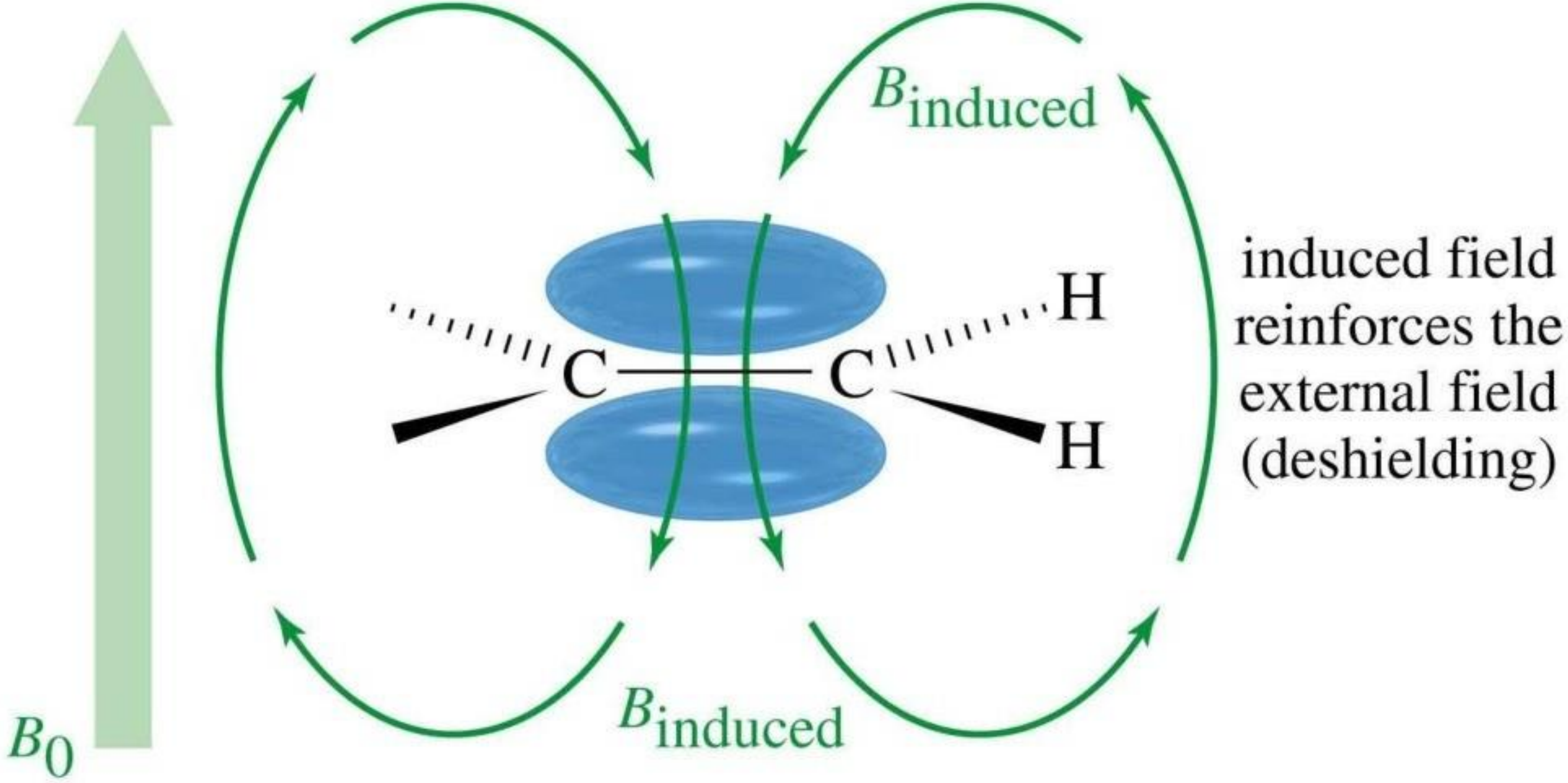
Vinyl Protons, δ5-δ6 In a magnetic field, the loosely held π electrons of the double bond create a magnetic field that reinforces the applied field in the vicinity of the protons.

The protons now feel a stronger magnetic field, and require a higher frequency for resonance. Thus the protons are deshielded



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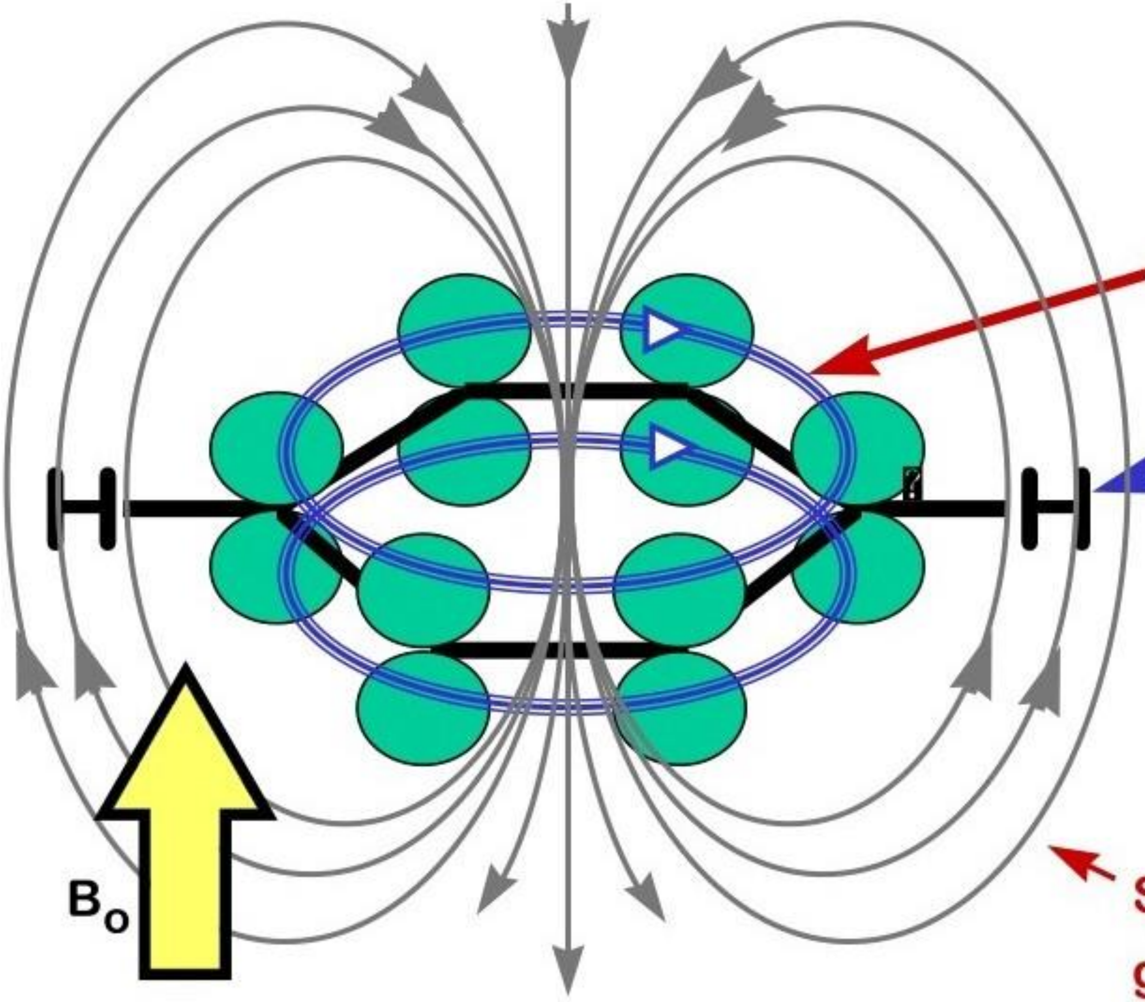
Ring Current in Benzene



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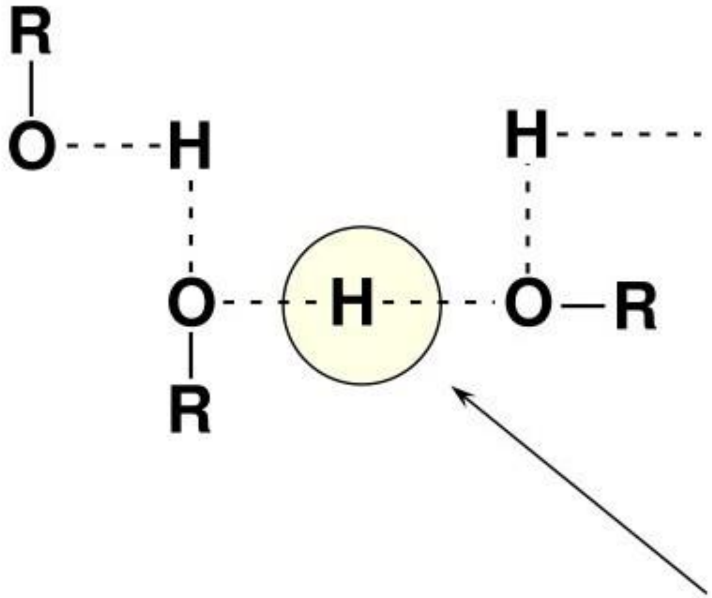
Circulating � electrons

Deshielded

fields add together

Secondary magnetic field generated by circulating � electrons deshields aromatic protons

3. HYDROGEN BONDING - DESHIELDS



The chemical shift depends
on how much hydrogen bonding
is taking place.

Alcohols vary in chemical shift
from 0.5 ppm (free OH) to about
5.0 ppm (lots of H bonding).

Hydrogen bonding lengthens the
O-H bond and reduces the valence
electron density around the proton
- it is **deshielded** and shifted
downfield in the NMR spectrum.

. Chemical Shift Values

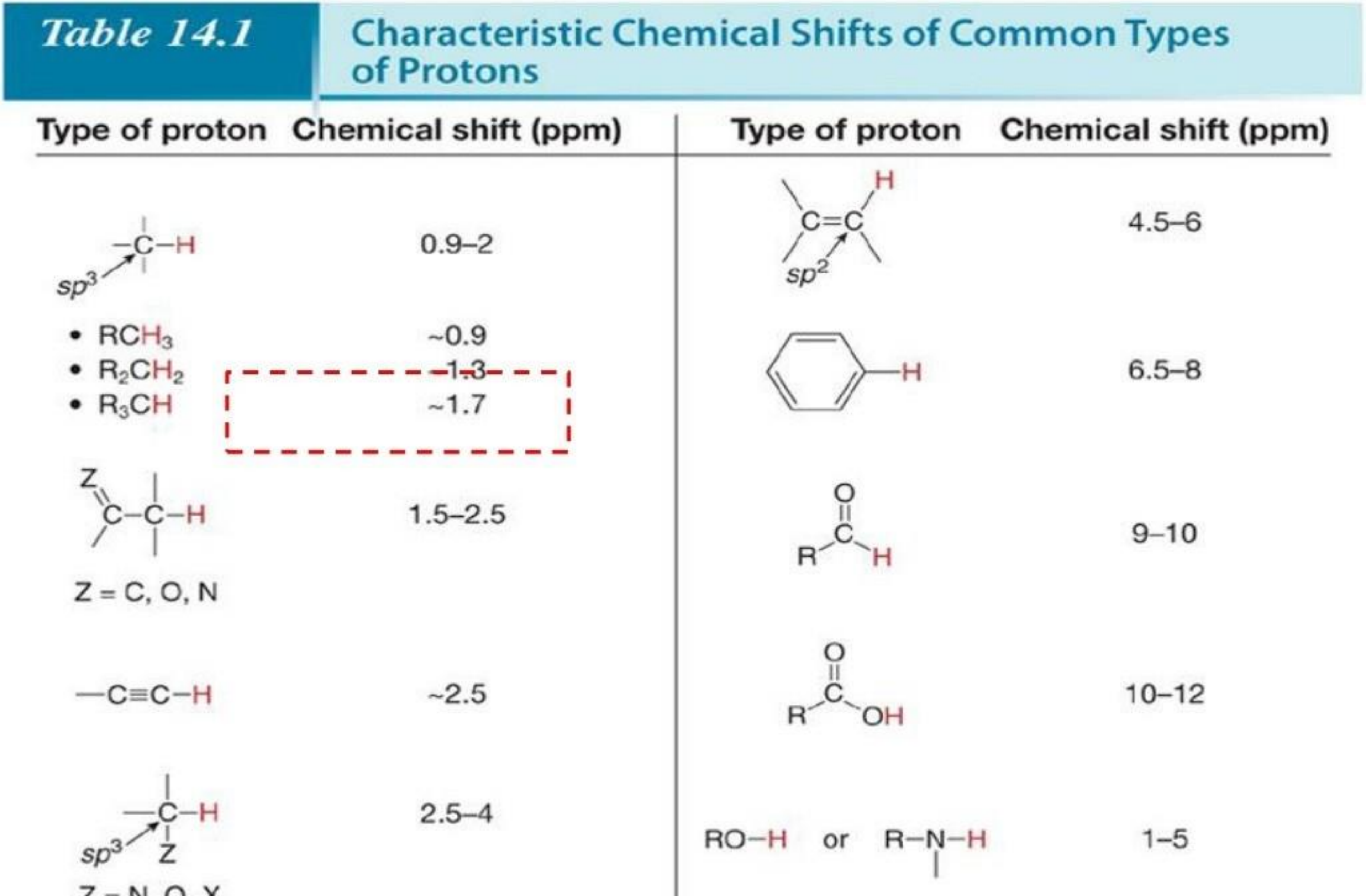


Table 14.1 Characteristic Chemical Shifts of Common Types of Protons

Type of proton	Chemical shift (ppm)	Type of proton	Chemical shift (ppm)
$-C-H$ (sp^3)	0.9–2	$C=C-H$ (sp^2)	4.5–6
• RCH_3 • R_2CH_2 • R_3CH	~0.9 ~1.3 ~1.7	C_6H_5-H	6.5–8
$Z=C-C-H$ Z = C, O, N	1.5–2.5	$RCHO$	9–10
$-C\equiv C-H$	~2.5	$RCOOH$	10–12
$-C(Z)-H$ (sp^3) Z = N, O, X	2.5–4	RO−H or R−N−H	1–5

INTENSITY -INTEGRATION OF A PEAK

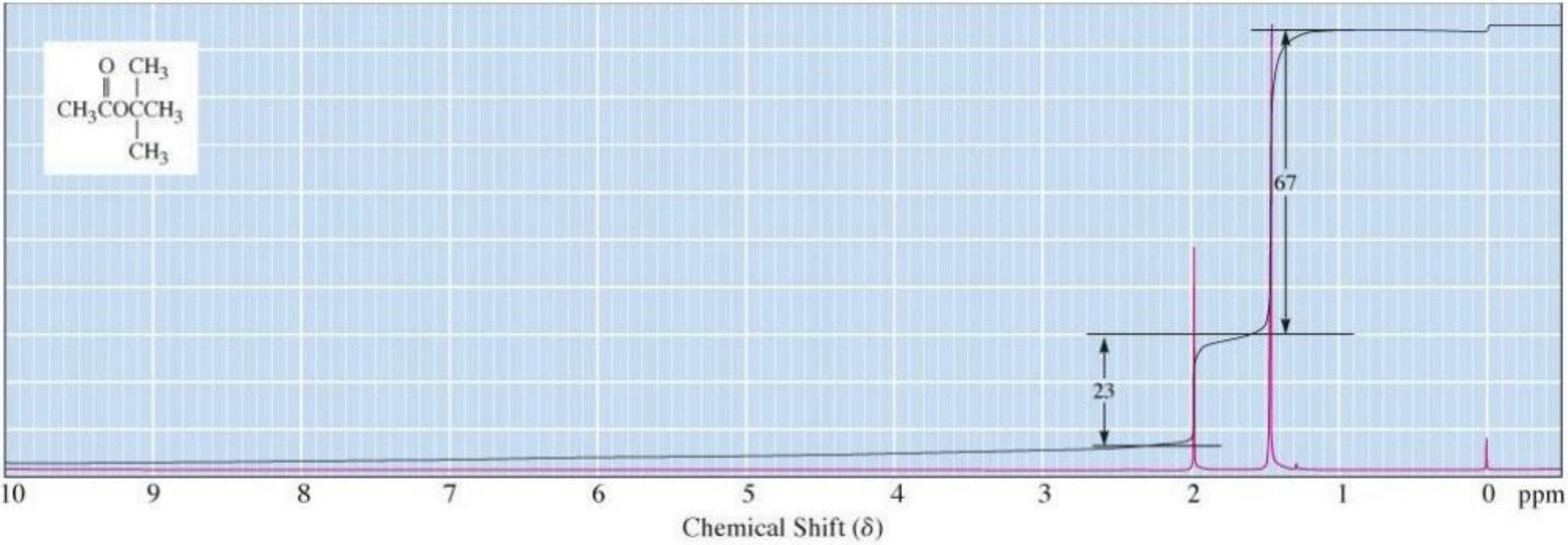
Not only does each different type of hydrogen give a distinct peak in the NMR spectrum, but we can also tell the relative numbers of each type of hydrogen by a process called integration.

Integration = determination of the area under a peak

The area under a peak is proportional to the number of hydrogens that generate the peak.

Signal Areas

- Relative areas of signals are proportional to the number of H giving rise to each signal, Modern NMR spectrometers electronically integrate and record the relative area of each signal.



SPIN-SPIN SPLITTING

•Often a group of hydrogens will appear as a multiplet rather than as a single peak.
This happens because of interaction with neighboring hydrogens and is called SPIN-SPIN SPLITTING.

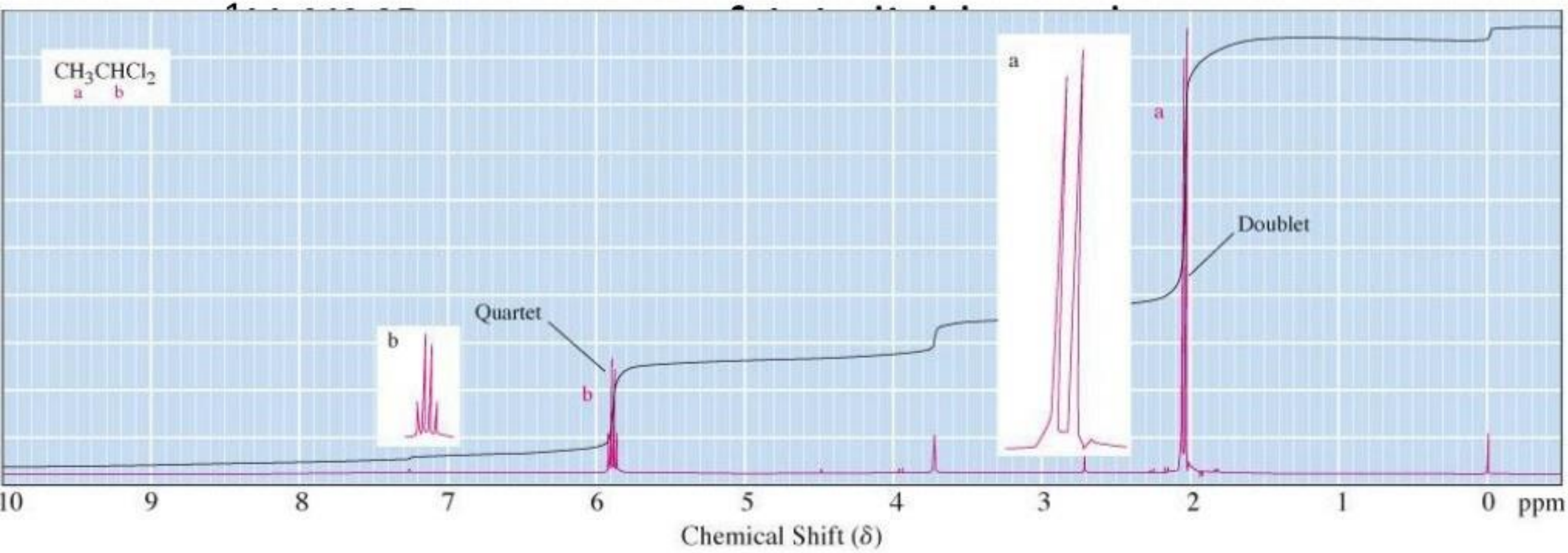
. Nonequivalent protons on adjacent carbons have magnetic fields that may align with or oppose the external field.
This magnetic coupling causes the proton to absorb slightly downfield when the external field is reinforced and slightly upfield when the external field is opposed.
All possibilities exist,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

.

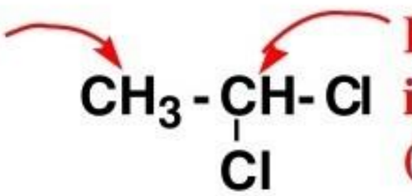
Signal Splitting; the (*n* + 1) Rule

- **Peak:** The units into which an NMR signal is split; doublet, triplet, quartet, multiplet, etc.
- **Signal splitting:** Splitting of an NMR signal into a set of peaks by the influence of neighboring nonequivalent hydrogens.
- **(*n* + 1) rule:** If a hydrogen has *n* hydrogens nonequivalent to it but equivalent among themselves on the same or adjacent atom(s), its ^{1}H-NMR signal is split into (*n* + 1) peaks.

Signal Splitting (n + 1)

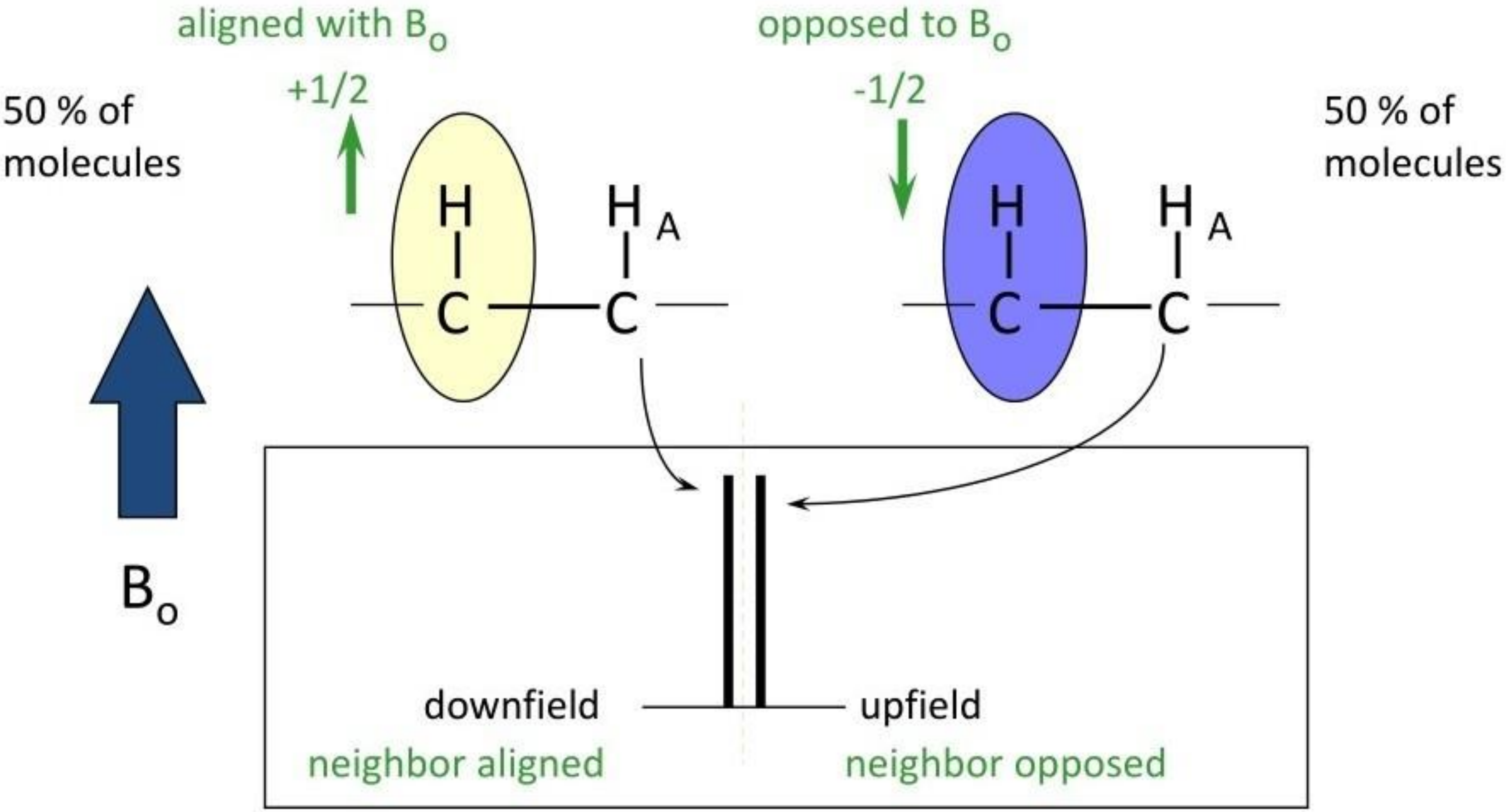


For these hydrogens, n = 1;
their signal is split into
(1 + 1) = 2 peaks; a doublet


$CH_3-\underset{\underset{Cl}{|}}{C}H-Cl$

For this hydrogen, n = 3;
its signal is split into
(3 + 1) = 4 peaks; a quartet

THE CHEMICAL SHIFT OF PROTON H_A IS AFFECTED BY THE SPIN OF ITS NEIGHBORS



50 % of molecules

aligned with B_o

+1/2

opposed to B_o

-1/2

50 % of molecules

B_o

downfield

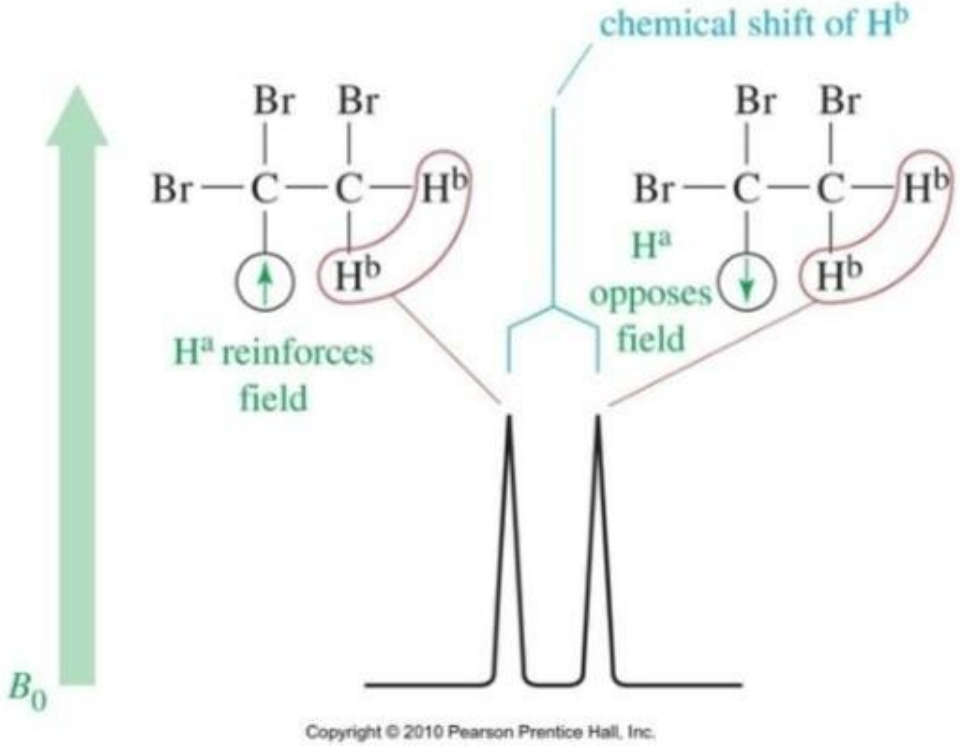
neighbor aligned

upfield

neighbor opposed

At any given time about half of the molecules in solution will have spin +1/2 and the other half will have spin -1/2.

Doublet: One Adjacent Proton



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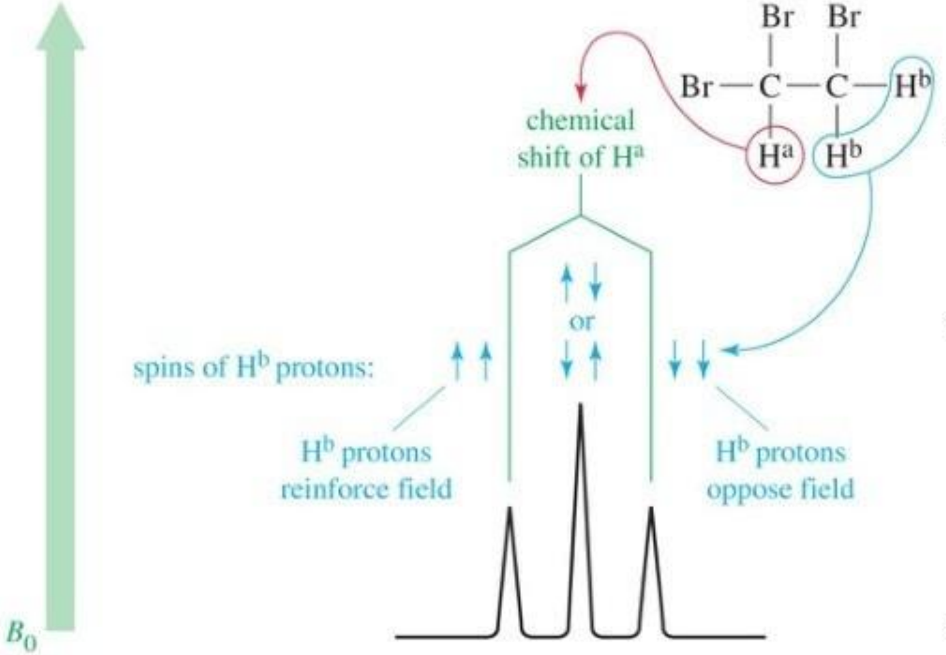
- H^b can feel the alignment of the adjacent proton H^a.
- When H^a is aligned with the magnetic field, H^b will be deshielded.
- When H^a is aligned with the magnetic field, H^b will be shielded.
- The signal is split in two and it is called a doublet.

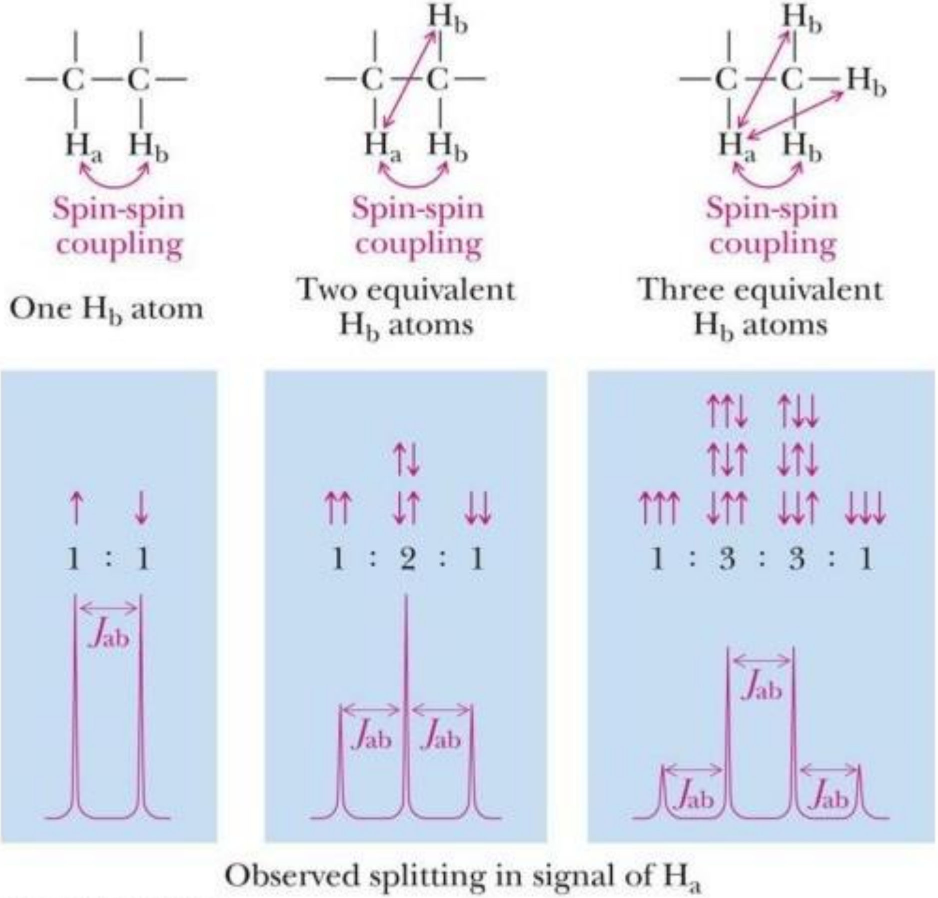
Triplet: Two Adjacent Protons



- When both H^b are aligned with the magnetic field, H^a will be deshielded.
- When both H^b are aligned with the magnetic field, H^a will be deshielded.
- It is more likely that one H^b will be aligned with the field and the other H^b against the field. The signal will be at its normal position.
- The signal is split in three and it is called a triplet.

Origins of Signal Splitting

- The origins of signal splitting patterns. Each arrow represents an H_b nuclear spin orientation.



Observed splitting in signal of H_a

Rules for splitting of proton signals

- Equivalent protons do not split each other.
- Protons bonded to the same carbon will split each other if they are nonequivalent.
- Protons on adjacent carbons normally will split each other.
- Protons separated by four or more bonds will not split each other.

PASCAL'S TRIANGLE

(Intensities of multiplet peaks)

The interior entries are the sums of the two numbers immediately above.

1 — singlet

1 1 — doublet

1 2 1 — triplet

1 3 3 1 — quartet

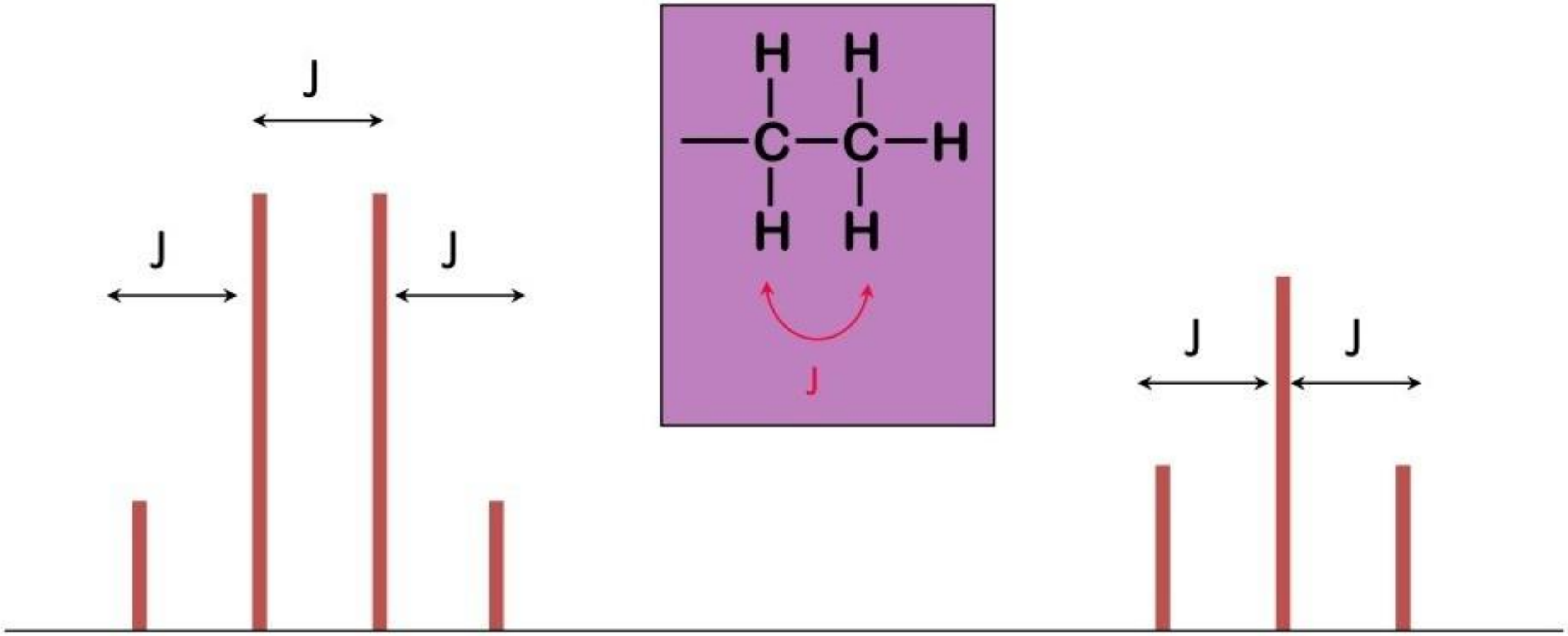
1 4 6 4 1 — quintet

1 5 10 10 5 1 — sextet

1 6 15 20 15 6 1 — septet

1 7 21 35 35 21 7 1 — octet

THE COUPLING CONSTANT



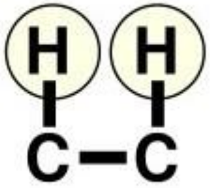
The coupling constant J is the distance (measured in Hz) between the peaks in a multiplet. Not dependent on strength of the external field

J is a measure of the amount of interaction between the two sets of hydrogens creating the multiplet.

NOTATION FOR COUPLING CONSTANTS

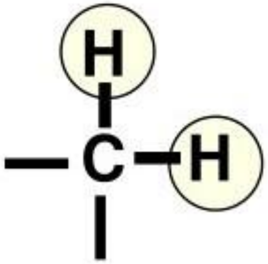
The most commonly encountered type of coupling is between hydrogens on adjacent carbon atoms.

^3J



This is sometimes called *vicinal* coupling. It is designated ^3J since three bonds intervene between the two hydrogens.

Another type of coupling that can also occur in special cases is



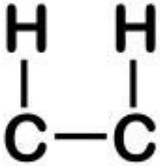
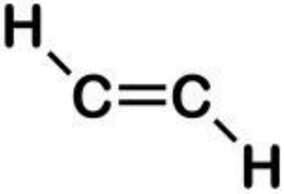
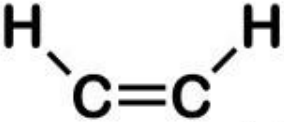
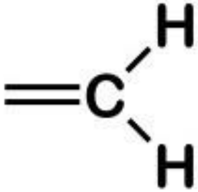
^2J

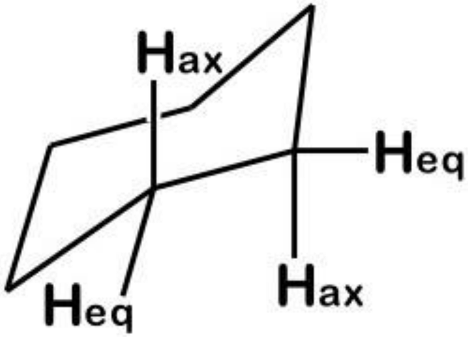
^2J or *geminal* coupling

(most often $^2J = 0$)

Geminal coupling does not occur when the two hydrogens are equivalent due to rotations around the other two bonds.

SOME REPRESENTATIVE COUPLING CONSTANTS

vicinal		6 to 8 Hz	three bond	^3J
trans		11 to 18 Hz	three bond	^3J
cis		6 to 15 Hz	three bond	^3J
geminal		0 to 5 Hz	two bond	^2J



H_{ax}, H_{ax} = 8 to 14

H_{ax}, H_{eq} = 0 to 7 three bond ^3J

H_{eq}, H_{eq} = 0 to 5

Steps for analysing NMR spectra

1. Look at the number of peak sets and hence the number of different environments
2. The chemical shift for each peak set
3. The relative number of protons in each peak set (from the relative peak area)
4. The number of fine peaks each major peak set is split into
5. Determine the relative number of hydrogens in each environment
6. The protons responsible for each peak set and the carbon to which they are bonded

NMR Problem

A compound has molecular formula $C_8 H_8 0$.

The proton NMR has three peaks;

singlet at d 2.2 (3H),

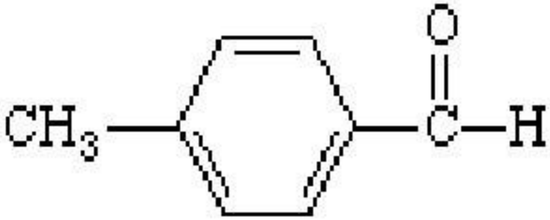
singlet at d 10 (1H)

two doublets centered around d 7.6.Assign the structure.

SOLUTION:

.The doublets centered at d 7.6 are in the aromatic region; the fact that two doublets are observed (2H each) suggests a 1,4-disubstituted aromatic compound. The peak at � 2.2 is in the region for a methyl group adjacent a mildly electronegative group. The singlet at � 10 is in the region observed for aldehydic protons. The presence of two doublets in the aromatic region is highly characteristic of 1,4-disubstitution.

Hence Structure is



Structure:

IUPAC Name: 4-methylbenzaldehyde