

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



Lecture1

# **Advanced Analysis for Pharmacy Students**

**By**

**Professor Dr. Mohie Sharaf El Din**



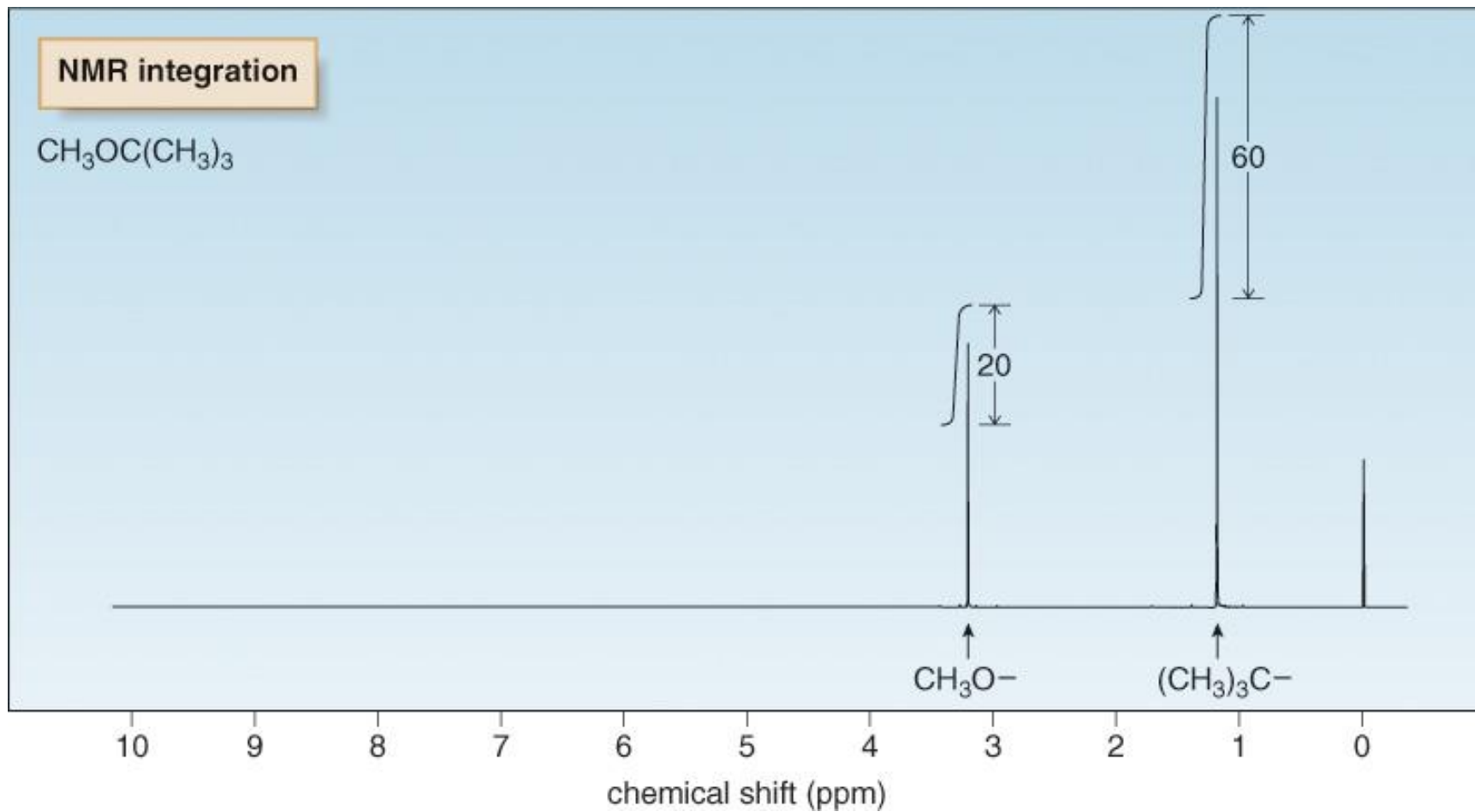
# Nuclear Magnetic Resonance Spectroscopy

## c-<sup>1</sup>H NMR—Intensity of Signals

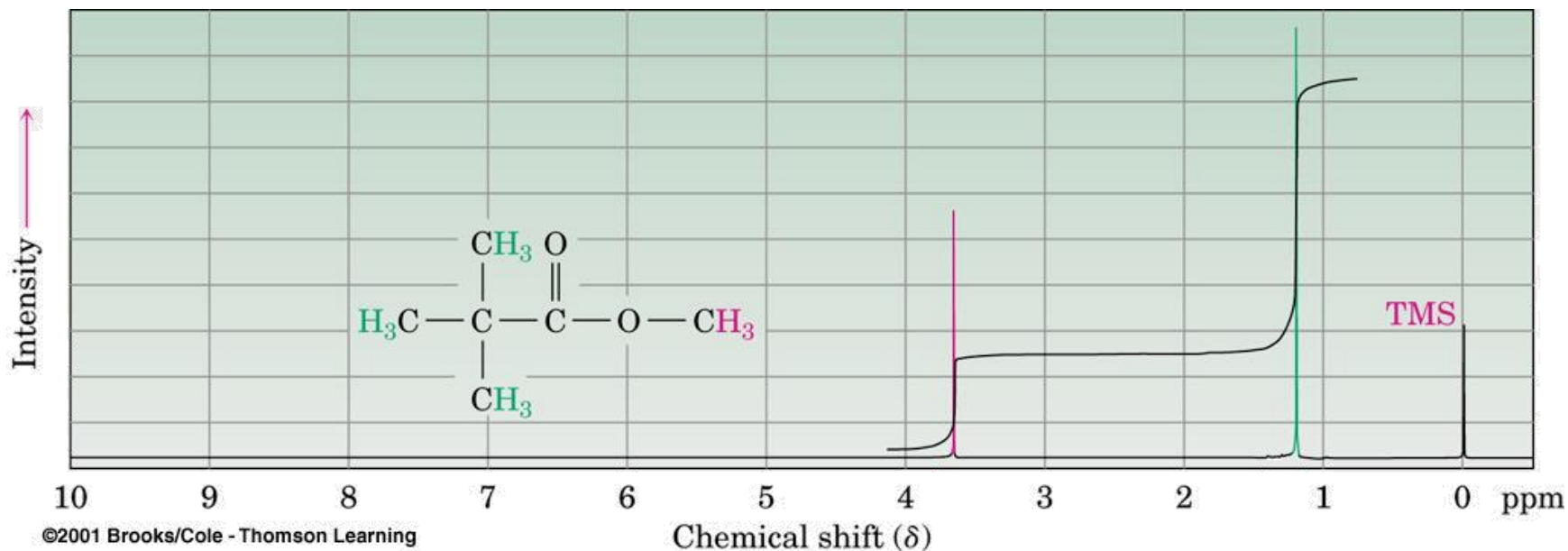
- The area under an NMR signal is proportional to the number of absorbing protons.
- An NMR spectrometer automatically **integrates** the area under the peaks, and prints out a stepped curve (**integral**) on the spectrum.
- The height of each step is proportional to the area under the peak, which in turn is proportional to the number of absorbing protons.
- Modern NMR spectrometers automatically calculate and plot the value of each integral in arbitrary units.
- The ratio of integrals to one another gives the ratio of absorbing protons in a spectrum. Note that this gives a ratio, and not the absolute number, of absorbing protons.

# Nuclear Magnetic Resonance Spectroscopy

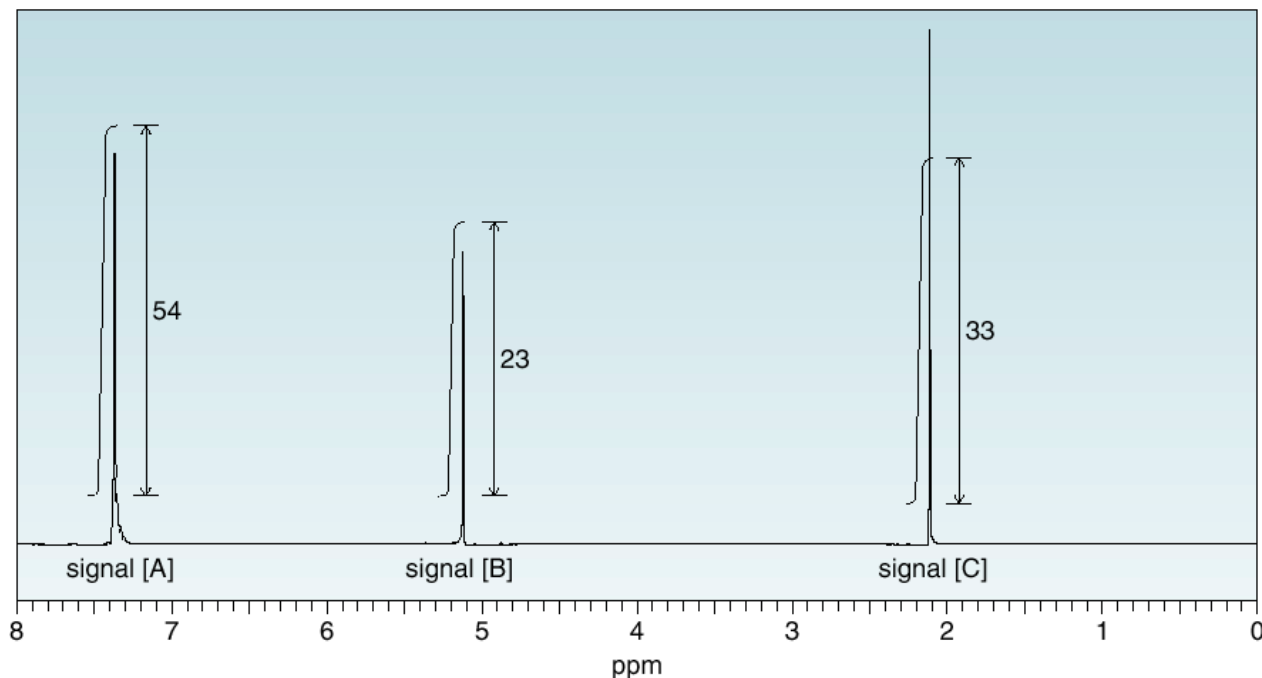
## $^1\text{H}$ NMR—Intensity of Signals



# Methyl $\alpha,\alpha$ -Dimethylpropionate



**Example** A compound of molecular formula  $C_9H_{10}O_2$  gives the following integrated  $^1H$  NMR spectrum. How many protons give rise to each signal?



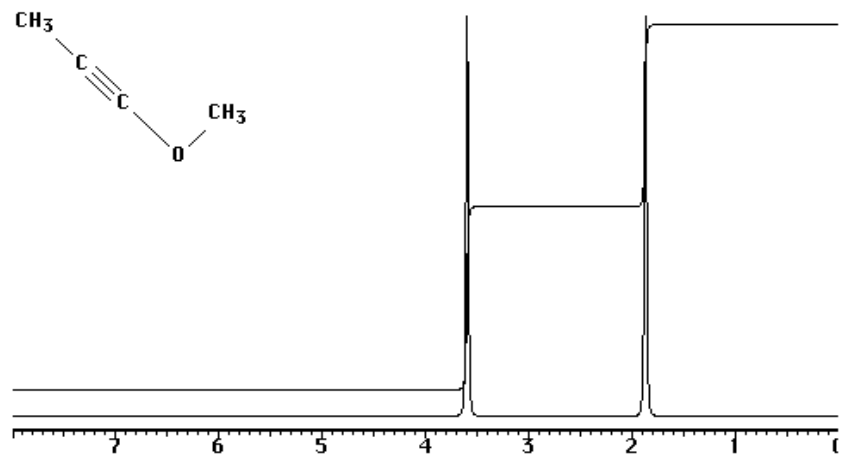
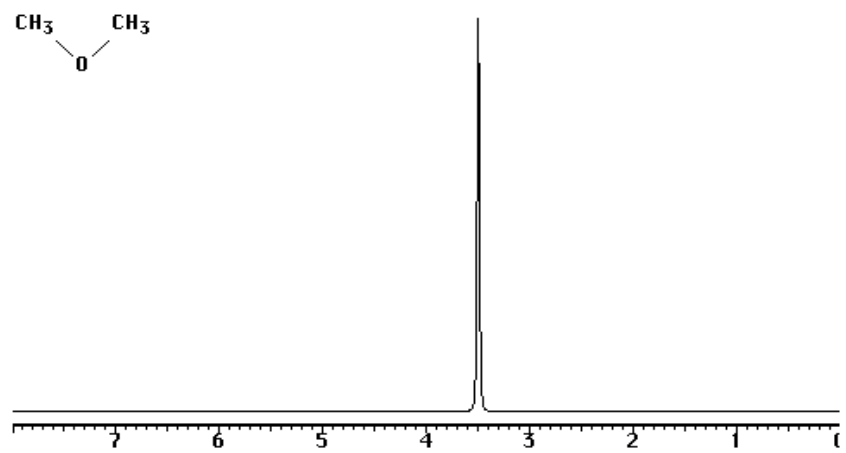
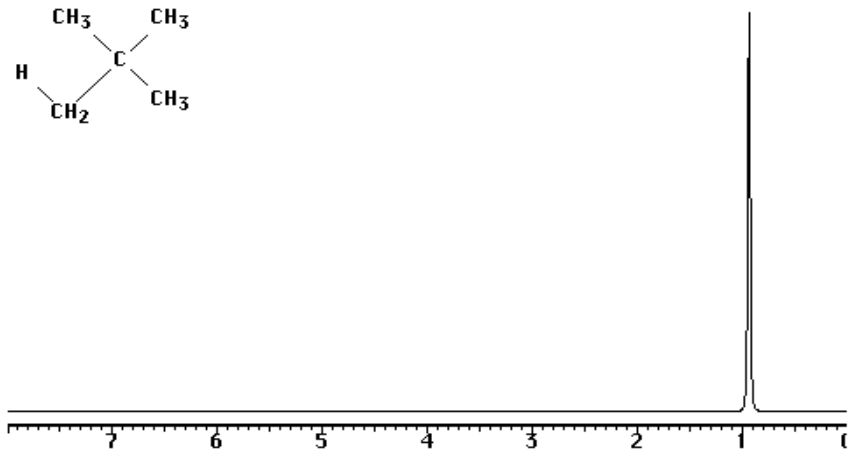
**Step [1]** Determine the number of integration units per proton by dividing the total number of integration units by the total number of protons.

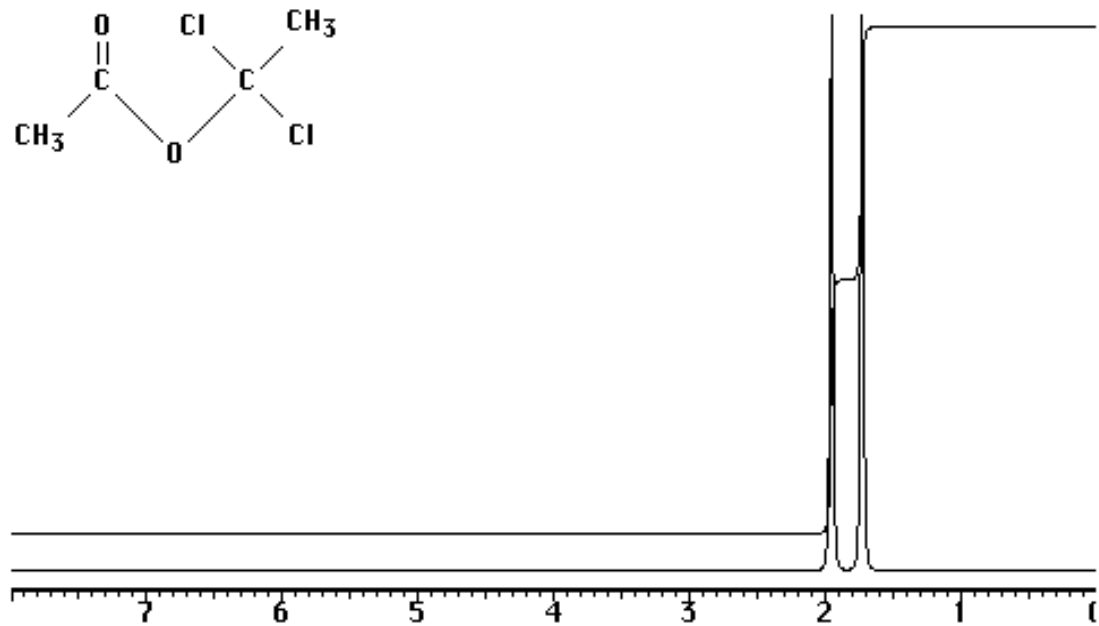
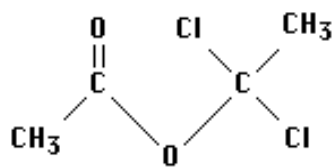
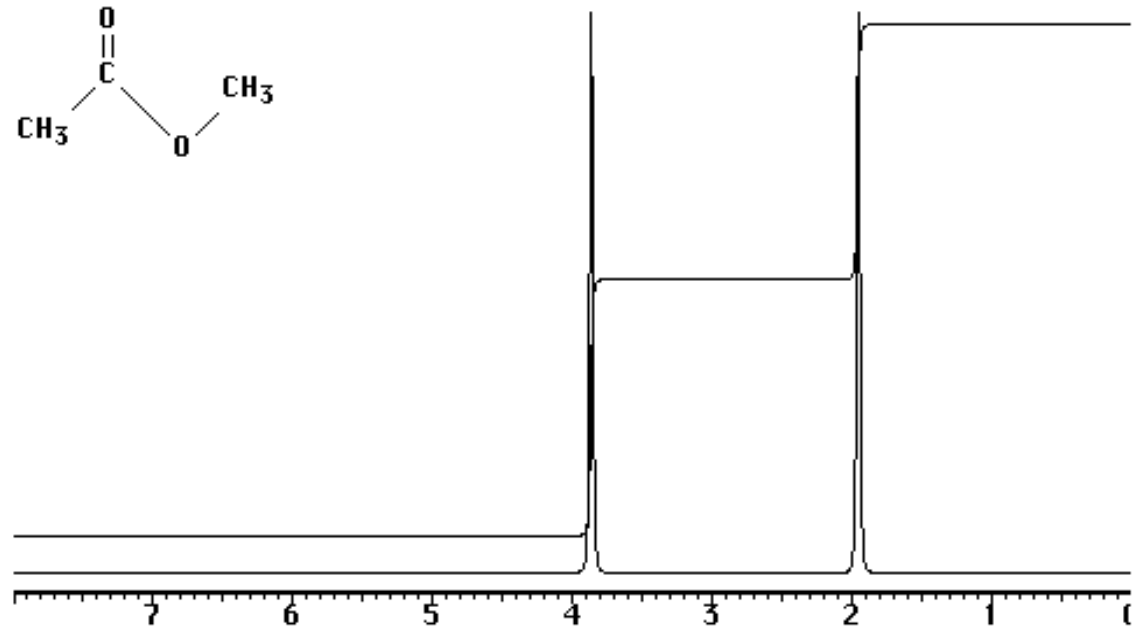
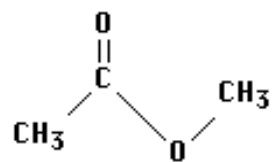
- Total number of integration units:  $54 + 23 + 33 = 110$  units
- Total number of protons = 10
- Divide:  $110 \text{ units} / 10 \text{ protons} = \mathbf{11 \text{ units per proton}}$

**Step [2]** Determine the number of protons giving rise to each signal.

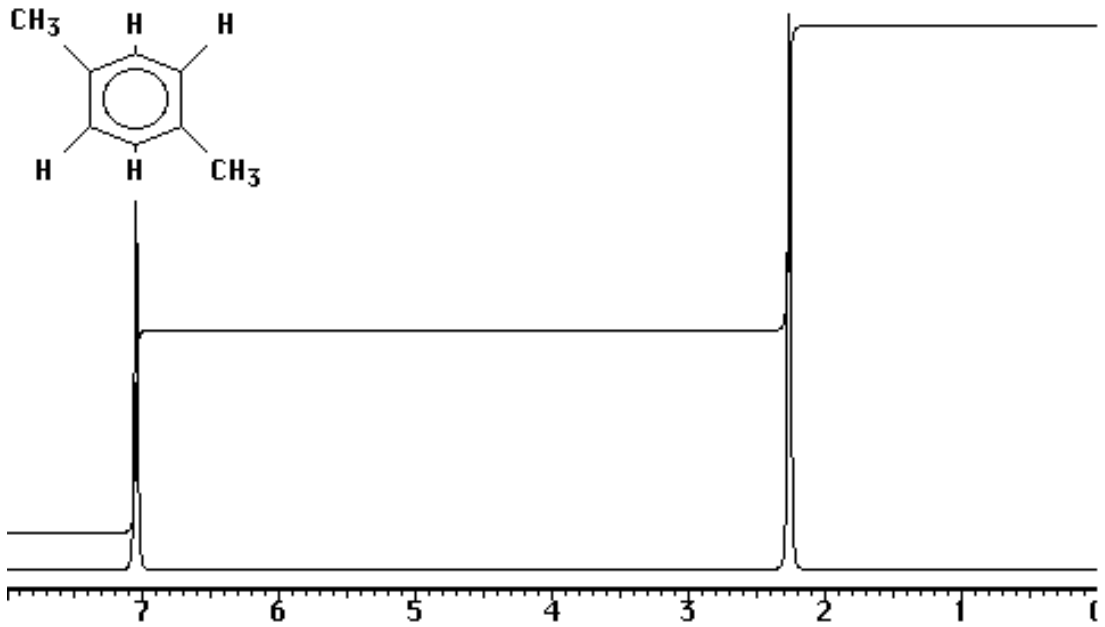
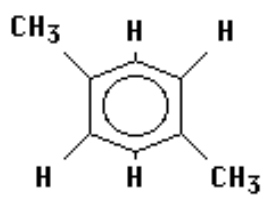
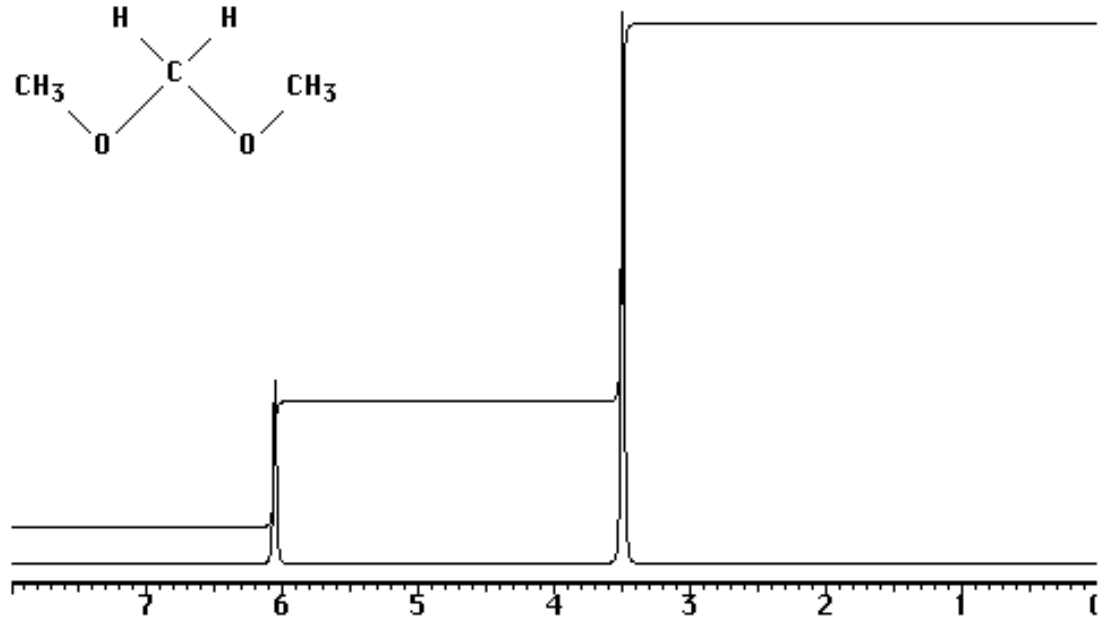
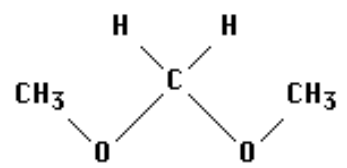
- To determine the number of H atoms giving rise to each signal, divide each integration value by the answer of Step [1] and round to the nearest whole number.

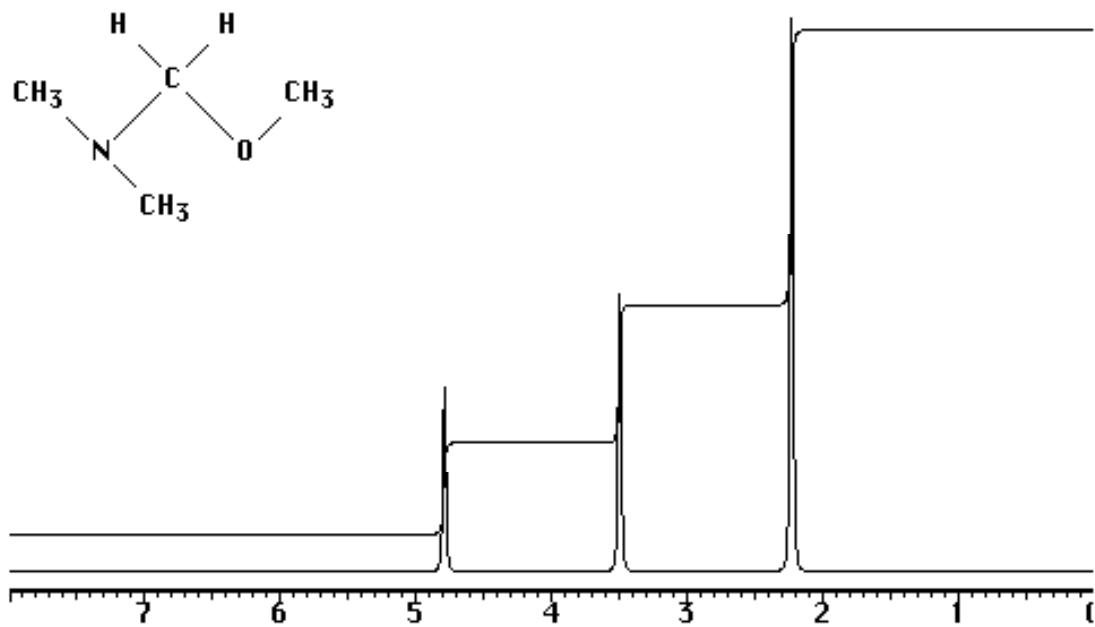
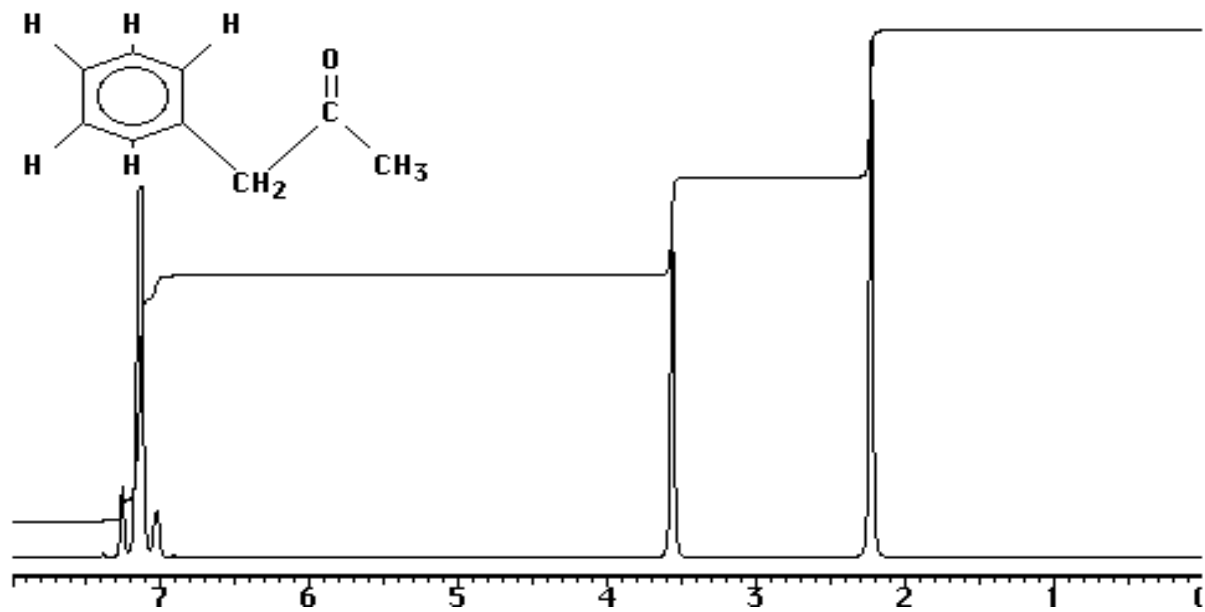
$$\text{Answer: } \frac{54}{11} = 4.9 \approx \mathbf{5 \text{ H}} \quad \left| \quad \frac{23}{11} = 2.1 \approx \mathbf{2 \text{ H}} \quad \left| \quad \frac{33}{11} = \mathbf{3 \text{ H}}$$







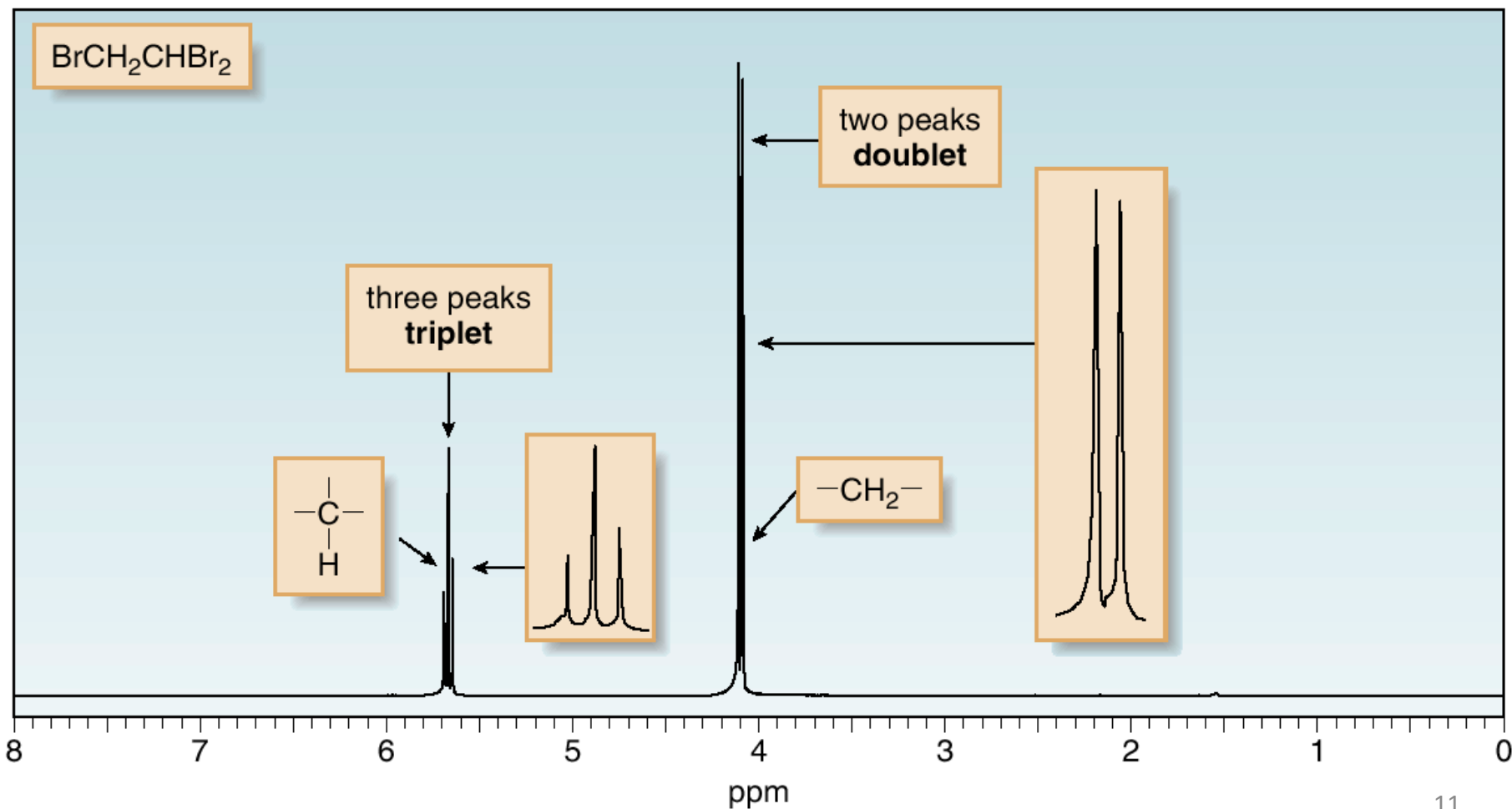




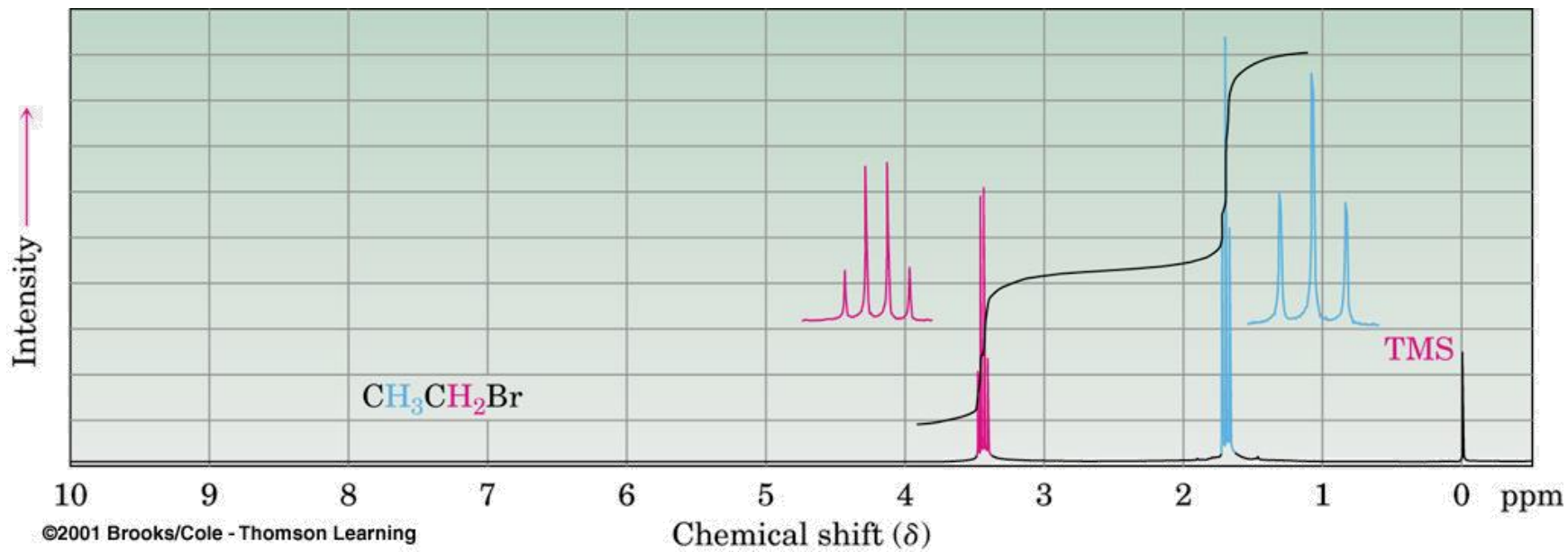
# Nuclear Magnetic Resonance Spectroscopy

## d- $^1\text{H}$ NMR—Spin-Spin Splitting

- Consider the spectrum below:

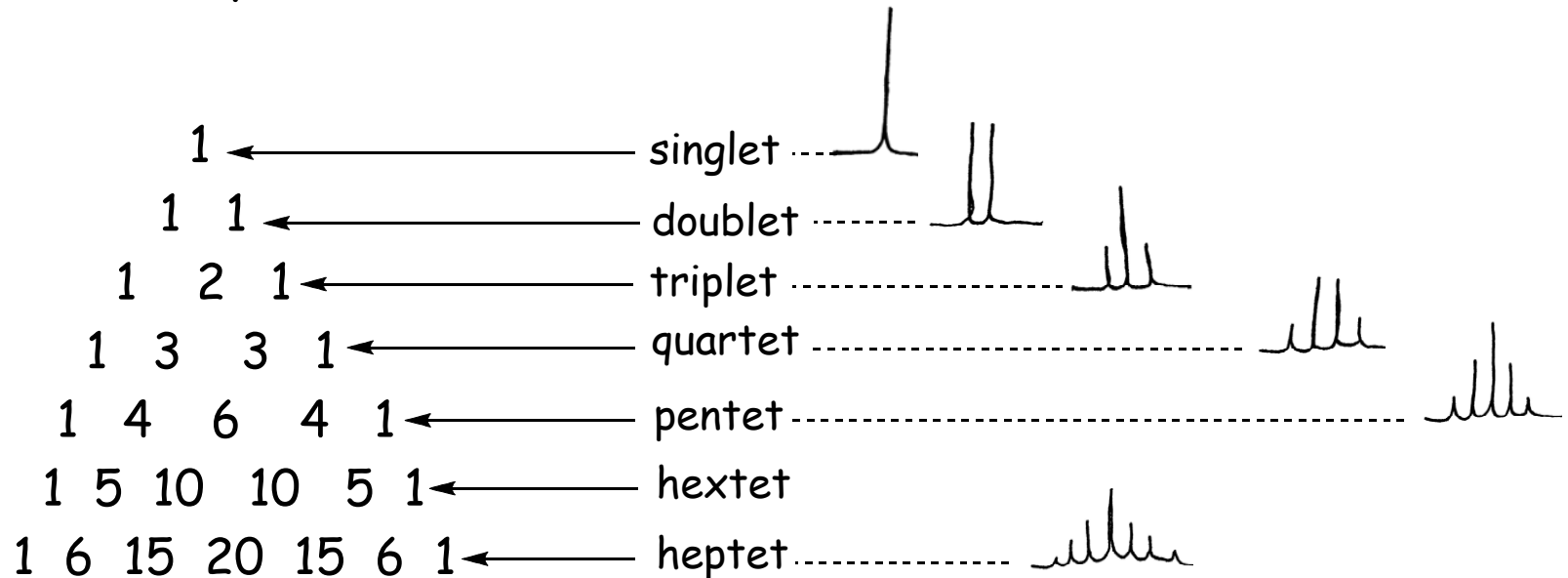


# Ethyl Bromide



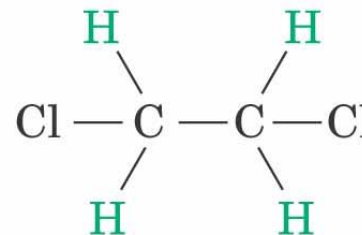
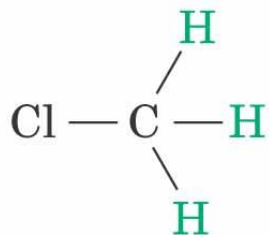
# Spin-Spin Splitting in $^1\text{H}$ NMR Spectra

- Peaks are often split into multiple peaks due to **magnetic interactions** between nonequivalent protons on adjacent carbons, The process is called **spin-spin splitting**
- The splitting is into one more peak than the number of H's on the adjacent carbon(s), This is the "**n+1 rule**"
- The relative intensities are in proportion of a binomial distribution given by Pascal's Triangle
- The set of peaks is a **multiplet** (2 = doublet, 3 = triplet, 4 = quartet, 5=pentet, 6=hextet, 7=heptet.....)



# Rules for Spin-Spin Splitting

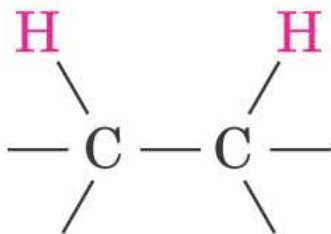
- Equivalent protons do not split each other



- Protons that Three C–H protons are chemically equivalent; no splitting occurs.

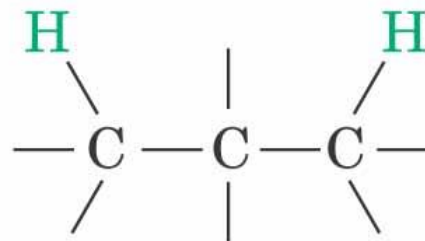
© 2004 Thomson/Brooks Cole

- Four C–H protons are it each other chemically equivalent; no splitting occurs.



Splitting observed

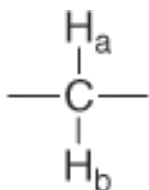
© 2004 Thomson/Brooks Cole



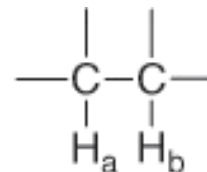
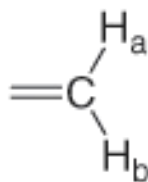
Splitting not usually observed

# $^1\text{H}$ NMR—Spin-Spin Splitting

If  $\text{H}_a$  and  $\text{H}_b$  are not equivalent, splitting is observed when:

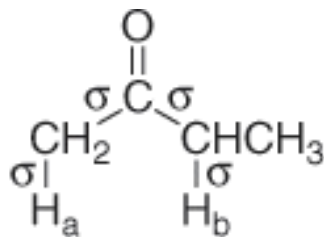


$\text{H}_a$  and  $\text{H}_b$  are on the **same** carbon.



$\text{H}_a$  and  $\text{H}_b$  are on **adjacent** carbons.

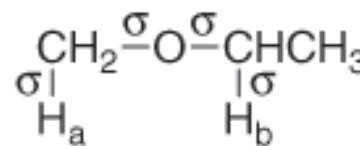
Splitting is not generally observed between protons separated by more than three  $\sigma$  bonds.



2-butanone

$\text{H}_a$  and  $\text{H}_b$  are separated by four  $\sigma$  bonds.

**no splitting** between  $\text{H}_a$  and  $\text{H}_b$



ethyl methyl ether

$\text{H}_a$  and  $\text{H}_b$  are separated by four  $\sigma$  bonds.

**no splitting** between  $\text{H}_a$  and  $\text{H}_b$

# The Origin of $^1\text{H}$ NMR—Spin-Spin Splitting

- Spin-spin splitting occurs only between nonequivalent protons on the same carbon or adjacent carbons.

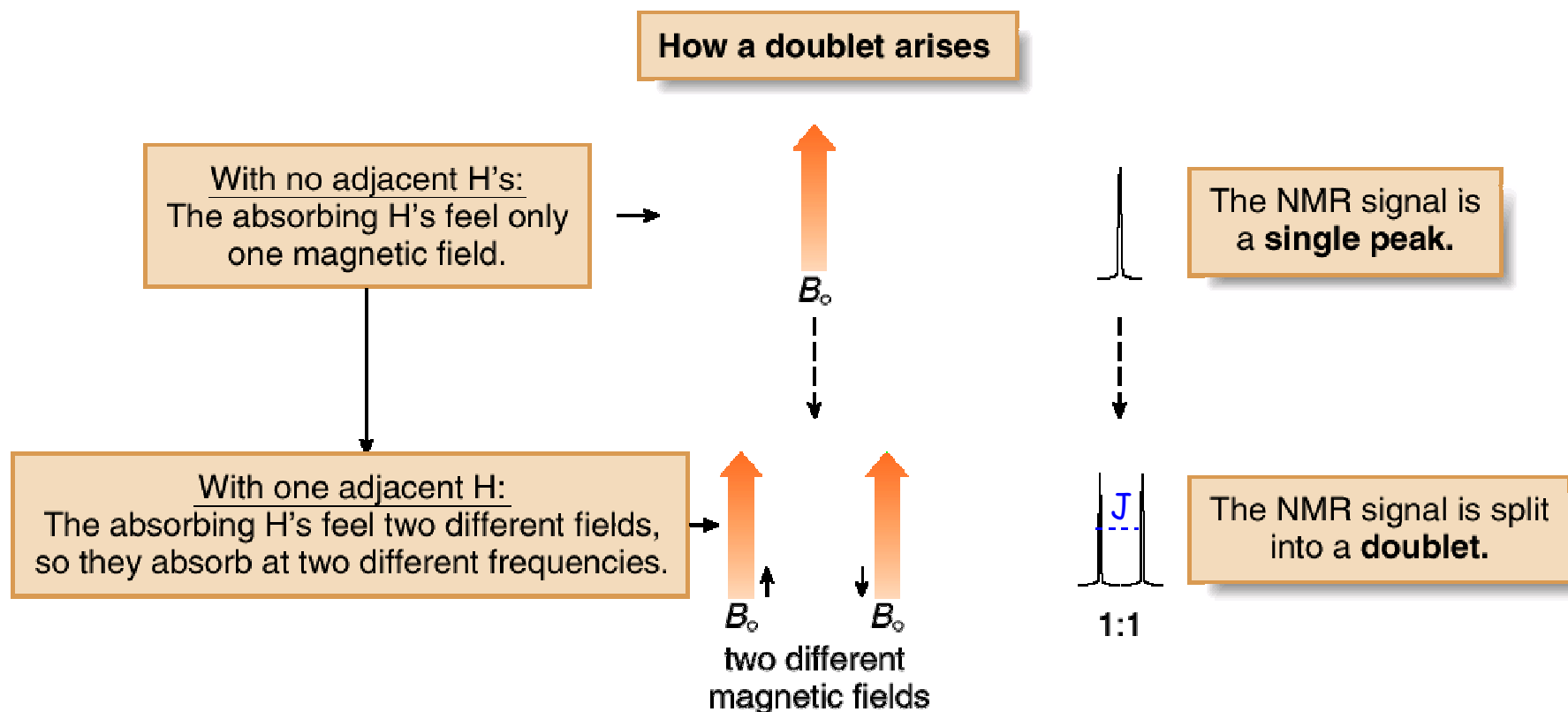
Let us consider how the doublet due to the  $\text{CH}_2$  group on  $\text{BrCH}_2\text{CHBr}_2$  occurs:

- When placed in an applied field, ( $B_0$ ), the adjacent proton ( $\text{CHBr}_2$ ) can be aligned with ( $\uparrow$ ) or against ( $\downarrow$ )  $B_0$ . The likelihood of either case is about 50% (i.e., 1,000,006 $\uparrow$  vs 1,000,000 $\downarrow$ ).
- Thus, the absorbing  $\text{CH}_2$  protons feel two slightly different magnetic fields—one slightly larger than  $B_0$ , and one slightly smaller than  $B_0$ .
- Since the absorbing protons feel two different magnetic fields, they absorb at two different frequencies in the NMR spectrum, thus splitting a single absorption into a doublet, where the two peaks of the doublet have *equal* intensity.



# The Origin of $^1\text{H}$ NMR—Spin-Spin Splitting

The frequency difference, measured in Hz, between two peaks of the doublet is called the **coupling constant,  $J$** .



- One adjacent proton splits an NMR signal into a doublet.

# The Origin of $^1\text{H}$ NMR—Spin-Spin Splitting

Let us now consider how a triplet arises:



- When placed in an applied magnetic field ( $B_0$ ), the adjacent protons  $\text{H}_a$  and  $\text{H}_b$  can each be aligned with ( $\uparrow$ ) or against ( $\downarrow$ )  $B_0$ .
- Thus, the absorbing proton feels three slightly different magnetic fields—one slightly larger than  $B_0$  ( $\uparrow_a \uparrow_b$ ), one slightly smaller than  $B_0$  ( $\downarrow_a \downarrow_b$ ) and one the same strength as  $B_0$  ( $\uparrow_a \downarrow_b$ ).

# The Origin of $^1\text{H}$ NMR—Spin-Spin Splitting

- Because the absorbing proton feels **three** different magnetic fields, it absorbs at **three** different frequencies in the NMR spectrum, thus splitting a single absorption into a **triplet**.
- Because there are **two** different ways to align one proton with  $B_0$ , and one proton against  $B_0$ —that is,  $\uparrow_a\downarrow_b$  and  $\downarrow_a\uparrow_b$ —the middle peak of the triplet is **twice** as intense as the two outer peaks, making the ratio of the areas under the three peaks 1:2:1.
- Two adjacent protons split an NMR signal into a triplet.
- When two protons split each other, they are said to be coupled.
- The spacing between peaks in a split NMR signal, measured by the  $J$  value, is equal for coupled protons.

# The Origin of $^1\text{H}$ NMR—Spin-Spin Splitting

## How a triplet arises

With no adjacent H's:  
The absorbing H feels only one magnetic field.



$B_0$



The NMR signal is a **single peak**.

With two adjacent H's:  
The absorbing H feels three different fields, so it absorbs at three different frequencies.



$B_0$

$\uparrow_a \downarrow_b$

or

$\downarrow_a \uparrow_b$

$\downarrow_a \downarrow_b$

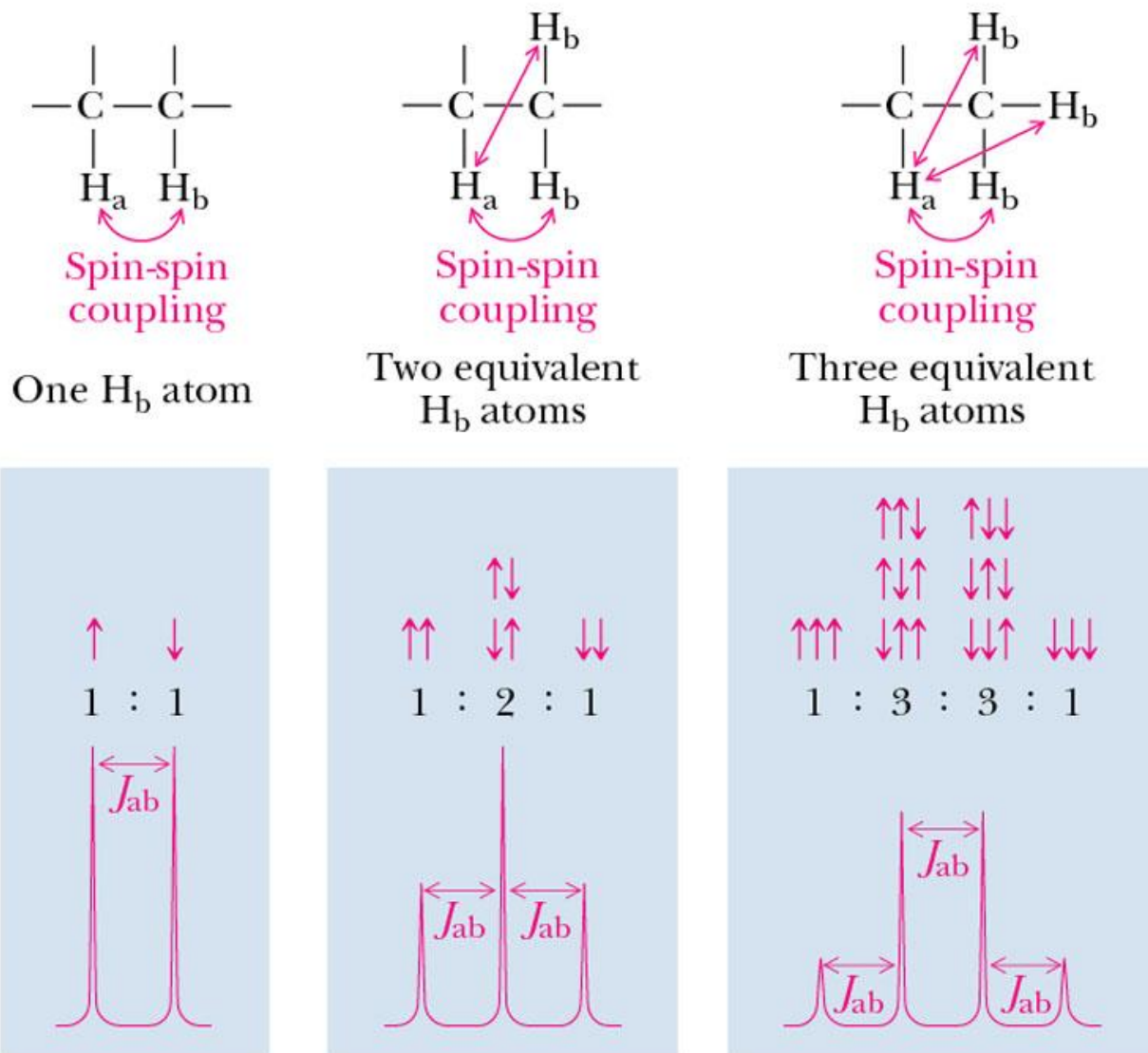
three different magnetic fields



1:2:1

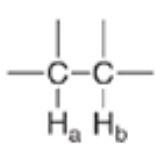

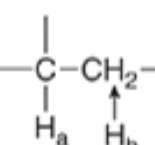
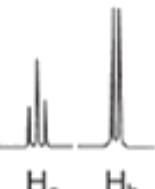
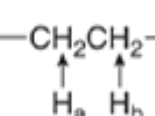
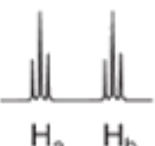
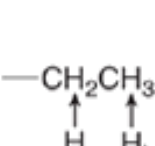

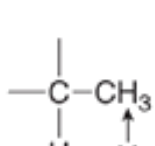

The NMR signal is split into a **triplet**.

# The Origin of $^1\text{H}$ NMR—Spin-Spin Splitting



Observed splitting in signal of  $\text{H}_a$

**Table 14.4****Common Splitting Patterns Observed in  $^1\text{H}$  NMR**

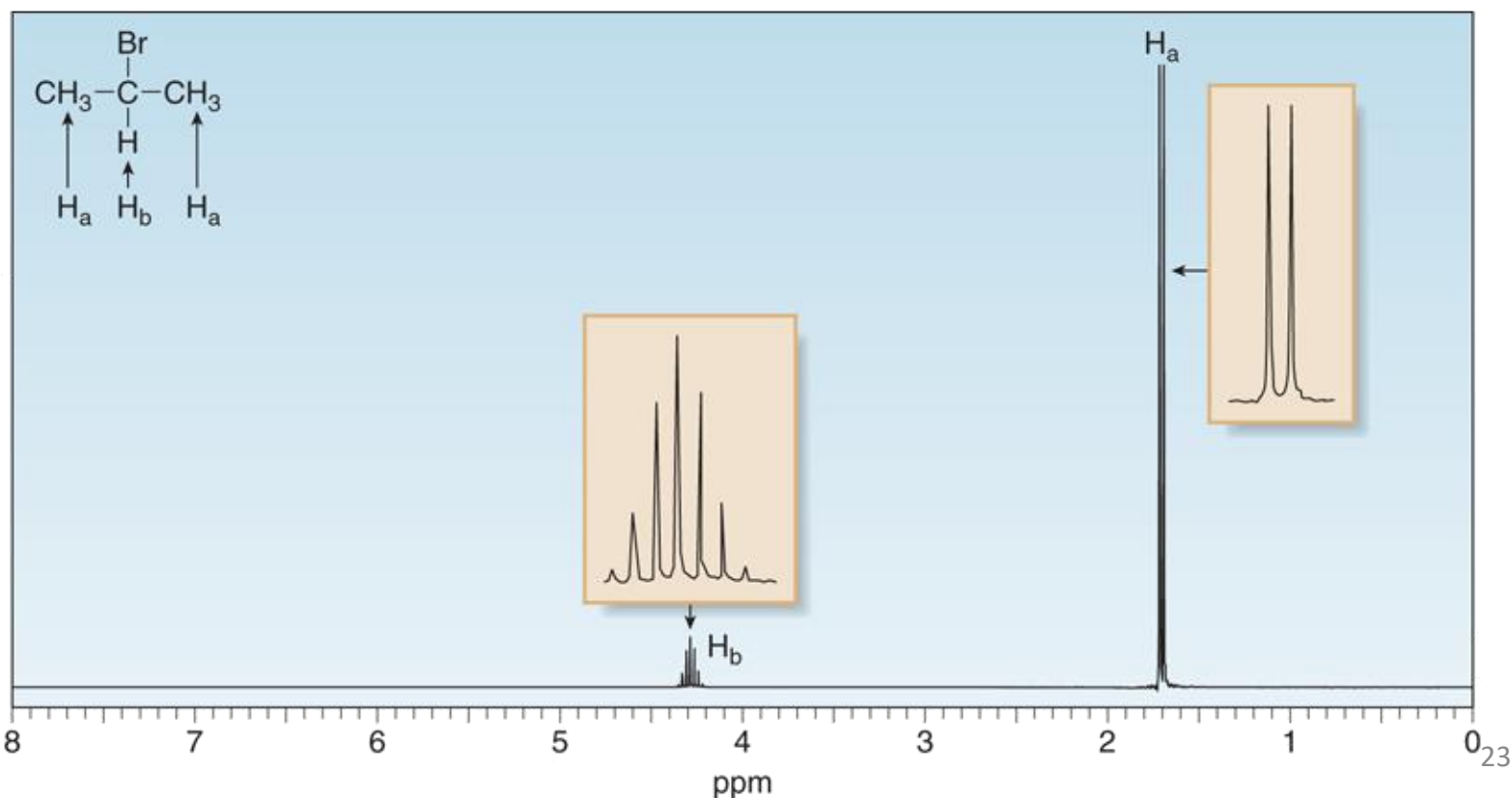
Example	Pattern	Analysis ( $\text{H}_a$ and $\text{H}_b$ are not equivalent.)
[1] 		<ul style="list-style-type: none"> <li><math>\text{H}_a</math>: one adjacent <math>\text{H}_b</math> proton <math>\dashrightarrow</math> two peaks <math>\dashrightarrow</math> a <b>doublet</b></li> <li><math>\text{H}_b</math>: one adjacent <math>\text{H}_a</math> proton <math>\dashrightarrow</math> two peaks <math>\dashrightarrow</math> a <b>doublet</b></li> </ul>
[2] 		<ul style="list-style-type: none"> <li><math>\text{H}_a</math>: two adjacent <math>\text{H}_b</math> protons <math>\dashrightarrow</math> three peaks <math>\dashrightarrow</math> a <b>triplet</b></li> <li><math>\text{H}_b</math>: one adjacent <math>\text{H}_a</math> proton <math>\dashrightarrow</math> two peaks <math>\dashrightarrow</math> a <b>doublet</b></li> </ul>
[3] 		<ul style="list-style-type: none"> <li><math>\text{H}_a</math>: two adjacent <math>\text{H}_b</math> protons <math>\dashrightarrow</math> three peaks <math>\dashrightarrow</math> a <b>triplet</b></li> <li><math>\text{H}_b</math>: two adjacent <math>\text{H}_a</math> protons <math>\dashrightarrow</math> three peaks <math>\dashrightarrow</math> a <b>triplet</b></li> </ul>
[4] 		<ul style="list-style-type: none"> <li><math>\text{H}_a</math>: three adjacent <math>\text{H}_b</math> protons <math>\dashrightarrow</math> four peaks <math>\dashrightarrow</math> a <b>quartet*</b></li> <li><math>\text{H}_b</math>: two adjacent <math>\text{H}_a</math> protons <math>\dashrightarrow</math> three peaks <math>\dashrightarrow</math> a <b>triplet</b></li> </ul>
[5] 		<ul style="list-style-type: none"> <li><math>\text{H}_a</math>: three adjacent <math>\text{H}_b</math> protons <math>\dashrightarrow</math> four peaks <math>\dashrightarrow</math> a <b>quartet*</b></li> <li><math>\text{H}_b</math>: one adjacent <math>\text{H}_a</math> proton <math>\dashrightarrow</math> two peaks <math>\dashrightarrow</math> a <b>doublet</b></li> </ul>

\*The relative area under the peaks of a quartet is 1:3:3:1.

# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Spin-Spin Splitting

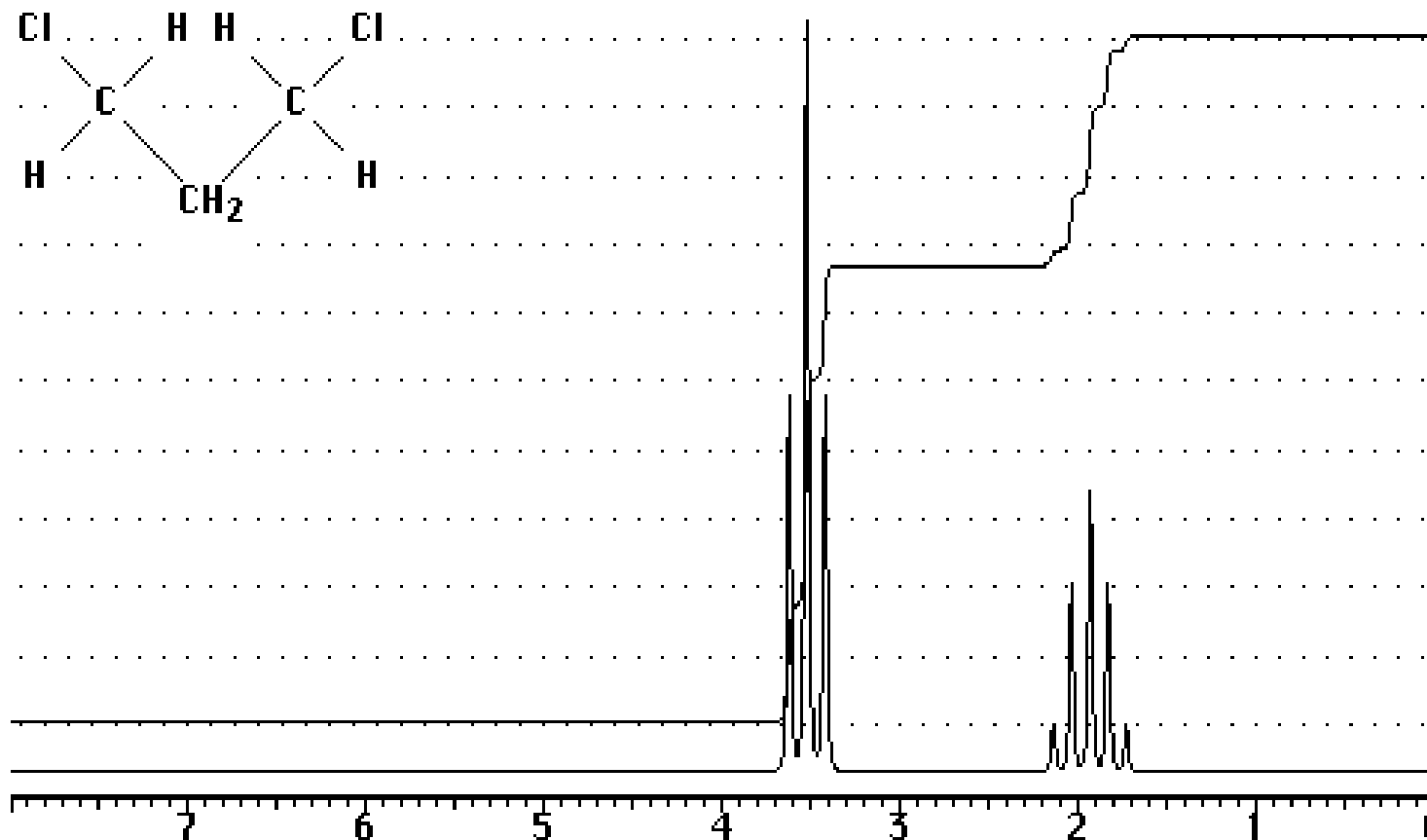
Whenever two (or three) different sets of adjacent protons are equivalent to each other, use the  $n + 1$  rule to determine the splitting pattern.



# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Spin-Spin Splitting

Whenever two (or three) different sets of adjacent protons are equivalent to each other, use the  $n + 1$  rule to determine the splitting pattern.



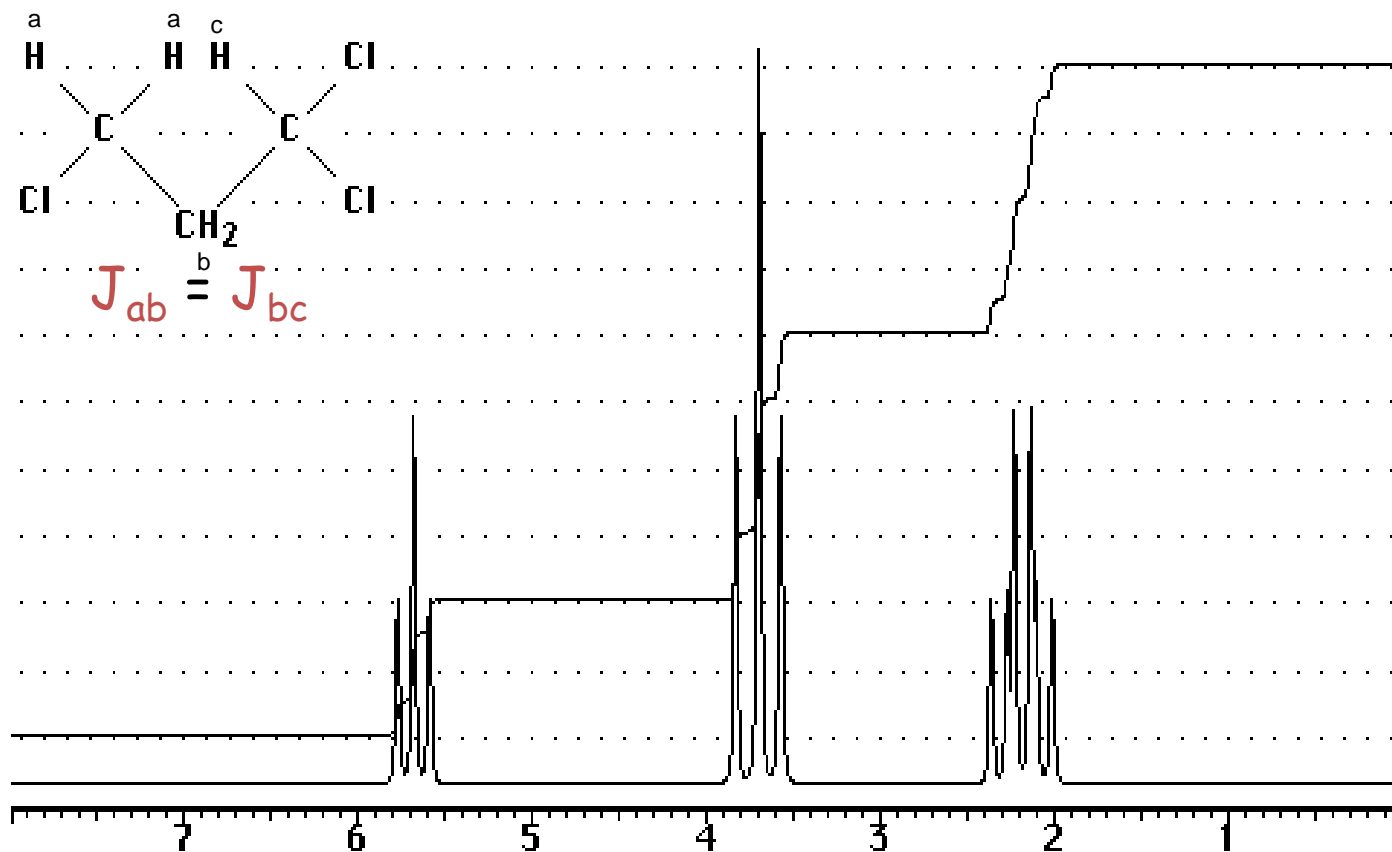


# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Spin-Spin Splitting

Whenever two (or three) different sets of adjacent protons are **not equivalent** to each other, use the  $n + 1$  rule to determine the splitting pattern only if the **coupling constants ( $J$ ) are identical**:

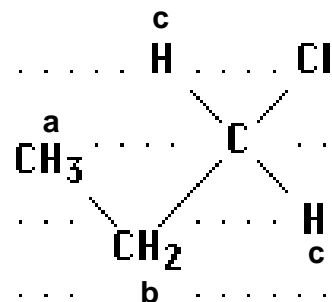
**Free rotation around C-C bonds averages coupling constant to  $J = 7\text{Hz}$**



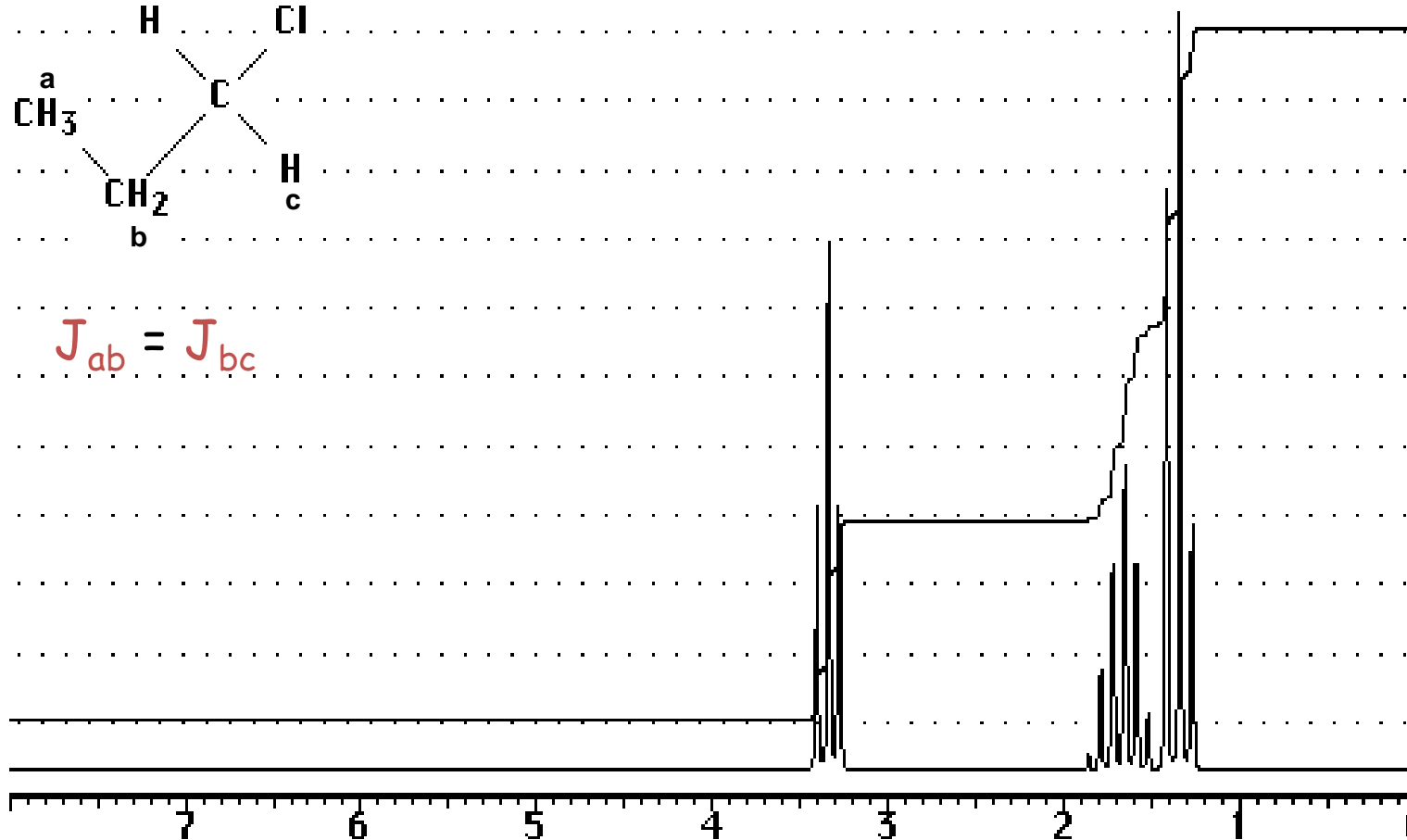
# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Spin-Spin Splitting

Whenever two (or three) different sets of adjacent protons are **not equivalent** to each other, use the  $n + 1$  rule to determine the splitting pattern only if the **coupling constants ( $J$ )** are identical:



$$J_{ab} = J_{bc}$$

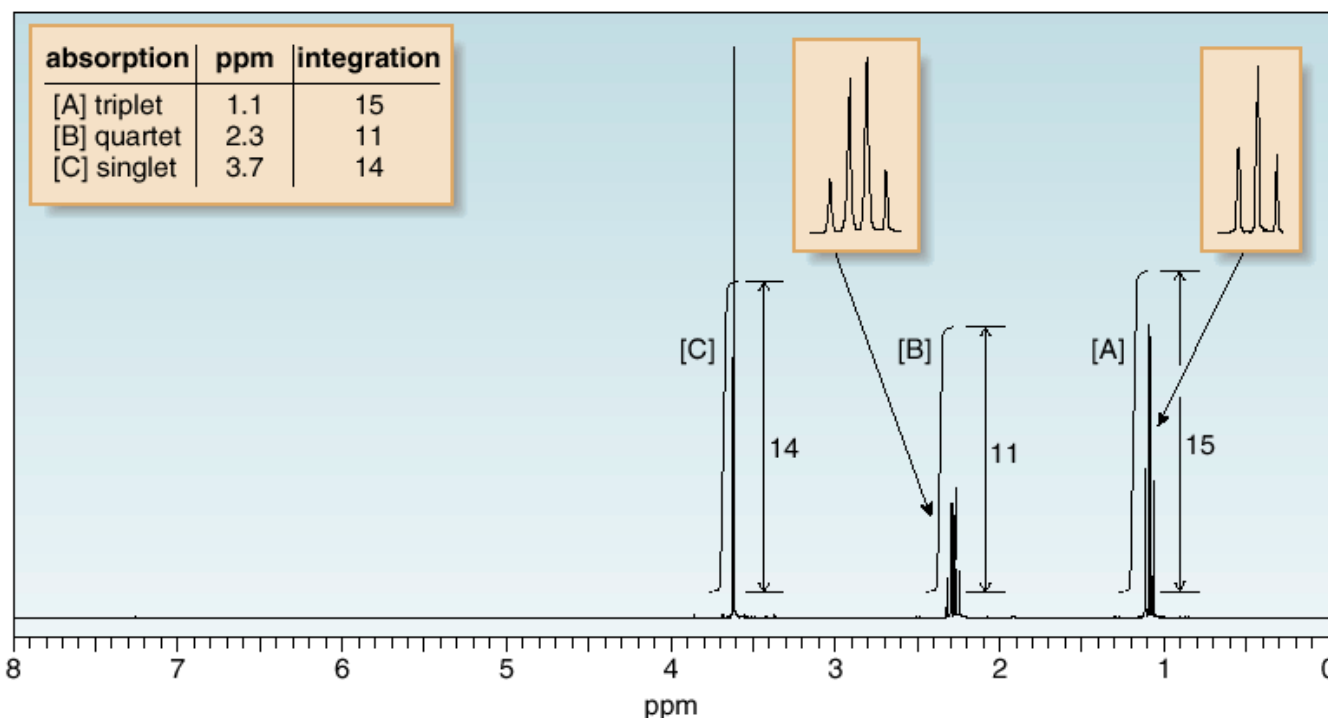


# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Structure Determination

### How To Use $^1\text{H}$ NMR Data to Determine a Structure

**Example** Using its  $^1\text{H}$  NMR spectrum, determine the structure of an unknown compound X that has molecular formula  $\text{C}_4\text{H}_8\text{O}_2$  and contains a  $\text{C}=\text{O}$  absorption in its IR spectrum.



**Step [1]** Determine the number of different kinds of protons.

- The number of NMR signals equals the number of different types of protons.
- This molecule has three NMR signals ([A], [B], and [C]) and therefore **three** types of protons ( $\text{H}_a$ ,  $\text{H}_b$ , and  $\text{H}_c$ ).

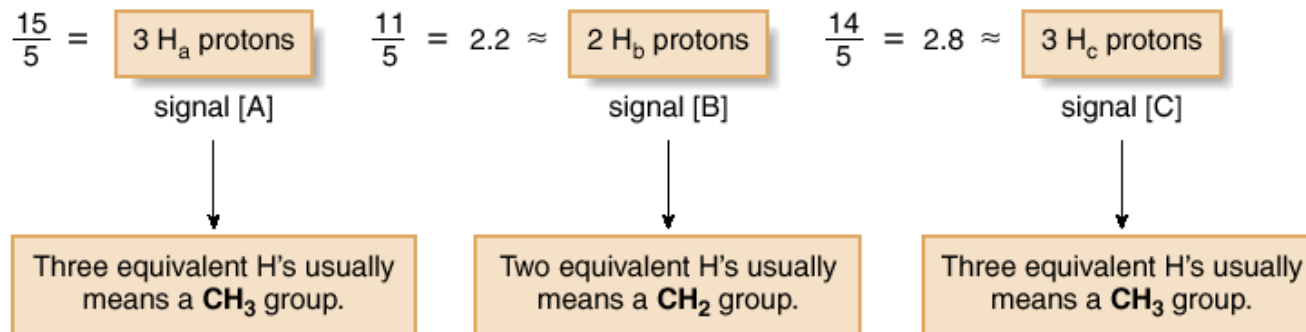
# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Structure Determination

### How To, continued . . .

Step [2] Use the integration data to determine the number of H atoms giving rise to each signal (Section 14.5).

- Total number of integration units:  $14 + 11 + 15 = 40$  units
- Total number of protons = 8
- Divide:  $40 \text{ units} / 8 \text{ protons} = 5 \text{ units per proton}$
- Then, divide each integration value by this answer (5 units per proton) and round to the nearest whole number.



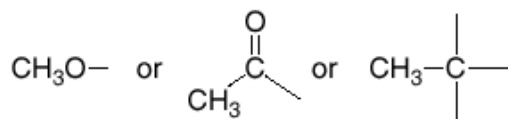
# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Structure Determination

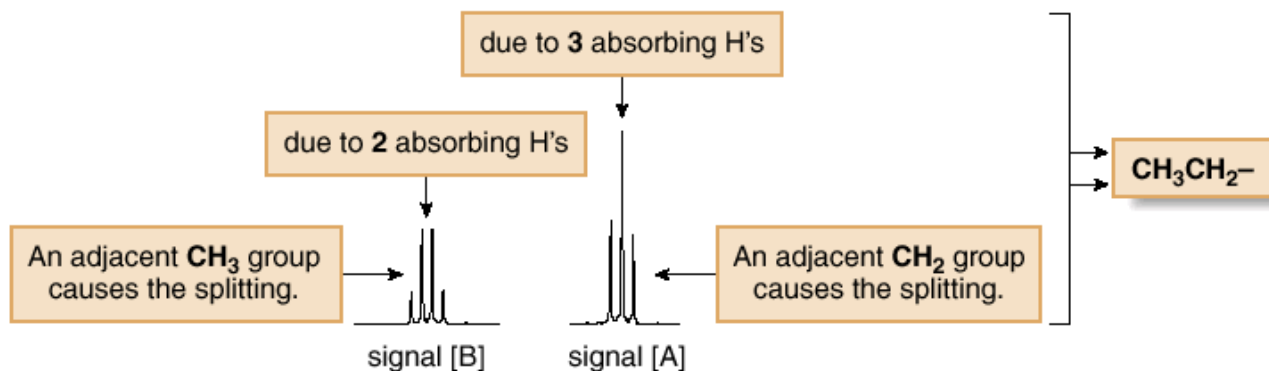
### How To, continued . . .

Step [3] Use individual splitting patterns to determine what carbon atoms are bonded to each other.

- Start with the singlets. Signal [C] is due to a  $\text{CH}_3$  group with no adjacent nonequivalent H atoms. Possible structures include:



- Because signal [A] is a **triplet**, there must be **2 H's** ( $\text{CH}_2$  group) on the adjacent carbon.
- Because signal [B] is a **quartet**, there must be **3 H's** ( $\text{CH}_3$  group) on the adjacent carbon.
- This information suggests that **X** has an **ethyl** group  $\text{---} \rightarrow \text{CH}_3\text{CH}_2\text{---}$ .



To summarize, **X** contains  $\text{CH}_3\text{---}$ ,  $\text{CH}_3\text{CH}_2\text{---}$ , and  $\text{C}=\text{O}$  (from the IR). Comparing these atoms with the molecular formula shows that one O atom is missing. Because O atoms do not absorb in a  $^1\text{H}$  NMR spectrum, their presence can only be inferred by examining the chemical shift of protons near them. O atoms are more electronegative than C, thus deshielding nearby protons, and shifting their absorption downfield.

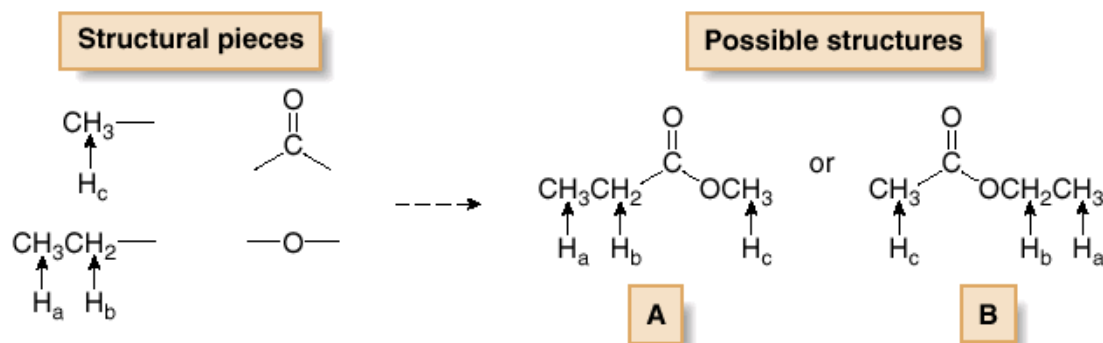
# Nuclear Magnetic Resonance Spectroscopy

## $^1\text{H}$ NMR—Structure Determination

### *How To, continued . . .*

Step [4] Use chemical shift data to complete the structure.

- Put the structure together in a manner that preserves the splitting data and is consistent with the reported chemical shifts.
- In this example, two isomeric structures (**A** and **B**) are possible for **X** considering the splitting data only:



- Chemical shift information distinguishes the two possibilities. The electronegative O atom deshields adjacent H's, shifting them downfield between 3 and 4 ppm. If **A** is the correct structure, the singlet due to the  $\text{CH}_3$  group ( $\text{H}_c$ ) should occur downfield, whereas if **B** is the correct structure, the quartet due to the  $\text{CH}_2$  group ( $\text{H}_b$ ) should occur downfield.
- Because the NMR of **X** has a singlet (not a quartet) at 3.7, **A is the correct structure**.