Nuclear Magnetic Resonance Spectrometry

Overview

A Deeper Look

11.1 Introduction

Nuclear magnetic resonance (NMR) spectrometry, together with mass spectrometry and infrared spectrometry, is of major importance in determining the molecular structure of organic compounds as well as macro-molecules, both man-made and biochemical.

NMR involves measuring the absorption of radiofrequency radiation by a sample material that is placed in a strong magnetic field. The radiation used is in the range of 100-MHz (the frequency of FM radio in the U.S.) up to nearly 1 GHz. The magnetic fields are large; the most sophisticated instruments use some of the largest constant magnetic fields that can be generated.

As in other spectrometries, the fraction of the power absorbed is proportional to the concentration of the absorbing species while the energy of the transition is determined by molecular and atomic properties. However, in NMR spectroscopy, the energy also depends on the magnitude of the magnetic field.

An instrument for NMR spectrometry measures the spectrum for one atomic species at a time. The most commonly measured atomic species are ^1H, ^{13}C, ^{19}F, and ^{32}P. By far, the most commonly measured is ^1H-NMR, usually called **proton NMR**. Not only is proton NMR the most frequently used, it is also inherently the most sensitive for two reasons. First, the proton's nuclear magnet is the largest and, second, nearly 100% of the natural isotopic abundance of hydrogen is ^1H. The next most commonly measured NMR nucleus is ^{13}C. However, ^{13}C-NMR (read "carbon thirteen NMR" or "C thirteen NMR") is inherently less sensitive, and, further, the natural abundance of ^{13}C—the isotope that is NMR active—is only 1.1%. For these reasons, good spectra require either ^{13}C-enriched samples, highly concentrated samples, or long data collection times (days). Nevertheless, in practice, ^{13}C-NMR has proven exceedingly useful.

In ^1H-NMR, either solvents containing no hydrogen (for example, CCl_4) or deuterated solvents (for example, D_2O) are generally used. Otherwise, the proton-NMR absorption from the solvent might overwhelm that of the sample. The solvent signal can be suppressed, but information might be lost by doing so.

In this chapter we focus primarily on the study of the structures of small, organic molecules in solution. However, great strides have been made in both the analysis of structures of proteins up to about 10,000 daltons and the analysis of the structures of solids and surfaces. The variety of experiments and the information they can provide can only be suggested by this brief chapter.

11.2 General Principles of NMR

The nuclei of many atoms possess a **magnetic moment**. This means that they act like small bar magnets—magnetic dipoles. In a magnetic field, a nucleus like ^1H or ^{13}C can point in only two directions relative to the field and none in between. In other words, the orientation of a nuclear dipole is quantized. As illustrated in Figure 11.1, the ground state is the energy level of the nucleus when the magnetic dipole is aligned along the magnetic field, and the excited state is the energy level when the magnetic dipole is aligned against the

(a)

FIGURE 11.1 ▶

The spectroscopic transition for nuclear magnetic resonance.

Energy is needed to reorient the nuclear magnets in a magnetic field. The energy is absorbed and reemitted at the frequency characteristic of the nucleus in its local environment. **(a)** The large arrow represents the external magnetic field. The two smaller arrows represent the dipole of a nucleus with a nuclear spin $I = \frac{1}{2}$. **(b)** An energy level diagram for the system in (a). The splitting is proportional to the field.

(b)
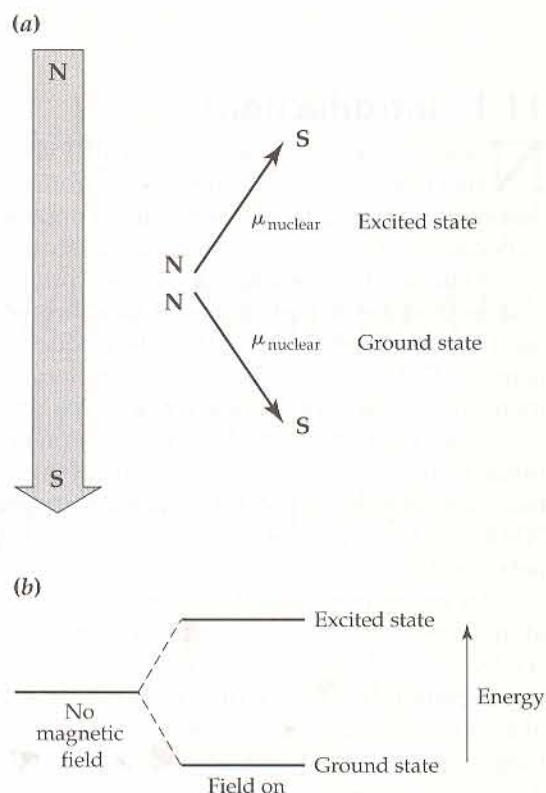
magnetic field. In an NMR experiment, when the radiofrequency radiation has the correct frequency, energy is absorbed by the nuclear magnets, which are thereby raised from their ground states to their excited states.

Spectra of radiofrequency energies are measured, and the spectra are plotted as narrow peaks rising from a baseline. The frequency at which the absorption of energy occurs in an NMR experiment depends on two factors:

1. The identity of the nucleus
2. The magnetic field strength

The relationship is written algebraically as

$$\nu_{\text{resonance}} = \left(\frac{\gamma}{2\pi}\right) \cdot H \qquad \text{(11-1)}$$

where $\nu_{\text{resonance}}$ is the **resonance frequency**, and H is the magnetic field strength at the nucleus. γ is a constant, the value of which depends on the identity of the nucleus; it is called the **magnetogyric ratio**. Table 11.1 lists the magnetic-field strengths and the resulting resonant frequencies of the five most commonly measured NMR nuclei. These five are, by far, the most easily investigated experimentally (although the instrument electronics is still quite sophisticated) and exhibit the most easily understood spectra. In this chapter, only the analytical uses of ^{1}H and ^{13}C NMR spectra are presented in some detail.

The early developers of the NMR techniques were hoping that NMR could be used to make highly precise measurements of the magnetogyric

Table 11.1 Field Strengths and Associated Resonance Frequencies of the Five Most Commonly Investigated Nuclei			
Isotope	Field strength H_0 in tesla[†]	Resonance frequency in MHz	Nuclear spin quantum number, I
^1H	1.41	60.0	$\frac{1}{2}$
	2.35	100.0	
	7.04	300.0	
	11.74	500.0	
	17.61	750.0	
	21.14	900.0	
^2H	2.35	15.3	1[*]
^{13}C	2.35	25.1	$\frac{1}{2}$
^{19}F	2.35	94.0	$\frac{1}{2}$
^{31}P	2.35	40.5	$\frac{1}{2}$

[†]Tesla is the SI unit. The unit of gauss is also used: 10^4 gauss = 1 tesla (abbreviation T).
[*]Deuterium, with a nuclear quantum number $I = 1$, has *three* different energy levels in a magnetic field.

ratios of nuclei. However, reality intervened, and they found, for a fixed frequency, the field at resonance varied in a manner that depended on the chemical environment of the nuclei. This effect was named the **chemical shift**. This "problem" is one of the reasons that NMR is such a useful analytical technique in chemistry. As you will see, the chemical shift is predictable from the specific chemical environment of the nucleus.

Through experiments, the chemical shift was found to be proportional to the magnitude of the local magnetic field. This result can be expressed algebraically by modifying Equation 11-1 with a magnetic field **shielding parameter**, σ. To include the chemical effects, we substitute $H_0(1 - \sigma)$ for H in Equation 11-1. H_0 represents the value of the applied external magnetic field. This substitution yields

$$\nu_{\text{resonance}} = \left(\frac{\gamma}{2\pi}\right) \cdot (H_0 - \sigma H_0) \qquad (11\text{-}2)$$

However, there is no way to know the field at the sample nucleus when there is no shielding, so we cannot find the value of σ by an NMR experiment. As a result, Equation 11-2 is of little practical use. This problem can be circumvented by using a *relative* frequency shift compared with a reference compound. The relative shift is also called the *chemical shift*, denoted by δ. The equation defining the chemical shift is

$$\delta \text{ (ppm)} = \frac{\nu \text{ (sample)} - \nu \text{ (reference)}}{\nu \text{ (reference)}} \times 10^6 \qquad (11\text{-}3)$$

Today, experiments are carried out exclusively with fixed fields. However, for historical reasons, spectra are displayed in terms of field shifts. Thus, the equation for δ is

$$\delta \text{ (ppm)} = \frac{H_0 \text{ (reference)} - H_0 \text{ (sample)}}{H_0} \times 10^6 \qquad (11\text{-}4)$$

as if the frequency remains fixed.

As indicated in Equations 11-3 and 11-4, the units of the chemical shift are **parts per million** (ppm). (This should not be confused with the ppm measure of chemical content.) A reference compound is used as an internal standard of the chemical shift. The internal standard for ^{1}H-NMR spectra of samples that dissolve in organic solvents is now mutually agreed upon: It is tetramethylsilane $(CH_3)_4Si$, abbreviated TMS. The reasons for this choice will become clear as the properties of NMR spectra are described below. On the chemical-shift (δ) scale, the tetramethylsilane ^{1}H resonance signal appears at $\delta = 0.00$ ppm *by definition*. In aqueous solvents, a common standard is trimethylsilylpropanesulfonate, with $\delta = 0.015$ ppm. Its formula is $(CH_3)_3SiCH_2CH_2CH_2SO_3^-$.

EXAMPLE 11.1

Assume that a spectrum is run at a frequency of 300 MHz and a field of 70,460 gauss with TMS as reference. The resonance of a proton from the sample appears at a frequency 1080 Hz higher than that of the standard. What is the chemical shift?

Solution

From the definition of the chemical shift in the form of Equation 11-3,

$$\delta = \left(\frac{1080}{300 \times 10^6}\right) \times 10^6 = \frac{1080}{300} = 3.6 \text{ ppm}$$

The value of 300×10^6 Hz is used in the denominator. This is the frequency of resonance of the standard. We could just as easily used the frequency of the sample's resonance—300,001,080 Hz. The calculated result is the same to well beyond the precision of the experiment.

The frequency differences due to the chemical-shift effect are relatively small. For a 100-MHz resonance frequency, the range of chemical shifts found for ^{1}H is only about 1000 Hz. As a relative change, this is only 10 ppm. Experimentally, it is quite difficult to keep the irradiation frequency, detector amplifiers, and magnetic field stable enough for these measurements to be made accurately and reproducibly. Nevertheless, such conditions must be maintained because in NMR, as in other spectrometries, the effective instrumental bandwidth must be significantly smaller than the inherent spectral linewidth (as small as 0.1 Hz) in order to obtain undistorted sample spectra.

NMR, like most other analytical techniques, was the product of the work of many people. During this development, two different chemical-shift scales arose: the delta (δ) scale and the tau (τ) scale. By definition, $\tau = 10.00$ ppm for TMS and runs *with* the field. The δ scale runs in the opposite direction. The relationship between these two scales is illustrated in Figure 11.2. Essentially all data in the chemical literature are now reported using the delta scale. When τ-values are found in the early literature, it is easy to convert between the two scales using $\delta = 10 - \tau$.

Signal Magnitude and Concentration

As in other spectrometric methods, the frequency (or wavelength) is determined by internal properties of molecules, and the magnitude of the energy absorption is determined by the concentration. In NMR spectrometry, the

The linewidth:bandwidth ratio is discussed in Section 8.5. For the effective bandwidth in Fourier-transform spectra, see Chapter 17.

$$\delta = \tau + 10, \text{TMS} = 0.00$$

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 0 | −1 | −2 | | δ scale |

| −2 | −1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | | τ scale |

TMS

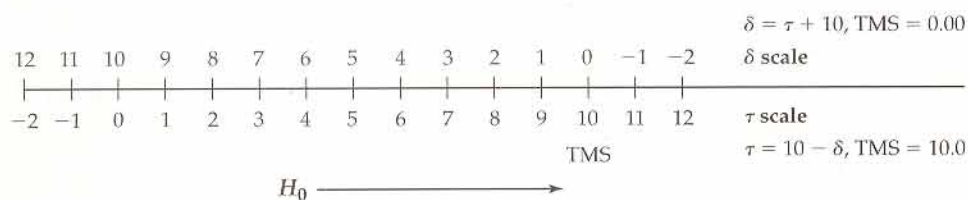$$\tau = 10 - \delta, \text{TMS} = 10.0$$

$H_0 \longrightarrow$

FIGURE 11.2 ▲

Illustration of the relationship between the two NMR chemical shift scales.

The resonance position of tetramethylsilane (TMS) is defined as $\delta = 0.0000$ ppm. The range shown here is typical for ^1H spectra. Other NMR nuclei, for example ^{19}F and ^{13}C, exhibit much wider chemical-shift ranges.

Benzyl acetate

Integral

44

30

72

TMS

| 8.0 | 7.5 | 7.0 | 6.5 | 6.0 | 5.5 | 5.0 | 4.5 | 4.0 | 3.5 | 3.0 | 2.5 | 2.0 | 1.5 | 1.0 | 0.5 | 0.0 |

ppm

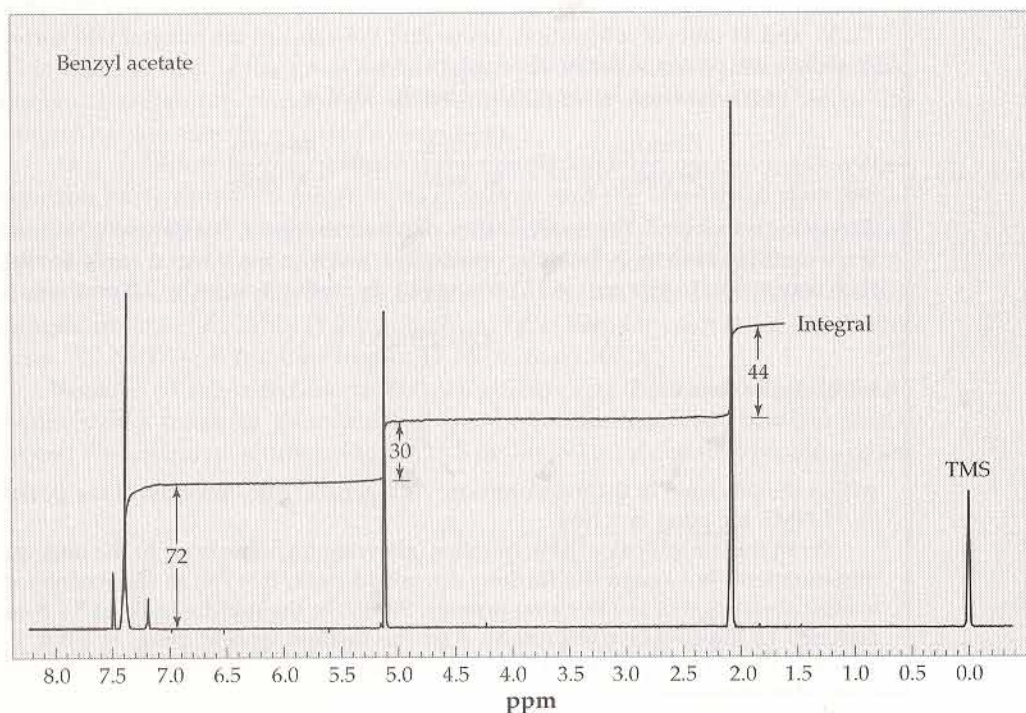FIGURE 11.3 ▲

A 250 MHz ^1H-NMR spectrum with integration of the peaks.

The sample is neat benzyl acetate with an internal standard of TMS.

areas under the peaks are measured by electronic integration. See Figure 11.3.

In NMR spectrometry, the integrated peak areas are only relative values, depending on the number of nuclei that contribute to the peak. This is one of the great strengths of the NMR method:

Each proton, when it absorbs energy, contributes equally to a simple NMR absorption spectrum.

The magnitude of the absorption is independent of the chemical shift when proper care is taken in the experiment. In effect, every proton has the same absorptivity. Obtaining absolute concentrations using NMR spectra is done using concentration standards.

EXAMPLE 11.2

Assign the three peaks of the benzyl acetate NMR spectrum shown in Figure 11.3.

Solution

The structure of benzyl acetate is

$$CH_3-\overset{\overset{\textstyle O}{\|}}{C}-O-CH_2-\hexagon$$
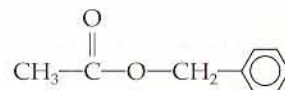
Recall that every proton contributes equally to a simple NMR spectrum. In benzyl acetate, five protons are on the ring, three on the methyl group, and two on the benzyl group. The area under each peak must be relatable to an integral number of protons. The integrated spectrum is the line with three steps of heights 72, 30, and 44 units of integration. Let us find the value of the integral that corresponds to *one* proton in the molecular formula.

Let us try dividing by the smallest height. That is

$$\frac{72\ \text{units}}{30\ \text{units}} = 2.40 \qquad \frac{30\ \text{units}}{30\ \text{units}} = 1.00 \qquad \frac{44\ \text{units}}{30\ \text{units}} = 1.47$$

The ratios here are not the desired ratios of whole numbers, but they will be close if we multiply each by 2. (In other words, 15.0 units on the integral curve would then correspond to one proton.) Carrying out the multiplication by 2.0 produces

$$4.80 : 2.0 : 2.93$$

which approximates

$$5 : 2 : 3$$

within the precision of the measurement. As a general rule, integrals of the peaks in ^{1}H-NMR are good to $\pm 10\%$.

From this simple calculation of ratios (allowing for some error in the integral measurement) we assign the furthest downfield peak, $\delta = 7.4$, as the resonance energy absorption of the five ring protons. Similarly, the middle peak, at 5.1 δ, is assigned to the two benzyl protons. And the furthest upfield peak, at 2.0 δ, is assigned to the three protons of the methyl group.

The quantitation of other nuclei, such as ^{13}C or ^{31}P, requires more attention to the experimental conditions since the integral areas per nucleus can depend on the local chemical environment. For example, the areas of ^{13}C tend to vary depending on the number of protons attached. Quite often, a carbon that has no protons, such as in $S^{13}CN^-$, will produce a peak much smaller than the peaks arising from the C—H, C—H$_2$, and C—H$_3$ groups. In other words, for an equimolar mixture of $^{13}CH_3OH$ and $S^{13}CN^-$, it is not at all likely that the ^{13}C resonance lines will have equal heights or areas.

11.3   Chemical Shifts: Origins and Values

Origin of σ, the Shielding Parameter

Equation 11-2 is an algebraic description of an experimental result: The instrument's magnetic field apparently is reduced by some opposing magnetic field that arises *inside* the sample. This reduction in the magnetic field at the nucleus is called **shielding**. We explain this internal field as being due to

the electron density around the nucleus: The greater the electron density, the larger the shielding.

Suppose we ran an experiment with a fixed radiofrequency and a variable magnetic field. Since the resonance occurs when the field and frequency reach the resonance condition—Equation 11-1—we must increase the external field to compensate for the reduction due to the shielding effect if we want to observe a resonance. The greater the electron density around a proton, the more the external magnetic field must be raised to observe resonance absorption. Since highly shielded protons require a larger field to achieve the resonance condition, the spectral lines are said to be **upfield** from less shielded protons. These relationships are illustrated in Figure 11.4. A most important general result is explained through this phenomenon:

> *The chemical shift of a resonant nucleus depends on the chemical structure near it.*

This leads to one of the great advantages of NMR spectra in chemistry. The chemical shifts for protons in different chemical environments occur in *ranges* that are specific to that environment.

As a result of the regularities of the chemical shifts, we can make a **correlation table** of the chemical shifts observed and the identity of the chemical groups in which the protons are bonded. Figure 11.5 shows some general correlations so that you can see what a few of the chemical regularities are. A more detailed figure of this type is shown in Figure 11.6. The chemical shift ranges are listed in Table 11.2 by chemical type. Similar correlations are seen with ^{13}C-NMR spectra. (See Figure 11.24 on page 505.)

Because of the correlations, the assignments of the benzyl acetate resonance peaks made in Example 11.2 can be made from the chemical shifts alone. The chemical shifts in Figure 11.5 or Table 11.2 show that protons of an
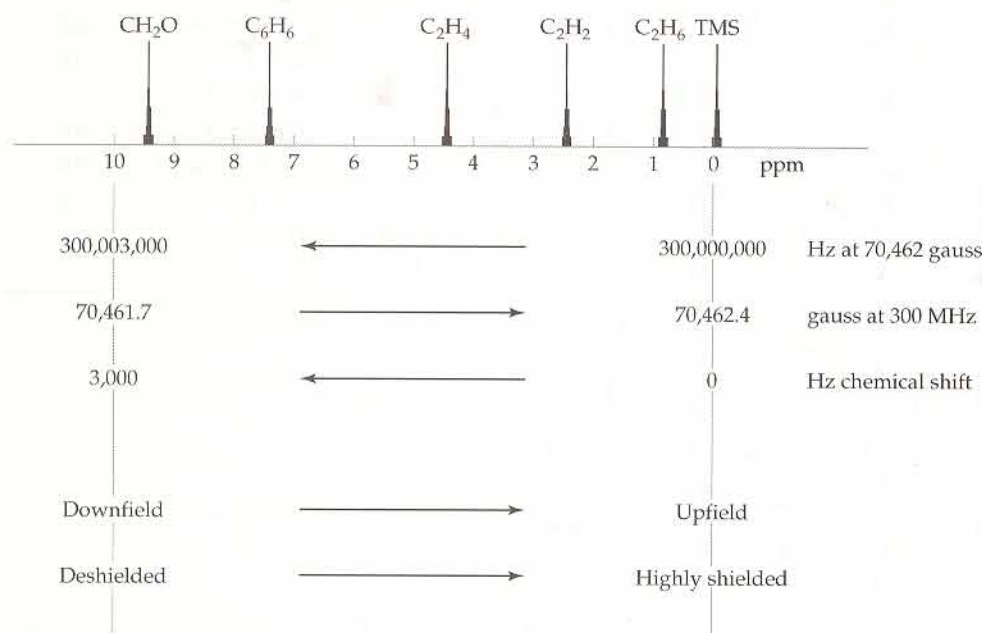

FIGURE 11.4 ▲

Some of the nomenclature and numerical values found for ^1H-NMR spectra.
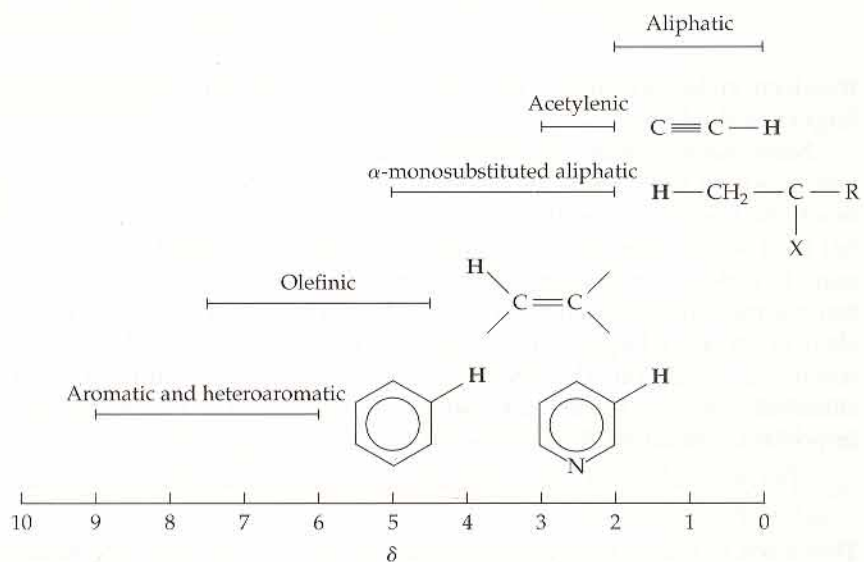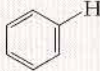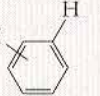
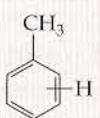The numerical values apply to 300 MHz instruments.

Aliphatic

Acetylenic

$C{\equiv}C-H$

α-monosubstituted aliphatic

$H-CH_2-\underset{X}{\overset{}{C}}-R$

Olefinic

Aromatic and heteroaromatic

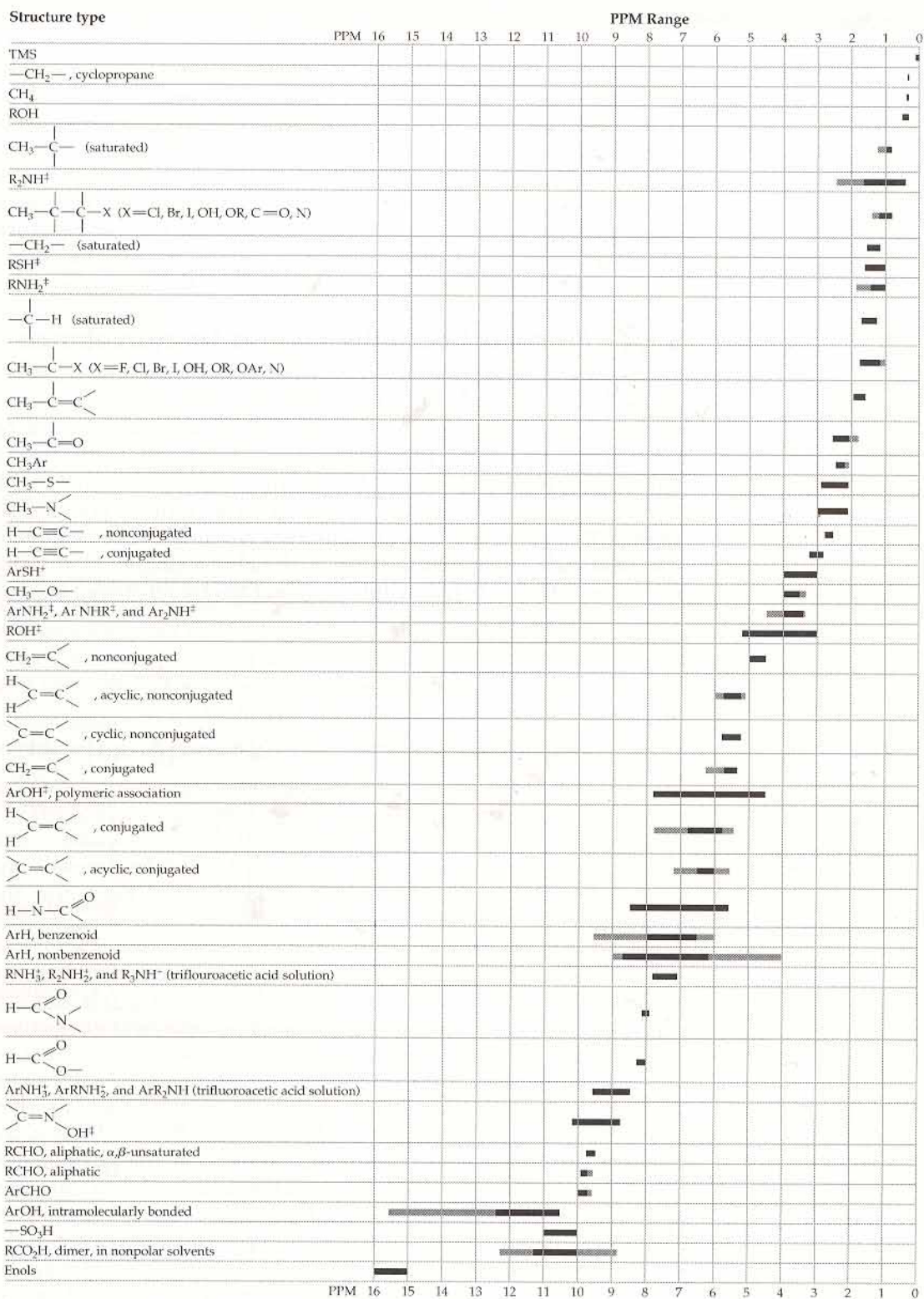10 9 8 7 6 5 4 3 2 1 0

δ

FIGURE 11.5 ▶
Typical ranges of the chemical shift of protons in organic molecules.

The substituent noted by X can be a group such as O, N, Cl, Br, and NO$_2$. More detail is presented in Figure 11.6 and Table 11.2.

Table 11.2 Approximate NMR Chemical Shifts of Protons (R— = alkyl group, Ar— = aromatic group)

CH$_3$— protons		—CH$_2$— protons		—CH— protons		Other groups	
Proton	δ (ppm)	Proton	δ (ppm)	Proton	δ (ppm)	Proton	δ (ppm)
CH$_3$—C	0.9	—C—CH$_2$—C	1.3	—C—CH—C	1.5	—C≡CH	5.3
CH$_3$—C—C≡C	1.1	—C—CH$_2$—C—C≡C	1.7	—CH—C—Br	1.9	—C≡CH—	5.1
CH$_3$—C—Cl	1.4	—CH$_2$—C—Cl	1.8	—CH—C—Cl	2.0	—C≡CH—(cyclic)	5.3
CH$_3$—C—O	1.4	—CH$_2$—C—Br	1.8	—C—CH—C—O	2.0	R—C≡CH	3.1
CH$_3$—C≡C	1.6	—C—CH$_2$—C—O	1.9	—C—CH—CO—R	2.7	—C≡CH—CO	5.9
CH$_3$—C—NO$_2$	1.6	—C—CH$_2$—C—NO$_2$	2.1	—C—CH—N	2.8	—CH≡C—CO	6.8
CH$_3$—C—Br	1.8	—C—CH$_2$—CO—O—R	2.2	—CH—Ar	3.0	R—CHO	9.9
—C≡C(CH$_3$)—CO	1.8	—C—CH$_2$—C≡C	2.3	—C—CH—O—R	3.7	Ar—CHO	9.9
CH$_3$—CO—N—R	2.0	—C—CH$_2$—CO—O—R	2.2	—C—CH—O—H	3.9	H—CO—O	8.0
CH$_3$—C≡C—CO	2.0	—C—CH$_2$—C≡C	2.3	—CH—Cl	4.0	H—CO—N	8.0
CH$_3$—CO—R	2.2	—C—CH$_2$—CO—R	2.4	—CH—Br	4.1	CHCl$_3$	7.25
CH$_3$—Ar	2.3	—C—CH$_2$—C≡C—CO	2.4	—C—CH—NO$_2$	4.7	H$_2$O	~5
CH$_3$—N	2.3	—C≡C(CH$_2$)—CO	2.4	—C—CH—O—CO—R	4.8		
CH$_3$—CO—O—Ar	2.4	—C—CH$_2$—N	2.5				
CH$_3$—CO—Ar	2.6	—C—CH$_2$—Ar	2.7	**Aromatic protons**			
CH$_3$—Br	2.7	—C—CH$_2$—O—R	3.4				
CH$_3$—Cl	3.0	—CH$_2$—Cl	3.4		7.2		
CH$_3$—O—R	3.3	—CH$_2$—Br	3.4				
CH$_3$—O—Ar	3.8	—C—CH$_2$—O—H	3.6		7.0–9.0		
CH$_3$—O—CO—R	3.7	—C—CH$_2$—O—CO—R	4.1				
		—C—CH$_2$—O—Ar	4.3		2.3		
		—C—CH$_2$—NO$_2$	4.4		7.1		

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Structure type	PPM Range

Structure type — PPM Range

TMS
—CH₂—, cyclopropane
CH₄
ROH
CH₃—C— (saturated)
R₂NH‡
CH₃—C—C—X (X=Cl, Br, I, OH, OR, C=O, N)
—CH₂— (saturated)
RSH‡
RNH₂‡
—C—H (saturated)
CH₃—C—X (X=F, Cl, Br, I, OH, OR, OAr, N)
CH₃—C=C
CH₃—C=O
CH₃Ar
CH₃—S—
CH₃—N
H—C≡C—, nonconjugated
H—C≡C—, conjugated
ArSH⁺
CH₃—O—
ArNH₂‡, Ar NHR‡, and Ar₂NH‡
ROH‡
CH₂=C, nonconjugated
C=C, acyclic, nonconjugated
C=C, cyclic, nonconjugated
CH₂=C, conjugated
ArOH‡, polymeric association
C=C, conjugated
C=C, acyclic, conjugated
H—N—C=O
ArH, benzenoid
ArH, nonbenzenoid
RNH₃⁺, R₂NH₂⁺, and R₃NH⁻ (triflouroacetic acid solution)
H—C=O, N
H—C=O, O—
ArNH₃⁺, ArRNH₂⁺, and ArR₂NH (trifluoroacetic acid solution)
C=N—OH‡
RCHO, aliphatic, α,β-unsaturated
RCHO, aliphatic
ArCHO
ArOH, intramolecularly bonded
—SO₃H
RCO₂H, dimer, in nonpolar solvents
Enols

‡ The absorption positions of these groups are concentration dependent; they shift toward zero ppm in more dilute solutions.

FIGURE 11.6 ▲

Typical ranges of NMR absorption chemical shifts for protons in various chemical environments.
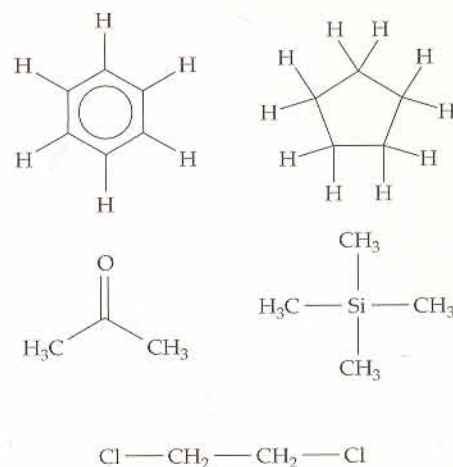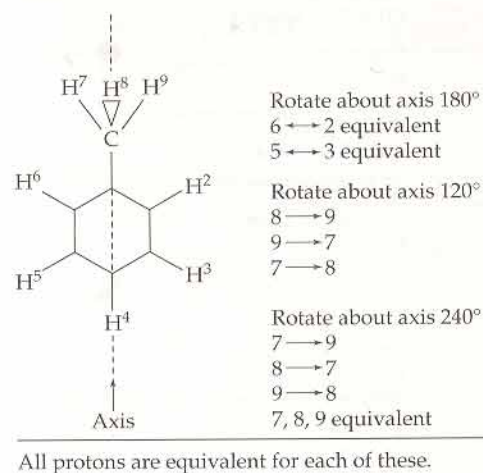
The lighter areas are extreme but possible regions. [Based on Dyer, J. R. 1965. *Applications to Absorption Spectroscopy of Organic Compounds.* Englewood Cliffs, NJ: Prentice-Hall. Reproduced with permission.]

aromatic ring with a carbon attached should be expected at around 7.1 ppm. The methyl protons of an acetate group are expected at around 2.0 ppm (CH_3—CO—O—Ar). The methylene protons are the only ones left, and they appear at 5.1 ppm.

As for other spectrometries, we may not have enough resolving power to separate all the spectral transitions, and that is the case here. For example, the single peak that appears from the five protons of the ring can be resolved into a complex set of peaks with instruments that can measure the spectra with higher resolution. This brings us to the subject of the next section.

11.4 Nuclear Equivalence and Inequivalence

You have seen how the peaks occurring in the NMR spectrum of benzyl acetate can be assigned to the three different types of protons in the molecule. In addition, in Figure 11.3 at $\delta = 0.00$, you can see a single peak from the internal standard TMS. The TMS molecule, $(CH_3)_4$ Si, has 12 protons, but only one peak appears in its NMR spectrum. When the resonances of two or more protons give rise to only *one* absorption peak, the protons are said to be **chemically equivalent**. Less formally, they are simply called **equivalent** protons, and their single peak is called, reasonably, a **singlet**. More examples of molecules with peaks from equivalent protons are illustrated in Figure 11.7.

Rotate about axis 180°
6 ←→ 2 equivalent
5 ←→ 3 equivalent

Rotate about axis 120°
8 —→ 9
9 —→ 7
7 —→ 8

Rotate about axis 240°
7 —→ 9
8 —→ 7
9 —→ 8
7, 8, 9 equivalent

All protons are equivalent for each of these.

FIGURE 11.7 ▶
Illustration of the concept of equivalence of protons.

The protons that are interchangeable are equivalent. The dotted line shows the axis about which the molecule is rotated.

A simple technique can be used to see if protons are equivalent. If it is possible to rotate the molecule in some way so that you cannot tell which protons were interchanged, then the protons are equivalent. Figure 11.7 illustrates the sorts of rotations that are used. The study of this subject is part of an area of mathematics called *group theory*. Further discussion of this topic is outside the subject area of this book.

Let us apply these ideas to benzyl acetate's structure and spectrum, shown in Figure 11.3. As its structure shows, only one position on the benzene ring is substituted. When we carry out the rotations of the ring in benzyl acetate to see which protons are equivalent, the rule given above suggests that the ring protons should fall into three groups: two *ortho*, two *meta*, and one *para* to the substitution. However, as you can see from the spectrum, only one peak is seen at 7.4 δ. The three lines are apparently not resolved. In other words, simply because chemically distinct protons *can* have different chemical shifts does not mean they *will* appear as separate in the spectrum. In this case, the instrument does not have enough resolving power to show the separate lines. We can, however, see evidence for this lack of spectral resolution; the linewidth of the 7.4 δ peak is broader than the other two. How can we tell that from these narrow-line spectra—especially from this small figure? The answer is to compare the heights and integrals of the lines. For example, the integrals of the 2.1 δ and 5.1 δ peaks vary by a factor of $1\frac{1}{2}$, as do their heights. This means that their linewidths are about equal. However, as you can see, the 7.4 δ and 5.1 δ peaks are about the same height, but the integrals differ by a factor of $2\frac{1}{2}$. The 7.4 δ line must be broader.

11.5   Nuclear Spin–Nuclear Spin Interaction

As noted above, the radiofrequency at which the NMR peak appears is determined by the size of the external magnetic field of the instrument and by the internal magnetic field due to the electrons surrounding the nucleus. However, one other magnetic field is present that has not been mentioned: the magnetic field from nearby protons. Since each proton nucleus is itself a magnet, it will affect the resonance frequency of the other protons nearby. The effect results in **split** lines, and it is called **spin-spin splitting** or **hyperfine splitting**. As you shall see, this effect is extraordinarily useful in determining chemical structures.

To explain the spin-spin splitting seen in the spectrum of Figure 11.8, first consider two protons attached to adjacent carbons. There are only two protons, but four lines are seen. What has happened is that each resonance line for a proton has been split into a **doublet**—two lines from the same proton(s). Each line of the doublet has half the area of the original line. The separation of the two lines (the spacing between their peaks) is called the **hyperfine coupling constant**. Usually the capital letter J is used to signify this value, and the magnitude is expressed in frequency units (Hz). For the two protons in Figure 11.8, the hyperfine coupling constant is the spacing, in Hz, for *both* doublets. That is, J is the splitting between the two lines on the left *and* the two lines on the right. This is a general result.

> *If the splitting by proton A of the resonance line of proton B is J_{AB}, then the resonance line of A will be split by the same amount.*

Algebraically, we write

$$J_{AB} = J_{BA} \qquad\qquad \textbf{(11-5)}$$
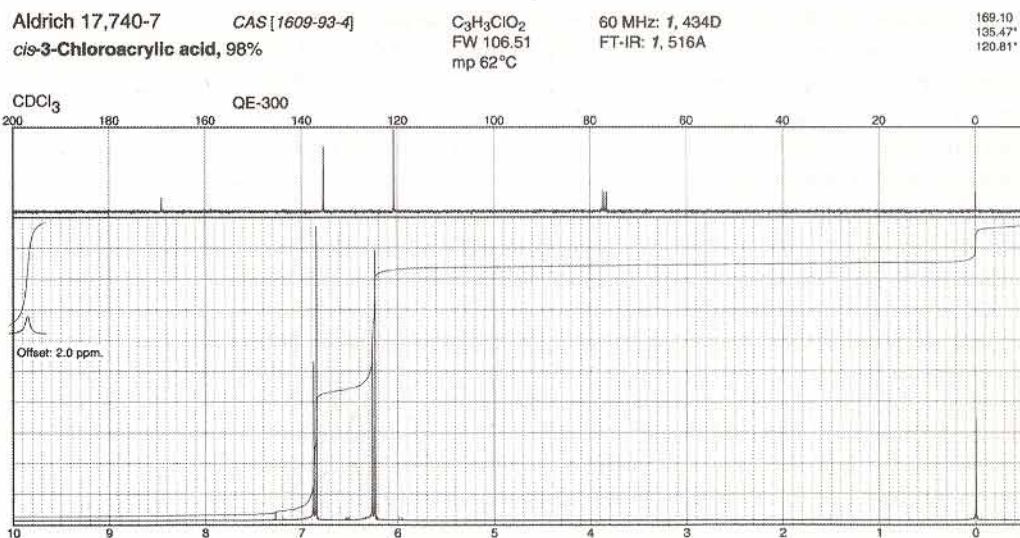
Aldrich 17,740-7   CAS [1609-93-4] $C_3H_3ClO_2$ 60 MHz: 1, 434D   169.10
cis-3-Chloroacrylic acid, 98%   FW 106.51   FT-IR: 1, 516A   135.47*
                                   mp 62°C   120.81*

FIGURE 11.8 ▲
The NMR spectrum of cis-3-chloroacrylic acid showing the effect on the spectrum of hyperfine splitting.

As seen in the [1]H (lower) spectrum, the protons on either side of the double bond have different chemical shifts: one at 6.2 ppm and one at 6.85 ppm. Each of the lines is split into two by the adjacent proton. The peak for the hydroxyl proton is moved onto the scale; it appears at 12.2 ppm. The top spectrum is the ^{13}C-NMR for the same compound and solvent.

Let us see in slightly more detail how the spin-spin splitting can be explained. Consider that the absorption being measured is that of proton H^a in Figure 11.9. Two factors must be considered.

1. The magnetic field position of each NMR absorption is determined by the internal (molecular) field. But now there is an extra magnetic field coming from an adjacent magnet, that of the nucleus of proton H^b. Thus, the line position depends on the proton's chemical shift and also the magnetic field of the adjacent proton (hyperfine interaction).

2. The other nuclear spin, due to H^b, is quantized in direction either along or away from the external magnetic field (recall Figure 11.1). The magnetic field of H^b can have only two different values.

These two factors explain the resonance spectrum because, when H^b is aligned along the field direction, the NMR absorption of H^a occurs at a lower external field than when H^b is not present. Conversely, when H^b is aligned against the external field, the NMR absorption of H^a occurs at a greater external field than if H^b is not present. The H^a absorption line appears to be split. No line appears at the unsplit line position, because the two orientations of the spin of H^b always shift the resonance.

Experimentally, J_{AB}, the size of the splitting of H^a by H^b, depends on a number of factors. But, overall, the magnitude of J decreases with distance between the protons. A general property is:

Unless there is a conjugated system, spin-spin coupling is rarely observed when more than three bonds separate the hydrogen nuclei.

A spectrum with spin-spin splitting is described as follows. The chemical shift is reported as if the spin-spin splitting did not occur. In the example of
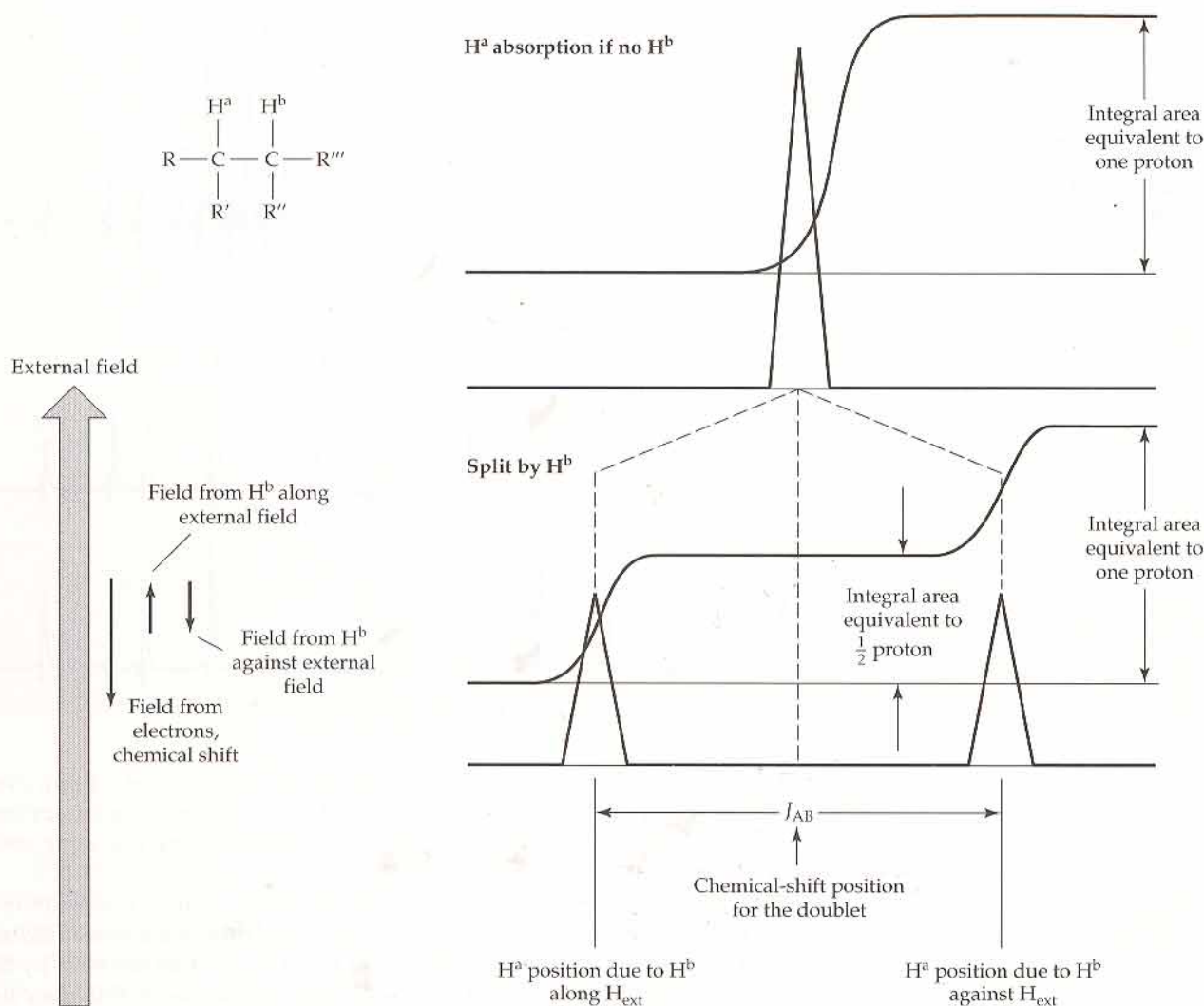
FIGURE 11.9 ▲
The origin of the intensities and positions of split NMR lines.

Shown here is the situation observed experimentally in the spectrum in Figure 11.8.
(At left) The positions of the resonance lines for proton H^a is determined by three interactions:
with the external magnetic field, with the field due to the electrons causing a chemical shift,
and with the two different alignments of the adjacent proton H^b. H^b is equally likely (in time)
to be in either orientation, so there are two peaks from H^a equal in magnitude from.

Figure 11.9, the chemical shift lies at the average position (midpoint) of the
doublet: the doublet's center of energy. For the spectrum in Figure 11.8, the
lines can be reported as

  a doublet at 6.2 ppm, with $J = 6.8$ Hz,
  a doublet at 6.9 ppm, with $J = 6.8$ Hz, and
  a singlet at 11.8 ppm.

Often, an even more compact notation is used: s for singlet, d for doublet,
t for **triplet** (three lines), and q for **quartet** (four lines). In this notation, the list
above is shortened to d 6.2 ($J = 6.8$ Hz); d 6.9 ($J = 6.8$ Hz); s 11.8.

Note that the chemical shifts are reported only to tenths of a ppm, even though the graph can be read more precisely. This is the limit of accuracy (not precision) of NMR instruments, as can be found by running the same spectrum repeatedly.

Now look at Figure 11.10, which shows NMR spectrum of acrylonitrile (CH_2CHCN) at three different field values (obtained on three different instruments). Observe the four lines on the right-hand side. They are split by the same amount *regardless of the size of the magnetic field*. However, the other resonance lines are shifted further away (in frequency units) from these four as the field gets larger. This set of spectra illustrates an important general result.

> *The frequency of the chemical shift, due to shielding by the electrons, depends on the external field; the spin-spin splitting, due to adjacent protons, does not.*

The Heights and Areas of Split Peaks

As illustrated in Figure 11.9, each of the peaks in the doublet has one-half the area that would be measured for the unsplit peak. The areas of the doublet peaks are essentially equal. A simple rule can be written about the area:

> *The integrated area per nuclear spin remains the same regardless of the number of lines into which it may be split.*

The Boltzmann distribution (Section 9A) explains the nearly equal areas caused by the two alignments of H^b.

This brings us to the next topic. What happens when more than one proton splits the resonance? The answer comes from an experimental spectrum, that of 1,1,2-trichloroethane (ClH_2CCHCl_2) shown in Figure 11.11. The integrated areas confirm the assignment of the spectrum. The resonance of proton H^a is split into three lines, a triplet, and the two equivalent protons H^b appear as a doublet.
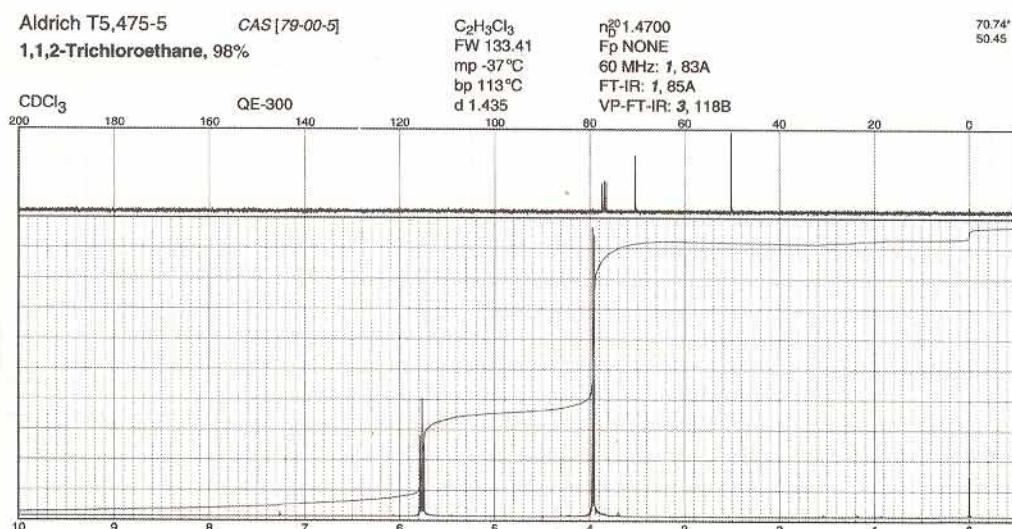
FIGURE 11.11 ▲
Splitting patterns.

When a proton resonance is split by more than one equivalent proton, different splitting patterns are observed. In the ¹H (lower) spectrum from 1,1,2-trichloroethane are resonances from two equivalent protons adjacent to a single proton. The pattern from the single proton (area = 1) is a typical NMR triplet. The spectrum from the two equivalent protons (area = 2) is a doublet with its splitting equal to the triplet's.

Figure 11.12 shows how we explain the triplet splitting and intensities. Either one or both of the two equivalent protons can be aligned along or against the external field, so that for the two protons there are four possible sets of orientations of equal probability. These four sets produce only three different magnetic fields because two sets produce the same (zero) magnetic field. Since *each* of these four sets of orientations is equally probable, the areas of the lines associated with each set of orientations are equal. Therefore, we expect an unshifted line twice as large as the two shifted lines since there are two orientations producing the unshifted line. The relative areas of the three lines are expected to be, as observed, 1:2:1. The total integrated area of the three lines is still that for one proton.

On the other hand, the two equivalent H^b protons are split into a doublet by the single H^a. The area of the doublet is that expected for two protons. Notice in the experimental spectrum that the splittings between the triplet lines equal the splitting between the doublet. Again, $J_{AB} = J_{BA}$.

When describing the NMR spectrum, the chemical shift of the triplet is, as before, reported as the frequency (or field) at which there is no contribution from the spin-spin interaction. When an *odd* number of lines exists in the **multiplet** (the general name for sets of lines: doublets, triplets, quartets, and so on), the chemical shift for the set is the position of the middle line.

General Splitting Patterns

Hyperfine splitting patterns become more complicated as the number of equivalent protons increases. A set of *n* equivalent protons splits adjacent
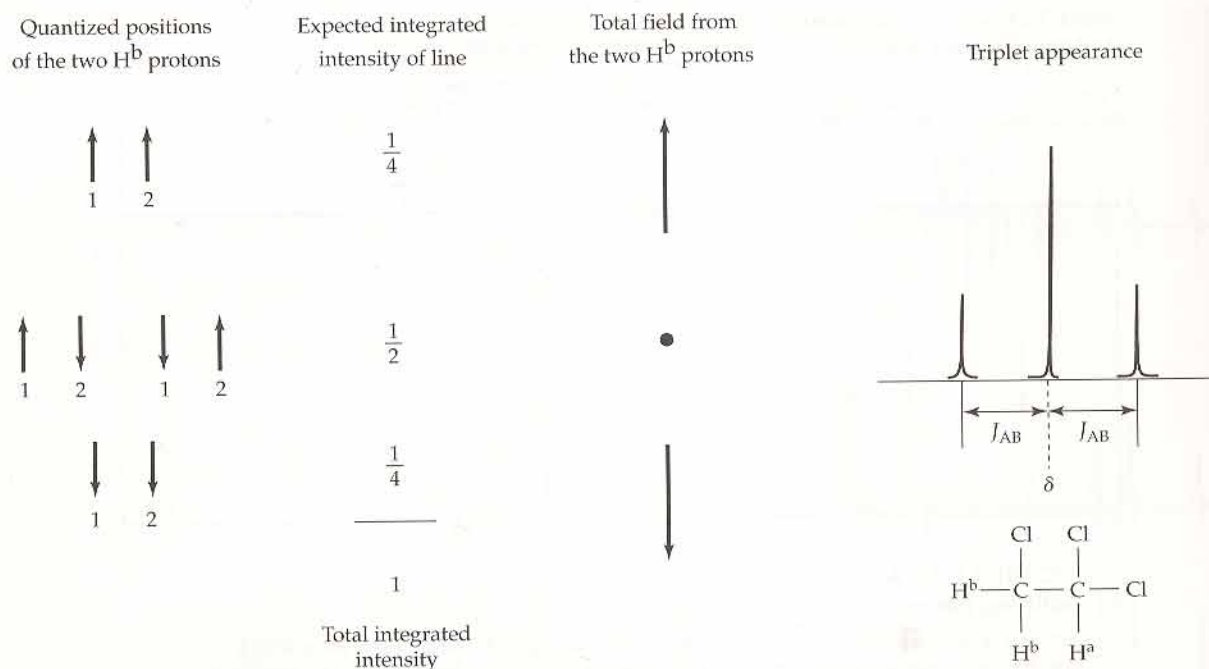
| Quantized positions of the two H^b protons | Expected integrated intensity of line | Total field from the two H^b protons | Triplet appearance |

FIGURE 11.12 ▲
The origin of a triplet splitting by two equivalent protons.

(left) The orientations with **(center)** probabilities produce the **(right)** total field to cause the **(far right)** observed triplet.

Number of equivalent protons causing splitting (n)	Number of peaks in multiplet ($n + 1$)	Multiplet name	Relative intensities of lines in multiplet
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

protons into ($n + 1$) lines. The number of lines in the splitting pattern called the **multiplicity**. The relative integrated area of each of the lines in multiplet can be calculated from probability theory or by drawing such grams as Figure 11.12. However, a far simpler way to obtain the relative ar is to use **Pascal's triangle**, illustrated in Figure 11.13. Each number in the angle is the sum of the two numbers above it (above left and above rig assuming zeros in the blanks. The areas of the individual lines in a multi are in the proportions given in the last column. Also, as before, the integr areas of all the lines of a multiplet together are proportional to the numbe protons absorbing there.

Let us review the rules to explain splitting patterns.

1. The number of lines in a multiplet equals $n + 1$, where n is the number of equivalent protons contribution to the splitting.
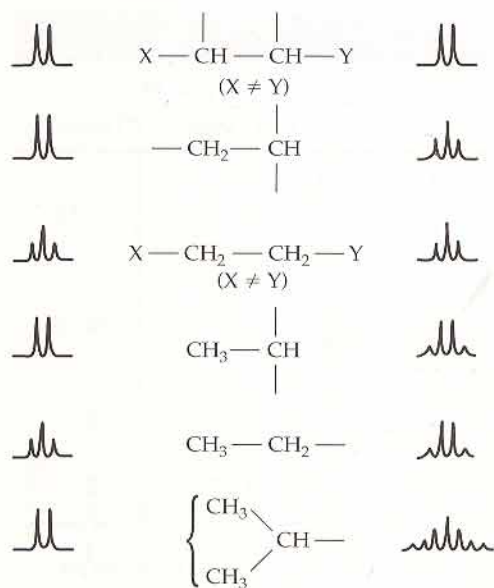
2. The expected integral area of each of the component lines in a
   multiplet is given by Pascal's triangle.
3. The total area of all the lines in a multiplet is proportional to the
   number of equivalent nuclei absorbing there.
4. The splittings $J_{AB} = J_{BA}$.
5. Seldom will there be splittings between protons that are more than
   three bonds removed.

   Figure 11.14 illustrates the splitting patterns for common organic groups.
Let us consider the information contained in such sets of multiplets.

a. The chemical environments of the nuclei are indicated by the value of δ.
b. The number of nuclei absorbing energy at a specific δ is proportional
   to the integrated area of each multiplet.
c. The number and intensities of lines in the multiplet tell you how
   many protons are adjacent.
d. The identity of the adjacent protons can be ascertained from their
   chemical shifts and splitting pattern.
e. The spin-spin coupling values can help confirm the above
   assignments. (See Table 11.3.)

^{1}H-NMR is, indeed, a powerful method for determining the structures of
molecules containing protons.

   Incidently, notice that the integral of the absorption peaks alone conveys
nothing about the analyte concentrations. The relative magnitudes of the
integrals indicate only the relative number of nuclei contributing to each
multiplet. A concentration standard is needed in order to obtain the analyte
concentrations, just as for any other spectrometric method.

Some Complications

You may have observed that a number of details that appear in the experi-
mental NMR spectra have been ignored. Some of these complications will be
pointed out next.

**Table 11.3** Spin-Spin Coupling Constants in Some Common Systems

System	$J_{H,H'}$, Hz Full range	Typical	System	$J_{H,H'}$, Hz Full range	Typical
H–C(H') (geminal)	0–25	12–15	H–C=C–H' (cis/trans)	0–12	7–10
CH–CH'	0–8	~7	C=C (H, H')	12–18	14–16
CH₃–CH₂'– Free rotation	6–8	~7	aromatic ring $J_{H,H'}$	6–10	8
(CH₃)₂CH'–	5–7	~6	$J_{H,H''}$	0–3	2
CH–C–CH'	0–1	0	$J_{H,H'''}$	0–1	1
C=CH–CH'	4–10	5–7	=C(H', H')	0–3.5	0–2
C=CH–CH'=C	6–13	10–13	–CH=C–CH'	0–3	0.5–2
CH–CH'O	0–3	2	CH–C=C–CH'	0–2	1
C=CH–CH'O	5–8	7	CH–C≡CH'	2–3	2–3

Source: Courtesy Varian Associates.

The first problem can be seen in Figure 11.15. Notice the small peaks on either side of the main peaks. These are not resonance peaks. They are **spinning side bands**, which originate as follows. Typically, the sample is contained in the bottom of a glass tube 5 mm in diameter and about 20 cm long. The tube containing the sample is mechanically spun around its long axis. Slight imperfections in the tube (out of round) or imperfections in the instrument settings cause side-band peaks to show up. The spinning side bands arise in pairs equidistant on each side of each absorption peak. It is easy to determine that they are not impurities from their symmetrical positions and from the fact that they change position with changes in the spinning rate as shown in the figure.

A second problem is that the chemical shift of a resonance may be pH dependent. An example is shown in Figure 11.16. This dependence appears for protons that are loosely held on a molecule. The chemical shift and shape of such peaks usually depend on the solvent and on pH. Such peaks tend to be broad.

Third, notice that the two lines in the doublets in the spectrum of Figure 11.8 on page 488 are not the same height (or area). The details of the

Slower spin rate   Faster spin rate
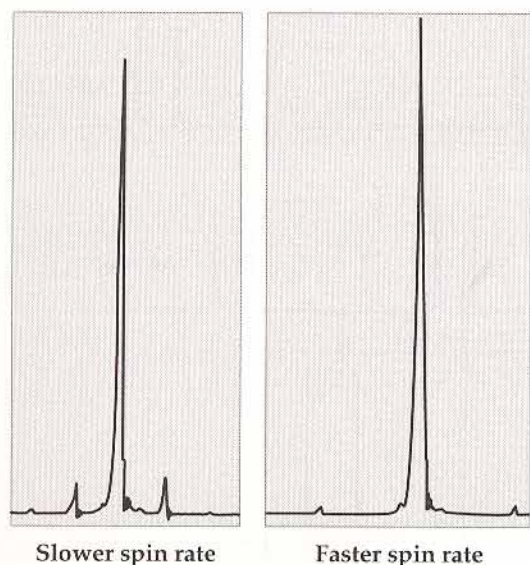
◀ FIGURE 11.15
Spinning side bands.

Spinning side bands occur when the
sample tube is not perfectly cylindri-
cal or the magnetic field is not well
adjusted. They appear symmetrically
placed on either side of each true
spectral line and appear further
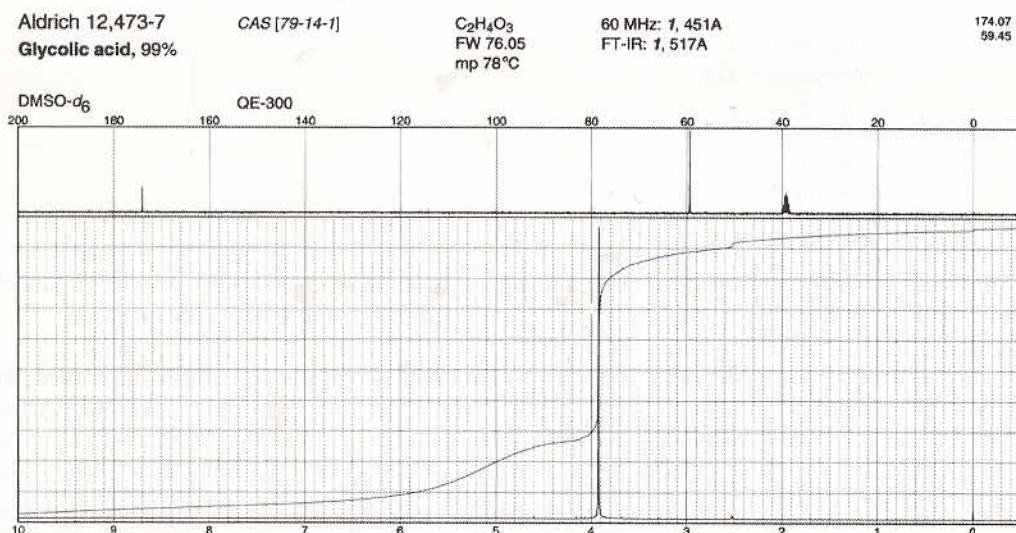away from the main peak as the
spinning rate is increased.

Aldrich 12,473-7	CAS [79-14-1]	$C_2H_4O_3$	60 MHz: *1*, 451A	174.07
Glycolic acid, 99%		FW 76.05	FT-IR: *1*, 517A	59.45
		mp 78°C		

DMSO-d_6 QE-300

FIGURE 11.16 ▲
Chemical exchange.

Illustration of one kind of effect that can be seen when a proton is not covalently bound to the
molecule. The hydroxy protons are **chemically exchanging** between a site on the molecule
and the solvent. The hydroxy resonance is broad, and it shows up only in the integral. Such
peaks shift position with pH. In addition, the exchanging hydroxy protons cause no hyperfine
splitting.

cause of this effect are beyond the level of this text. However, the effect can
be illustrated pictorially as in Figure 11.17. In addition to the obvious
changes in peak height, there is, less obviously, a change in the apparent
splitting values.

Fourth, spectra obtained with Fourier transform instruments have some
characteristics specific to them. For example, extra lines may show up in the
spectrum that depend on the instrument settings. It is usually pretty obvious
from their shape that these lines are not spectral lines. If there is any question,
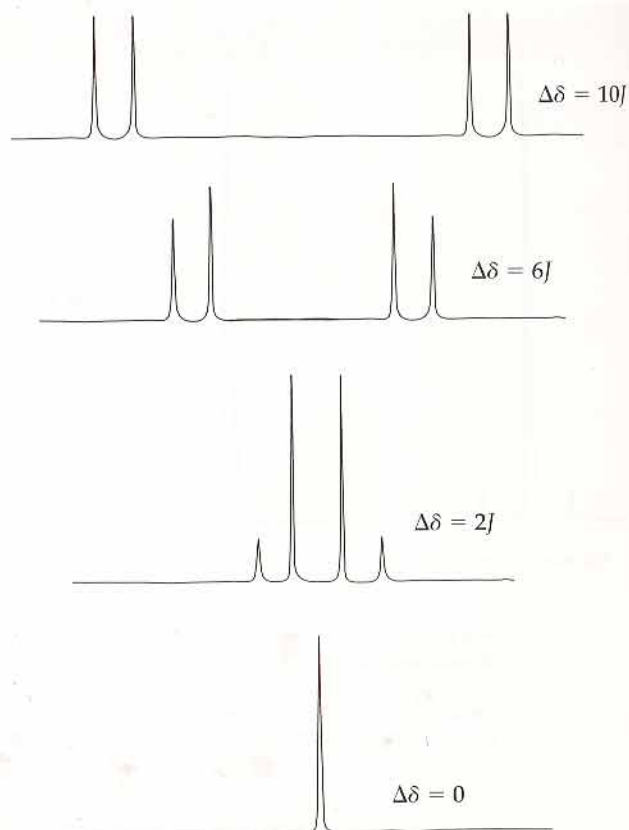their origin is still easy to recognize, since the extra lines will move around the

$\Delta\delta = 10J$

$\Delta\delta = 6J$

$\Delta\delta = 2J$

FIGURE 11.17 ▶
When $J_{AB} \approx \Delta\delta$.

Simulation of the effects on the peak intensities of two protons for which their mutual hyperfine splitting approximates the difference between their chemical shifts.

$\Delta\delta = 0$

spectrum when the appropriate instrument settings are changed, while the true spectral lines will remain in the same relative positions.

Splitting by More Than One Set of Equivalent Nuclei

If a nuclear resonance is split by more than one set of equivalent spins, the result is a multiplet of multiplets, as illustrated in Figure 11.18, the spectrum of 1-nitropropane. There is no guarantee that all the expected multiplet lines will show up in the complicated splitting patterns. In fact, it is usual to see fewer lines than expected. A typical case is the multiplet for the middle methylene group in 1-nitropropane. The lines are due to the protons labeled *b*. As shown in the figure, we expect a quartet of triplets: a total of 12 lines. But in the experimental spectrum only six lines appear. Fewer lines appear because the spin-spin splitting values J_{AB} and J_{CB} are nearly equal, and a number of peaks overlap as illustrated in the stick diagram. Thus, even for relatively simple molecules, it is necessary to be cautious and to be sure that the multiplet patterns and integrals make sense *as a whole* in order to assign structures. Notice that with four bonds separating them, the *a*- and *c*-protons do not show any spin-spin splitting.

NMR spectra easily become far more complicated in appearance. The complexity is most marked for molecules that are not highly symmetric or molecules in which more than two sets of equivalent protons contribute to spin-spin splittings. An example is shown in Figure 11.19, the spectrum of the relatively simple molecule 1,4-pentadiene. The same rules about splitting apply to this spectrum, but the various contributions to each multiplet are more difficult to pick out. This becomes especially hard when $J_{AB} \approx |\delta_A - \delta_B|$.
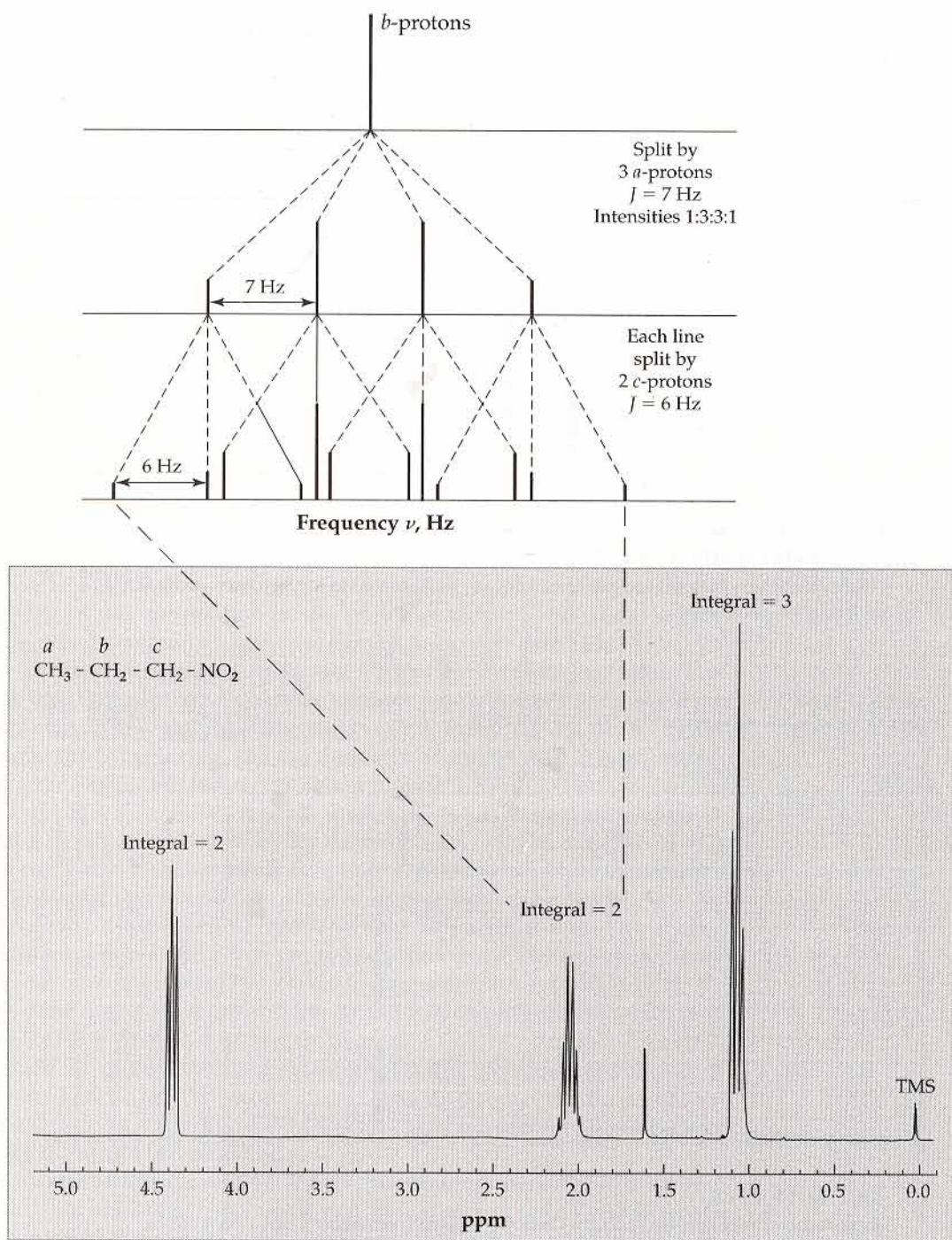
FIGURE 11.18 ▲
Multiplets of multiplets.
When a spectral line is split by more than one set of equivalent protons, the effects are
additive. This is illustrated by the 250-MHz ^{1}H spectrum of 1-nitropropane. The chemical
formula is shown. The stick figure at the top explains the splitting of the b-protons by the
a- and c-protons. The effect of the b-protons on the resonances of the a and c groups is
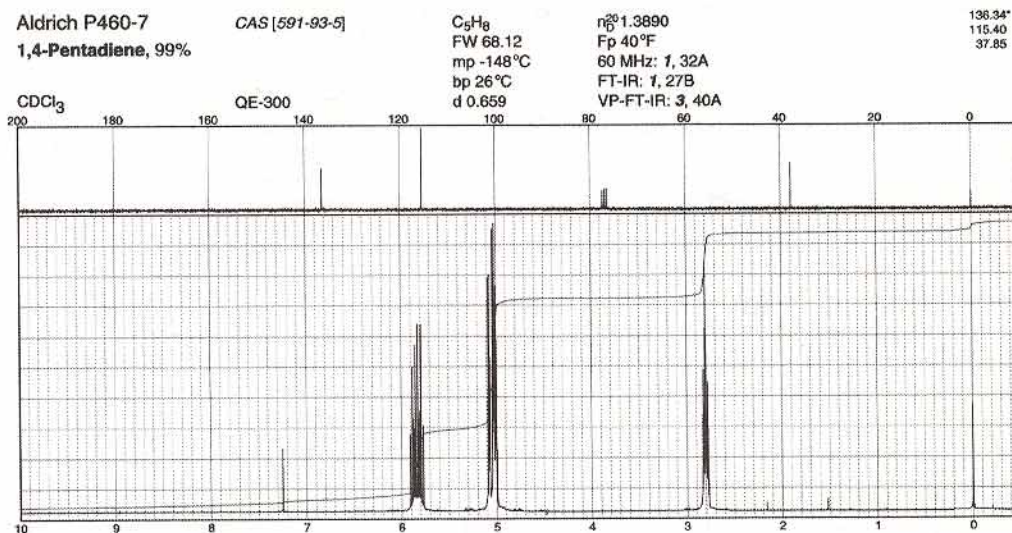straightforward: producing triplets for both.

Aldrich P460-7   CAS [591-93-5] C_5H_8 n_D^{20} 1.3890   136.34*

1,4-Pentadiene, 99%   FW 68.12   Fp 40°F   115.40

mp -148°C   60 MHz: *1*, 32A   37.85

bp 26°C   FT-IR: *1*, 27B

$CDCl_3$ QE-300   d 0.659   VP-FT-IR: *3*, 40A

FIGURE 11.19 ▲
Complicated splitting patterns.

The NMR splitting patterns can get quite intricate, as illustrated in the spectrum of the simple molecule 1,4-pentadiene.

Aldrich 17,781-4   CAS [353-83-3] $C_2H_2F_3I$ Fp NONE   60 MHz: *1*, 84B   128.84   -3.92

2-Iodo-1,1,1-trifluoroethane, 99%   FW 209.94   FT-IR: *1*, 90C   125.22   -4.41

bp 55°C   VP-FT-IR: *3*, 122C   121.60   -4.89

d 2.130   117.97   -5.37

$CDCl_3$ QE-300 n_D^{20} 1.4009
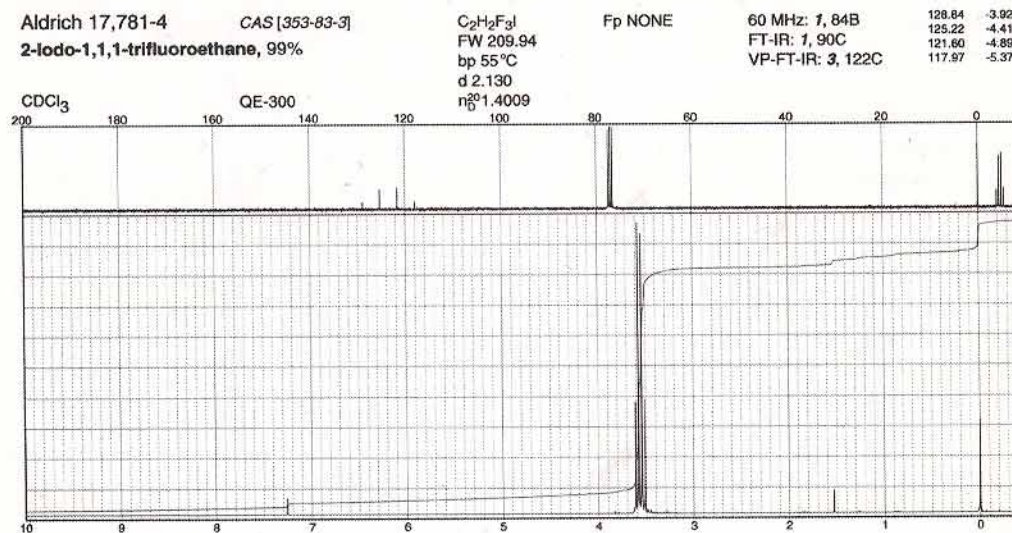
FIGURE 11.20 ▲
Resonance lines can be split by nuclei that are themselves not observable.

Here, three equivalent ^{19}F atoms ($I = \frac{1}{2}$) split the spectral line of the protons in 2-iodo-1,1,1-trifluoroethane.

In those situations, either the spectra can be compared with standard spectra for qualitative identification or computer programs can be used to fit the spectra. Further information on the latter method can be found in Suggestions for Further Reading.

These overlapping lines are fully amenable to assignment to the appropriate nuclei by running various types of **multidimensional NMR** exper-

iments. The dimensionality of the NMR refers to the number of different chemical-shift or frequency variables that are collected and subsequently used for the display. The most common NMR spectra, such as those shown so far in this chapter, are one-dimensional. They show a single chemical shift axis (the x-axis), with the magnitude of the NMR signal as the y-axis. Two-dimensional NMR experiments and their spectra are discussed briefly in Section 11.8.

Spin-Spin Splitting from Nonresonant Nuclei

After your first look at the spectrum shown in Figure 11.20, you might immediately start searching for some other multiplet which has an integral corresponding to three equivalent nuclei with nuclear spin $I = \frac{1}{2}$. However, you would not find it even if you searched past the normal spectral range. This is because the splitting is being caused by nuclei that are not themselves near their resonant frequency. In the case of 2-iodo-1,1,1-trifluoroethane, the ^{1}H splittings are from ^{19}F. Even though the ^{19}F nuclei are not at resonance, they still are effective as internal nuclear magnets and cause spin-spin splitting. Suggesting the expected ^{19}F-NMR spectrum of this compound is left to you as Exercise 11.7.

11.6 ^{13}C-NMR

Following from the information in the previous section, you might ask, If nuclei other than protons can split the spectral lines, why don't the ^{13}C nuclei do so in all these molecules? After all, ^{13}C has an $I = \frac{1}{2}$ nuclear spin. The answer is that the splitting *does* occur. However, only 1% of the carbons are ^{13}C. If a molecule has, say, only one carbon in it, then only 1% of the molecules exhibit the ^{13}C splitting, and the doublets are quite small, relative to the peak from the 99% of the molecules containing ^{12}C ($I = 0$).

The results of Case Study 11-1 depend on ^{13}C-NMR.

The low natural abundance of ^{13}C makes ^{13}C-NMR more difficult experimentally. As mentioned before, either the molecules must be enriched in ^{13}C, much more sample must be used, or a longer time must be spent collecting the data to obtain a good spectrum; that is, a spectrum with a sufficient S/N. Nevertheless, ^{13}C spectra can be measured routinely with appropriate instruments. The resonances also show patterns of chemical shifts that depend on the chemical type of carbon. The general regions are shown in Figure 11.21. Notice that the range of chemical shifts is quite large in comparison with that of ^{1}H: around 200 ppm as opposed to 10 ppm. It follows that the ^{13}C chemical shifts are far more sensitive to changes in local molecular environment than are the ^{1}H spectra. (As before, "local" usually denotes the immediately adjacent atoms.) As a result, ^{13}C-NMR is an extremely powerful method to investigate the structures of molecules.

One reason for this power is that all the protons connected to a carbon cause spin-spin splitting. This means that by looking at the ^{13}C-NMR spectrum, the number of protons (which are not at resonance) bonded to a carbon can be observed merely by noting the multiplet number of each carbon resonance. This effect can be seen in the upper spectrum in Figure 11.22: quartets for —CH$_3$, triplets for —CH$_2$—, and a singlet for C=O. However, the spectra quickly get so complicated that even for relatively simple molecules with a few similar groups, it becomes difficult to interpret.

The problem is remedied by using the technique of **proton noise decoupling**. The details of the method are beyond the level explored here. However, the effect is that the proton-carbon spin-spin coupling can be eliminated, and the multiplets of ^{13}C lines "collapse" into a single line for each
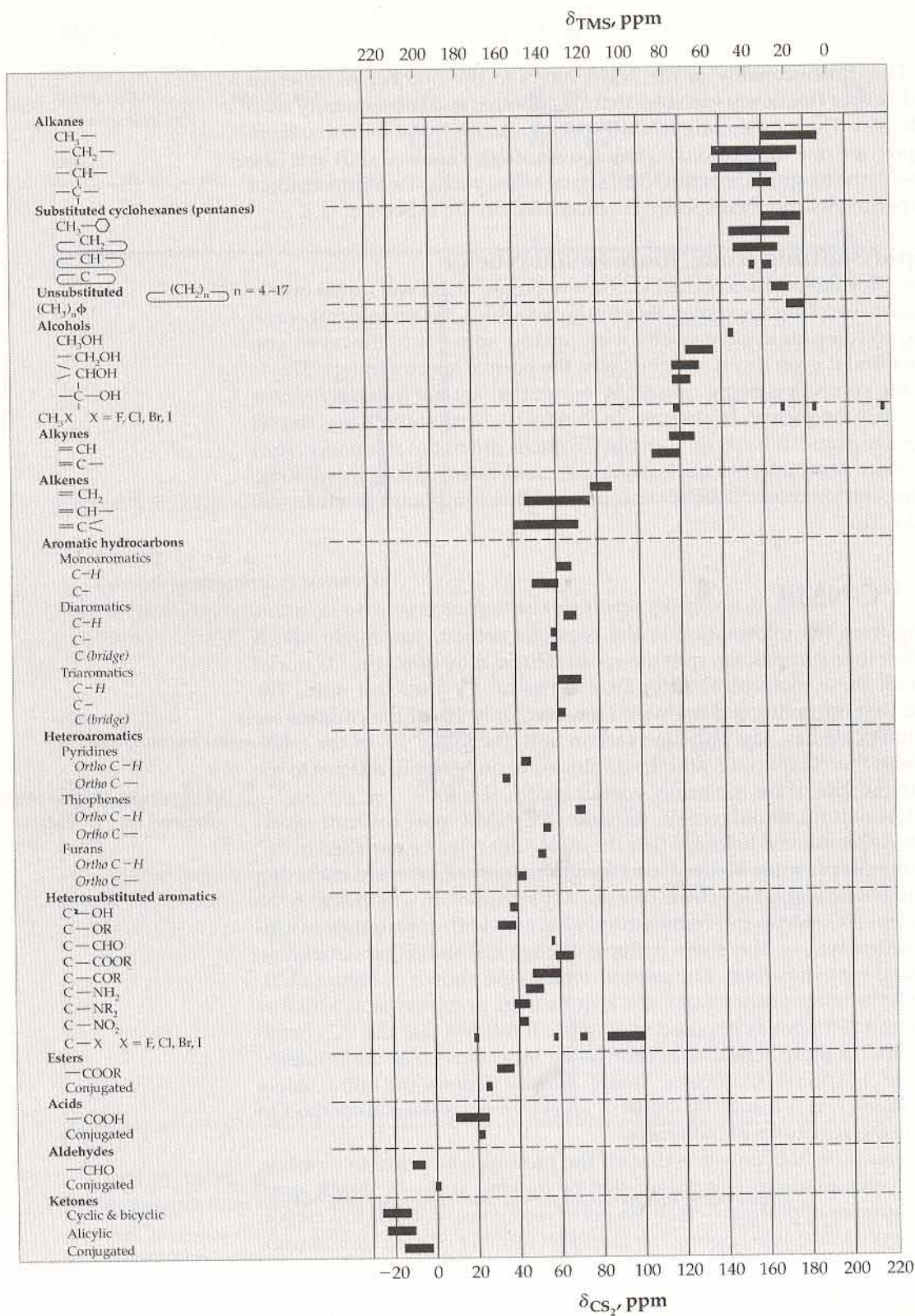
FIGURE 11.21 ▲

¹³C-NMR chemical shift correlation table.

Shown are typical ¹³C-NMR chemical shift ranges of organic materials relative to two standards: **(bottom)** the $^{13}CS_2$ standard and **(top)** the ^{13}C-TMS. [Ref: Jensen, R. K., Petrakis, L. 1972. *J. Magn. Reson.* 6, 105–106. Reproduced with permission, courtesy Academic Press.]
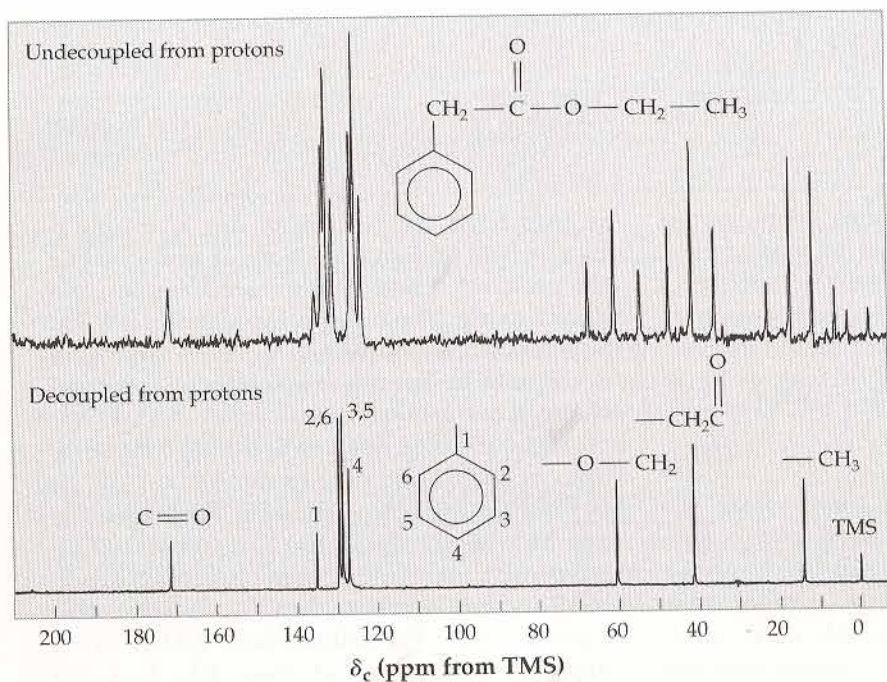
FIGURE 11.22 ▲
Nonresonant ¹H nuclei can split ¹³C-NMR peaks.

Only the protons bound directly to a carbon produce a splitting. This can aid in assignment of the spectrum to a specific molecule. The ^1H-^{13}C splitting can be removed by decoupling the protons. The lower spectrum shows the effects of decoupling. [Ref: Moore, J. A., Dalrymple, D. L. 1982. *Experimental Methods in Organic Chemistry*, 3rd ed. Philadelphia: W. B. Saunders & Co.]

carbon. A proton noise-decoupled spectrum is shown in the lower part of Figure 11.22. As noted at the end of Section 11.2, quantitation by integration of ^{13}C spectra tends to be inaccurate. You can see the effect here by comparing the heights of the peaks in Figure 11.22. They are far from proportional to the number of carbons. The same is true for the peak areas.

11.7   Quantitation

Unlike other spectrometries, concentrations in ¹H-NMR can range from pure liquids to quite dilute solutions without worrying about nonlinear concentration effects. Further, each nucleus contributes essentially equal signals to an NMR spectrum; every proton has the same molar absorptivity in ¹H-NMR spectrometry. Nevertheless, the instrument must be calibrated for the spectral conditions since the peaks' integrated areas report only relative numbers of protons. A standard is needed, and an internal standard is best.

EXAMPLE 11.3

The NMR spectrum for pure toluene was compared with the spectrum of an unknown mixture of toluene and *p*-xylene. The signals for the methyl protons overlap, as do those for the protons in the aromatic region. The following integrated peak areas were found.

CASE STUDY 11-1
Rub a Dub Dub, Clean Air for the Sub

For many years, amine solutions, ethanolamine among them, have been used to scrub carbon dioxide from enclosed atmospheres, such as in submerged submarines. The aqueous-solution reactions are

$$2\,RNH_2 + CO_2 \longrightarrow RNHCO_2^- + RNH_3^+$$

$$\xrightarrow{H_2O} RNH_3^+ + HCO_3^- + RNH_2$$
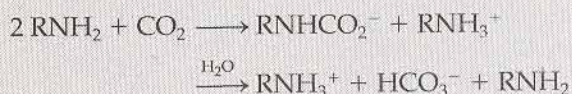
The CO_2 subsequently is driven from the solution by heating, and the gas is vented to allow another absorption cycle. In a submarine, a drawback to using ethanolamine ($HOCH_2CH_2NH_2$), is that volatile decomposition products can form at the heating elements immersed in the solution.

In a search for better compounds, the absorption and desorption chemistry and kinetics of the CO_2 were followed directly by ^{13}C-NMR. The solutions could be measured directly and without ^{13}C enrichment since the components' concentrations are on the order of molar, even when the solutions were diluted to dissolve any precipitates that formed. The concentrations were simply corrected for the dilution. Two ^{13}C spectra are shown below on the left.

In the spectra, the letters labeling the lines refer to the carbons with the same letters.

Signals from the carbamate are **a**, **b**, and **c**. Bicarbonate and carbonate are **d** and **e**, while the protonated and deprotonated amines produce **f** and **g**. The top spectrum shows a 2.5 M aqueous solution saturated with CO_2, and, after desorption by heating, the solution's spectrum changed to the lower one.

The time dependences and final concentrations of the CO_2 uptake from 4.7% CO_2 in air depend on the amine solution used. (See the graphs below on the right.) Fast uptake of CO_2 is essential, and so AMP is not as effective as 2-MAP and EA. However, the CO_2 capacity is also a crucial factor. The solution with 2-MAP eventually absorbs more CO_2 than EA; however (not shown here), in the 2-MAP solution the residual CO_2 at the end of a cycle is greater than EA, so the CO_2 capacity of the 2-MAP per cycle is less overall.

The author concluded that these amines and other, similar ones that were tested would not improve the submarine scrubbers substantially. Some might be suitable for industrial process scrubbers that have CO_2 loads higher than the ~1% of submarines.

[Ref: HOOK, R. J. 1997. "An Investigation of Some Sterically Hindered Amines as Potential Carbon Dioxide Scrubbing Compounds." *Ind. Eng. Chem. Res.* 36, 1779–1790.]
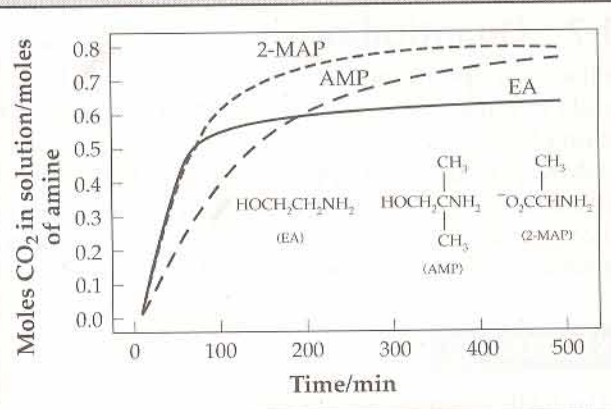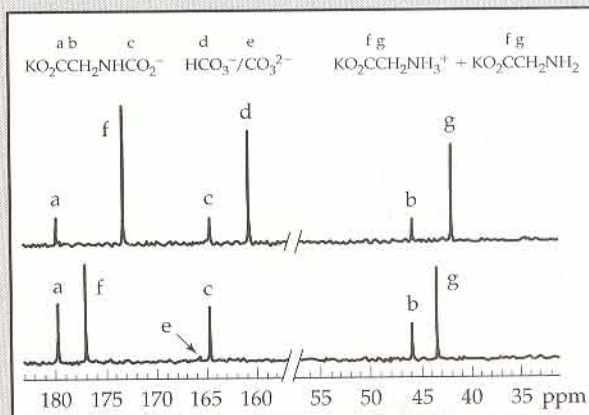
	Integrated areas	
	Methyl region	**Aromatic region**
Toluene	3.7_0	6.1_5
Mixture	4.8_0	5.8_0

(The second decimal is written as a subscript to indicate the measurement's precision of $\pm 5\%$.) What are the mole fractions of xylene and toluene in the mixture?

Solution

This example mirrors any mixture problem in spectrometry where the information in hand is the absorbance at two wavelengths for two components. Since each proton contributes equally, let us find a single response factor relating the integrated areas to concentration. The numbers of protons of each type are

	Number of protons	
	Methyl region	**Aromatic region**
Toluene	3	5
p-Xylene	6	4

Call the integrated signal S, and set up the two equations to be solved for the two frequency regions of the integral—just as for any other mixture.

$$S_{methyl} = 6\,R\,X_{xylene} + 3\,R\,X_{toluene}$$

$$S_{aromatic} = 4\,R\,X_{xylene} + 5\,R\,X_{toluene}$$

where R is the response factor that scales the signal per proton. The X-values are the mole fractions.

Next, R can be found from the toluene's methyl signal and the number of protons (3).

$$R = 1.23 = 3.70/3.$$

The same value of R is obtained from the aromatic protons.

$$1.23 = 6.15/5$$

The mole fractions of p-xylene and toluene are the only two unknowns, and the equations can be solved. In fact, the amount of information provided is more than is needed to solve the problem, since we also know that

$$X_{xylene} = 1 - X_{toluene}$$

for this two-component solution. Let us call this the *mole-fraction equation*. Any two of the three equations can be used to obtain an answer. For example, let us use the mole-fraction equation and one of the integrated-intensity equations to solve the problem. Substitute the mole-fraction relation into the equation for S_{methyl}.

$$S_{methyl} = 6 \cdot 1.23\,(X_{xylene}) + 3 \cdot 1.23\,(1 - X_{xylene}) = 4.80$$

Now, we can solve for X_{xylene} and substitute that value into the mole-fraction equation to find

$$X_{xylene} = 0.30; \quad X_{toluene} = 0.70$$

The unused integrated-signal equation can be used to check the answer to see whether some third component might be contributing to the spectrum. From the

mole-fraction equation and the other integrated-signal equation, you will find

$$X_{xylene} = 0.28; \quad X_{toluene} = 0.72$$

The agreement between the two calculations lies within the ±5% experimental error that might be expected for integrated peak areas in proton NMR for these concentrated solutions.

The quantitation of other nuclei, such as ^{13}C or ^{32}P, requires more attention to the experimental conditions, since the integral areas per nucleus can depend on the local chemical environment. As noted in the previous section, the areas of ^{13}C peaks tend to vary depending on the number of protons attached.

11.8 NMR of Solids

To obtain optimal NMR spectra, the analyte molecules should be tumbling rapidly relative to the magnet. Part of the reason for this requirement is that the motions average the interactions with neighboring molecules. In solids, however, the tumbling does not occur so the effects of the molecular environment do not average within the sample. As a result, the spectra tend to be broad and nearly featureless, with little chemical information. (See Figure 11.23.)

However, spectra of moderate resolution can be obtained through two fundamental tricks. The first is to spin the sample at a specific angle—54° 44′, which is called the **magic angle**—at a rate in excess of 200 KHz. This technique is called **magic-angle spinning** (MAS). The second is to average the magnetic interactions from the material surrounding the analyte molecules

FIGURE 11.23 ▶

Solid-state ^{1}H-NMR spectra of calcium acetate hemihydrate, Ca(CH$_3$COO)$_2$ · $\frac{1}{2}$H$_2$O.

(a) The solid-state spectrum run in the same manner as a solution spectrum would be run. (b) The spectrum obtained when the proton spin-spin interaction is decoupled. (c) The spectrum obtained when, in addition to the decoupling, the sample is spun around an axis set at the magic angle to the magnetic field. The magic angle is 54°44′, and the rate of magic angle rotation is greater than 2 kHz. [Ref: Harris, R. K. 1983. *Nuclear Magnetic Resonance Spectroscopy.* London, Pitman. With permission.]
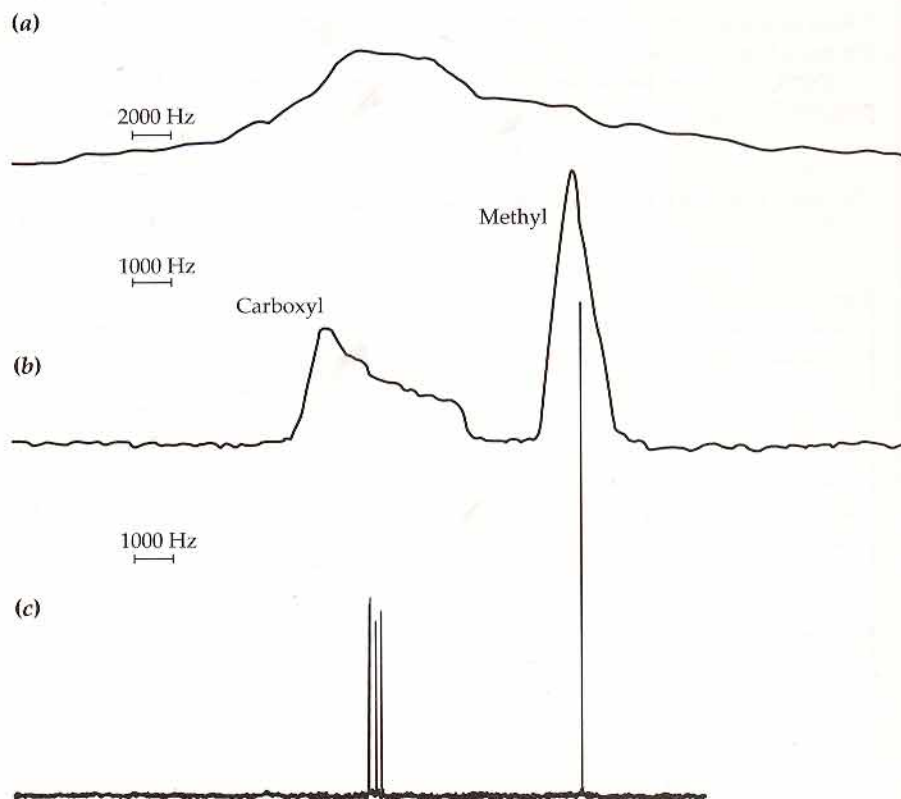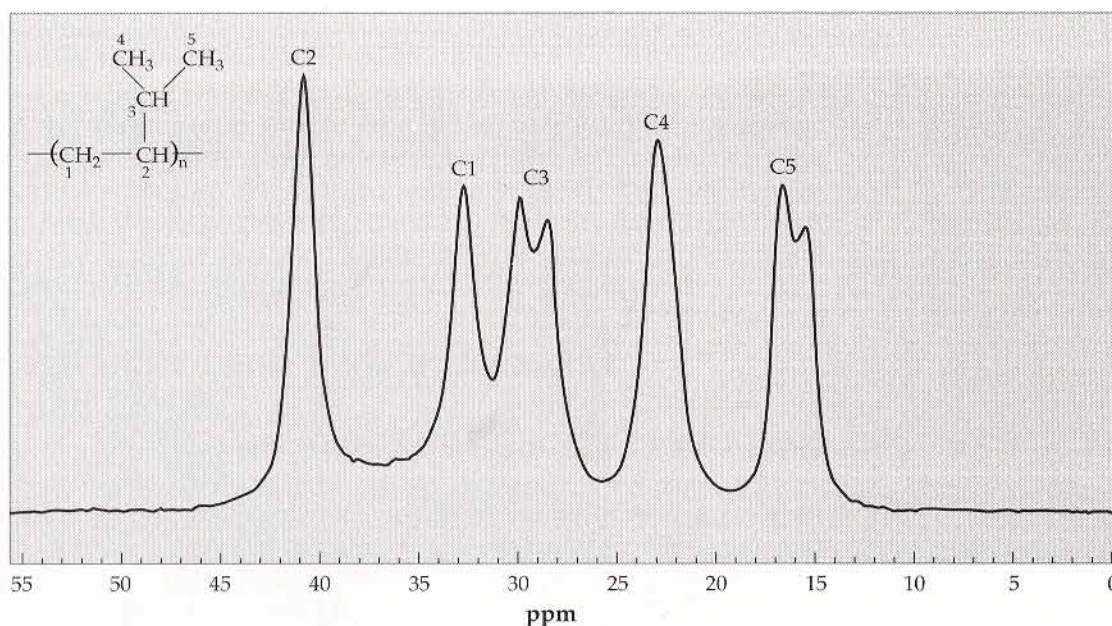
FIGURE 11.24 ▲
A ^{13}C CP/MAS NMR spectrum of a solid polymer, poly(3-methyl-1-butene).
The peaks are labeled with the numbers of the carbons from which they arise. The solid-state structure can be deduced from two factors. First, the C4 and C5 carbons are chemically different because of the solid structure's influence. Also, the C3 and C5 peaks are split as a result of molecular packing. This spectrum is the sum of 3350 transients, ^{13}C at 75.468 MHz, MAS frequency 3 kHz, ^{1}H decoupling field 55.5 kHz. [Ref: Reprinted with permission from Borriello, A., Busico, V., De Rosa, C., Schulze, D. 1995. *Macromolecules* 28, 5679–5680. Fig. 3. Copyright 1995 American Chemical Society.]

by using strong radiofrequency fields at specific frequencies related to the resonance frequencies of the H nuclei present in the sample. This is another instance of proton decoupling, which you also saw in the ^{13}C spectra of Figure 11.22. For the solid, the method to remove the spin-spin interaction is called **cross-polarization** (CP). A narrowed spectrum from a solid sample is shown in Figure 11.23. A relatively large sample is needed to run a narrowed solid-state NMR spectrum: around 100 mg. CP/MAS solid-state spectra also have been obtained from ^{13}C. An example is shown in Figure 11.24, where significant structural information could be deduced from the differences between the spectrum in the solid and that in solution for a similar polymer.

11.9   Multidimensional NMR

The simple, one-dimensional NMR spectra shown throughout the chapter display the magnitude of resonance (vertical) as it changes with a scale related to frequency (horizontal). The horizontal measure, ppm, is parts per million of the radiofrequency. A 2-D NMR spectrum, such as the contour plot shown in Figure 11.25, shows the resonance changes related to two frequency scales, here called F1 and F2. In this case, both the horizontal scale and the vertical scale are identical and show the 7.5–9.0 ppm frequency range for both the F1 and F2 frequencies. This plot shows a small part of a typical, full 2-D ^{1}H spectrum of 0–10 ppm on both axes.

One characteristic of 2-D spectra is that the usual 1-D spectrum runs along the diagonal from lower left to upper right. The off-diagonal peaks
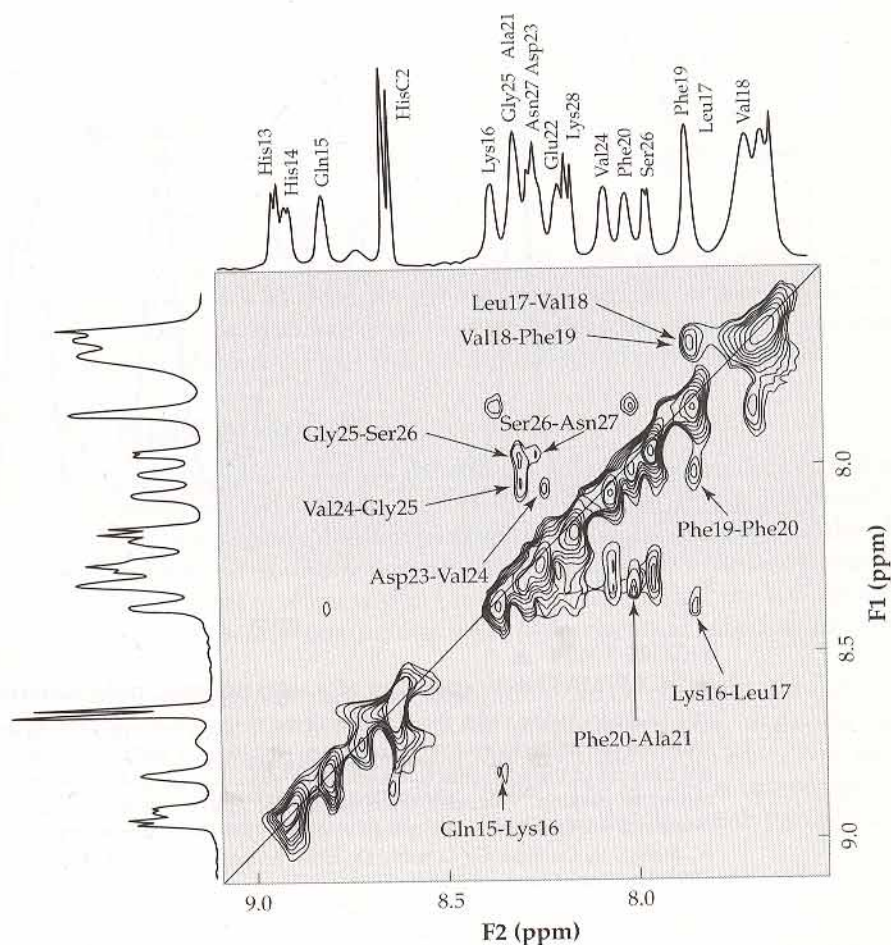
FIGURE 11.25 ▲
An example of a 2-D NMR spectrum.

This is the type called a COSY, which is short for correlated spectroscopy. The spectrum is a ^{1}H-NMR of a polypeptide 17 amino acids long in a solvent that is 60% d_2-trifluoroethanol/ 40% H_2O. The region shown is that where the amide protons show up. The 1-D spectrum in the region is shown on two sides of the 2-D spectrum. The 2-D spectrum consists of a large number of peaks along the diagonal, which, if plotted along the diagonal line, would also show the 1-D spectrum. The off-diagonal peaks show up when a spin-spin interaction occurs between two protons. For example, the furthest lower-left off-diagonal peak, labeled Gln 15-Lys16, results from the amide proton on glycine 15 interacting with the amide proton on Lysine 16. This cross peak lies at the intersection of the positions of the 1-D peaks for glycine 15 on the left 1-D spectrum and lysine 16 on the top 1-D spectrum. A corresponding peak lies across the diagonal. Its position lies at the intersection of the peak for lysine 16 on the left 1-D spectrum and glycine 15 at the top spectrum. The COSY spectrum is expected to show a mirror image across the diagonal. [Ref: Jayawickrama, D., Zink, S., Vander Velde, D., Effiong, R. I., Larive, C. K. 1995. "Conformational analysis of the beta-amyloid peptide fragment, beta(12–28)." *Journal of Biomolecular Structure & Dynamics.* 13, 229–244.]

are called **cross peaks**. Where cross peaks occur, they show which of the peaks of the 1-D spectrum are magnetically connected: since their nuclei interact magnetically, we deduce they are spatially close. A cross peak appears at the intersection of a vertical projection from one 1-D peak on the diagonal and a horizontal projection from another diagonal peak. They are

labeled with the assignments of the two peaks that are connected. The spectrum is symmetrical reflected across the diagonal.

Two-dimensional NMR allows dissection of complex spectra and assignments of the resonances to specific chemical groups. Additionally, groups close in space that influence each other can be linked, and, by doing so, the 2-D spectrum can lead to detailed structural information. Both small molecules and large molecules, such as proteins up to molecular weight about 10,000, can be analyzed this way. Relatively large amounts of sample are needed, and the results are qualitative in a chemical analysis sense.

11A   A Deeper Look: NMR Instruments and Samples

The NMR signal from a typical sample has approximately the same magnitude as the random noise due to the thermal motions of the molecules in the sample itself in the instrument. As a result, obtaining even routine NMR spectra requires sophisticated electronics. The methods of construction and optimization of NMR instrument response is a topic of advanced study. Thus, only the more general points of operation are described here.

NMR instruments use pulses of radiofrequency radiation to excite the protons in the sample. The data that is collected appears as shown in Figure 11A.1a. The decay in the amplitude is approximately exponential with time, and the name **free induction decay** (FID) is given to such data. Nearly all of the NMR spectra in this chapter are retrieved from FIDs. The mathematical relationship between the FIDs' time-dependent signal and the spectra displayed with frequency (as ppm) is a Fourier transform. As a result, NMR instruments that excite with radiofrequency pulses, collect free induction decays, and transform this data into a spectrum are called **Fourier-transform NMR** spectrometers, abbreviated FT-NMR.

The major features of an NMR instrument are illustrated in Figure 11A.2. The instrument consists of a radiofrequency source that is extremely stable in both frequency and power, a highly sensitive radiofrequency receiver, a magnet that produces a steady, strong field, and, of course, a method of recording the data. The data is collected as amplitudes of the signal at equal intervals for times up to a few seconds. The length of time the data is collected depends on the type of nucleus (for example, ^{1}H, ^{13}C), the environment (solids, solutions), the type of molecule (small, protein), and the concentration of the analyte. The number of data points taken over that time must be a power of two, and 16,384 is a common number. For most samples, a number of such free induction decays are collected and summed. The signals add with each FID, but the noise increases only as the $\sqrt{N}$, where N is the number of FIDs summed. Therefore, the signal-to-noise ratio increases as $\sqrt{N}$. The sum of FIDs is then converted into the familiar spectrum. In addition, the pulses are produced with regularly changing phases, and so the summing also compensates for imperfections in the amplifiers and other components of the radiofrequency system.

As noted earlier, the linewidth of an NMR-absorption peak can be as narrow as 0.1 Hz. Given that the resonance frequencies are on the order of 10^8 Hz (100 MHz), and since the field and frequency are directly related (Equation 11-1), the width of the line must reflect the precision of the magnetic field in which the sample sits. As a result, the magnetic field must be homogeneous to a few parts in 10^9. Making the field this homogeneous requires careful construction. Regardless of the type of magnet used (permanent magnets,
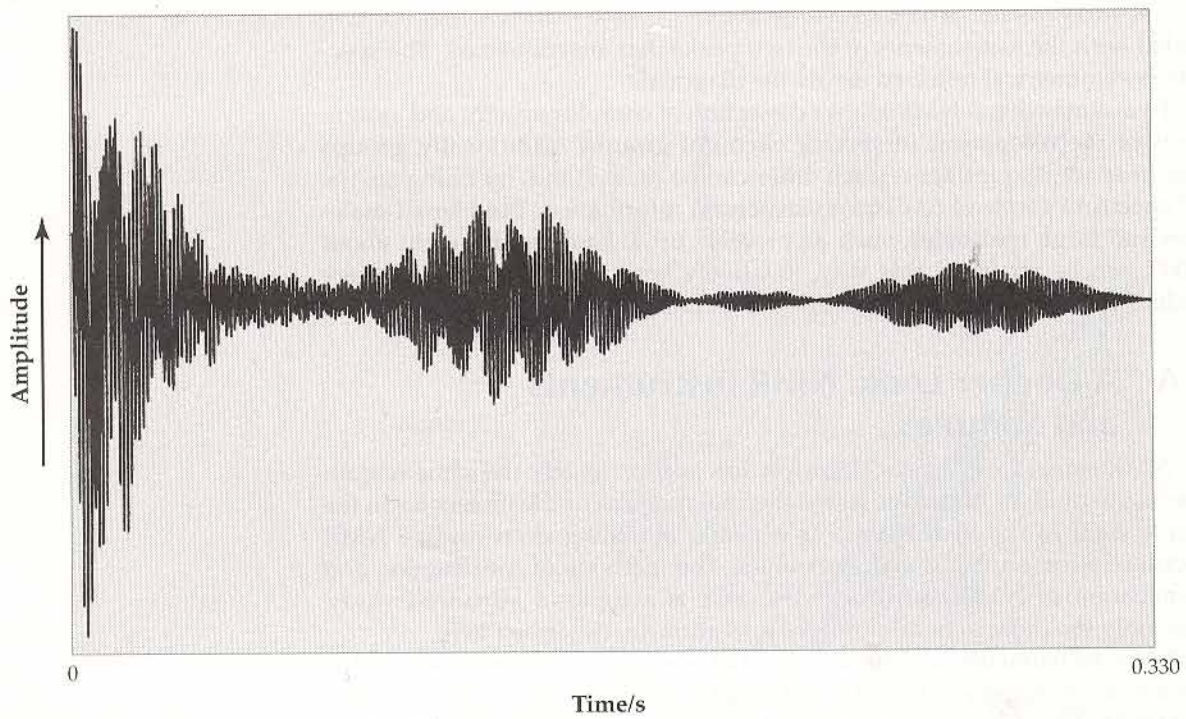
The technical details of this spectrum are explained in Chapter 17.

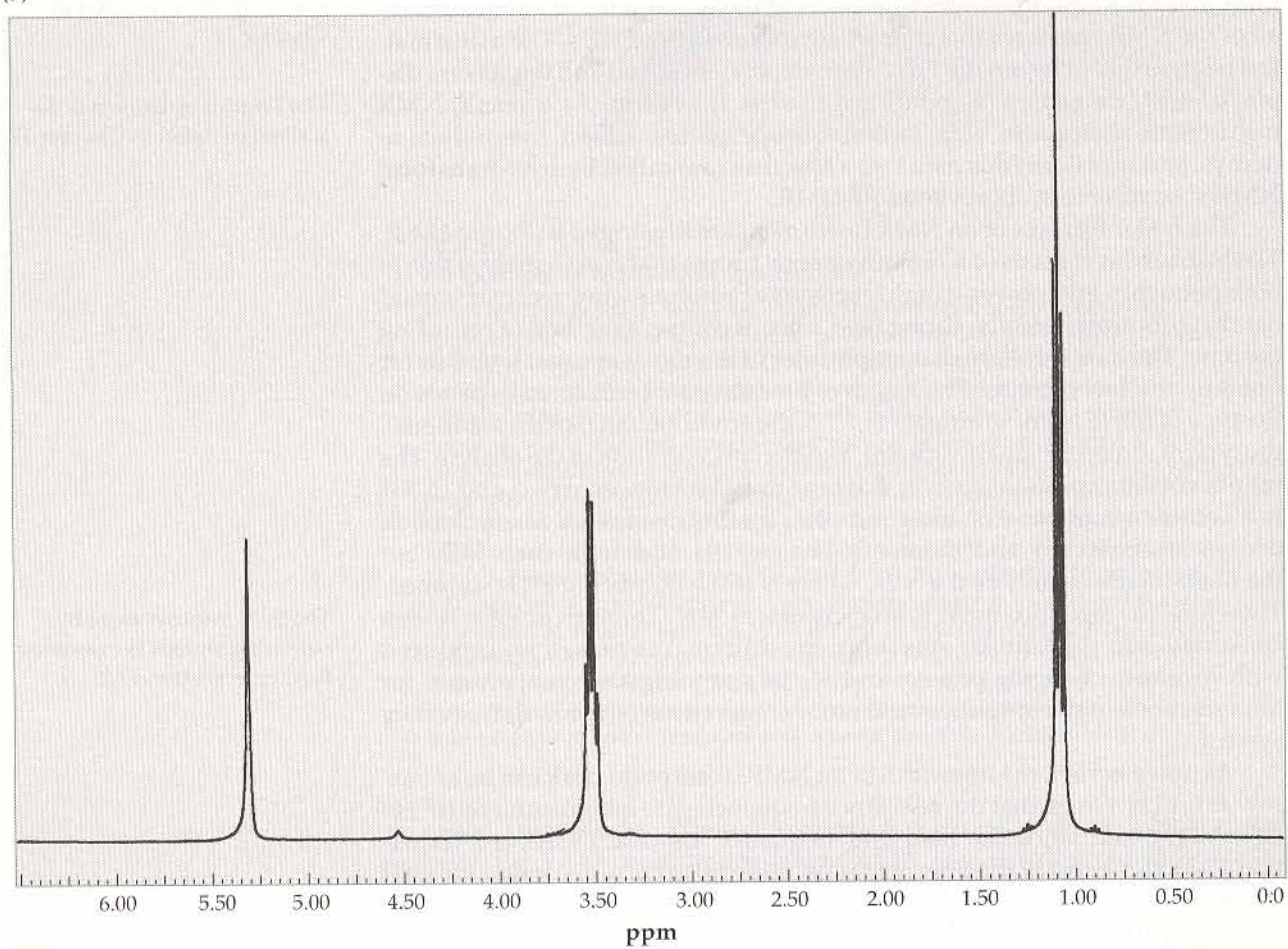The Fourier transform is described in detail in Chapter 17.

The S/N improvement by summing signals is examined further in Section 17.2.

(a)

Amplitude

0   0.330

Time/s

(b)

6.00   5.50   5.00   4.50   4.00   3.50   3.00   2.50   2.00   1.50   1.00   0.50   0:0

ppm

508

◀ FIGURE 11A.1
The proton free induction decay and spectrum of ethanol at 360 MHz.

(a) This FID was obtained from a sample of absolute ethanol in a 5 mm tube in a 360 MHz instrument (8.5 T field). The pulse width was 3 μs. The number of transients (that is, FIDs collected) = 8. The acquisition time for each FID was 3.4079 seconds, collecting 16K data points (that is, $16,384 = 2^{14}$). The FID plot displays the averaged data collected in the time frame 0–0.330 seconds. The fast oscillation of the signal is due to the difference between the radiofrequency of the instrument and the frequency of the resonance. The slower waves in the amplitude are due to the resonances of the different protons constructively and destructively interfering. (b) The resulting spectrum. The chemical shifts are referenced to TMS at 0.0 ppm. The small peak at 4.6 ppm is from residual water. The small peaks at either side of the large ones are the ^{13}C satellite peaks due to hyperfine coupling from the molecules with the 1% $I = \frac{1}{2}$ carbon randomly distributed. To obtain this spectrum, the FID of 16K points in time was zero filled to 32K data points. An exponential windowing followed and then the data was Fourier transformed to produce the spectrum. The windowing broadened the linewidth by 0.5 Hz. Windowing and zero filling are explained in Chapter 17. (Data courtesy of Dr. N. Reo)
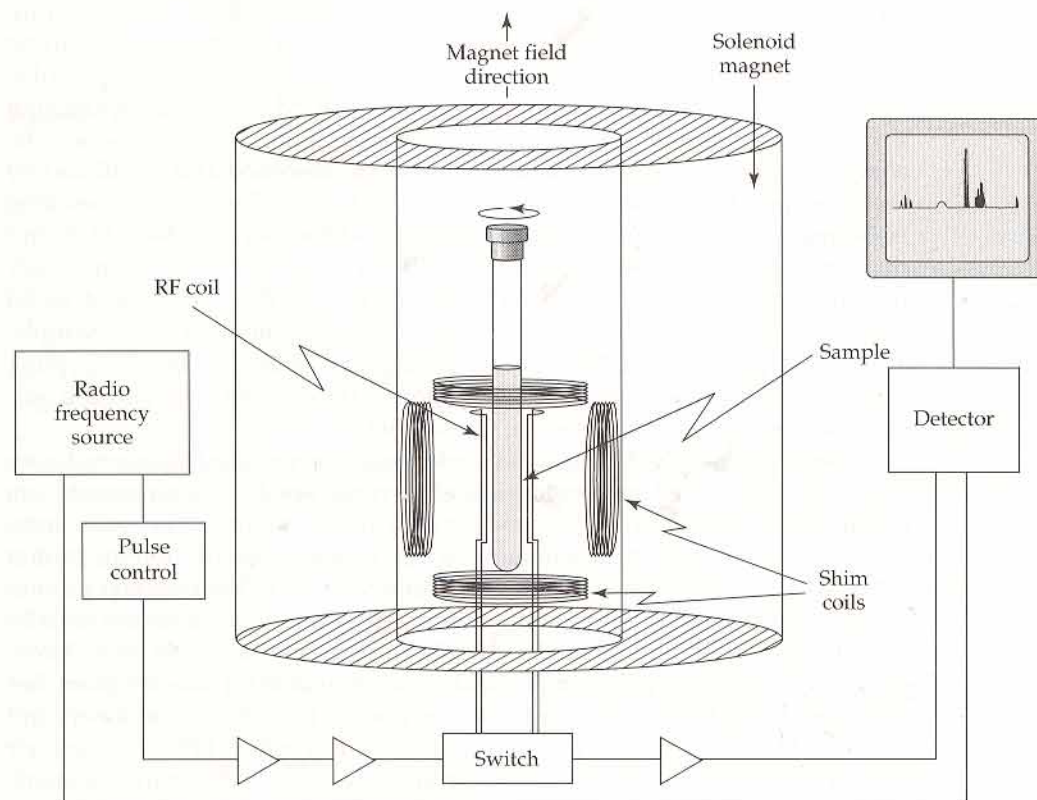
FIGURE 11A.2 ▲
Figure of a general FT-NMR machine.

The radiofrequency excitation is transmitted to the sample through a loop. The loop is shaped so that the magnetic field generated by the radiofrequency current is homogeneous and perpendicular to the DC magnetic field, which here lies along the axis of a large solenoid. The radiofrequency emission is then picked up by the same coil when it is connected to the detection circuitry after a delay of a few microseconds after the RF pulse ends. The magnetic field from the solenoid is made homogeneous through the use of shim coils around the sample area. The sample tube is spun around its axis in order to average out the residual inhomogeneities of the magnetic field that could not be removed by the shims.

electromagnets, or superconducting magnets, depending on the quality of the instrument), the field must be made more homogeneous by using small coils called shims or shimming coils. The shims lie between the main magnet and the sample, and they produce small magnetic field gradients of different shapes to compensate for slight imperfections in the main field. Further, to

keep the fields constant, the magnets are thermostatted. Then, as a final step, the sample is spun at a rate of a few cycles s^{-1} along the long axis of the sample tube. This spinning produces a final averaging of the magnetic field.

Precise control (parts in 10^8) is required in the radiofrequency source. The source frequency is controlled by the vibrations of a quartz crystal that is carefully thermostatted. Recall that we want an accurate measurement of the chemical shift; this is a ratio of the frequency of the irradiation relative to the field. The two cannot be allowed to drift relative to each other. This drift is reduced to insignificance by adding a circuit—the **field-frequency lock**—to the instrument, which holds the frequency relative to an internal NMR standard, usually deuterium ^2H. The lock is accomplished by having, in effect, a second, simple NMR spectrometer that measures the resonance of the deuterium in the field and keeps the instrument correctly tuned. The deuterium to "lock on" is usually in deuterated solvent or in a deuterium-containing species in a smaller, concentric tube lying along the axis of the sample tube.

The radiofrequency radiant energy is transmitted to the sample through a coil surrounding the sample tube, as shown in Figure 11A.2. In essence, the energy of the pulse of radiofrequency energy is absorbed and reradiated by the sample. This reradiation is detected and amplified by sophisticated noise-rejecting amplifiers, stored as a summed (of many pulses) FID, and mathematically brought into the spectral form desired. In contemporary instruments, a single coil is used both to irradiate the sample and as an antenna to pick up the much weaker free induction generated by the sample. The coil is perpendicular to the fixed magnetic field. For the decoupling experiments, such as mentioned for ^{13}C proton decoupling, a second coil outside the first is present to irradiate the sample.

The samples in NMR consists of relatively concentrated solutions (compared to most other spectrometries). The most sensitive instruments can obtain a spectrum in a few seconds with a mg of sample. The more commonly available instruments require 10 mg of sample in solution for proton NMR. The solutions are loaded to a depth of 2–3 cm in the bottom of a precisely cylindrical tube 5 mm in diameter. Larger diameter tubes are usually used for the less sensitive nuclei. Standard tubes are 20–25 cm long. Many different but less common sample holders are available for smaller quantities of material. Solids for solid-state NMR are packed into plastic containers and covered. The rotors are driven by compressed air to spin at kHz rates on their axis, which is aligned along the magic angle relative to the static magnetic field.

Suggestions for Further Reading

SILVERSTEIN, R. M., BASSLER, G. C., MORRILL, T. C. 1991. *Spectrometric Identification of Organic Compounds*. 5th ed. New York: John Wiley & Sons.

A practical book at an introductory but rigorous level with mass, infrared, and UV spectrometry, and proton and carbon NMR. Now an excellent chapter on elementary 2-D NMR is included. Not a book on instrumentation. Highly recommended for organic compounds. A good next place to read.

BRUCH, M. D. 1996. *NMR Spectroscopy Techniques*. New York: Marcel Dekker.

The approach of this book is to give descriptions of what molecular information various types of NMR experiments (solids, 2-D in liquids, etc.) can provide and how the spectra show it. A unique and highly useful approach at a reasonable level of sophistication with excellent figures.

Lambert, J. B., Shurvell, H. F., Lightner, D. A., Cooks, R. G. 1998. *Organic Structural Spectroscopy*. Upper Saddle River, NJ: Prentice Hall.

Covers much the same area experimentally as this chapter but with much more extensive organic chemical interpretation and application. The 2-D NMR has a pretty steep learning curve.

Sanders, J. K. M. 1993. *Modern NMR Spectroscopy, A Guide for Chemists*. 2nd ed. Oxford: Oxford University Press.

About as clear an explanation of the multidimensional NMR experiments and the information that can be acquired is found here. However, the structure is progressive, so you cannot simply look up, say, COSY or NOESY and understand their details.

Breitmaier, E., Bauer, G. 1984. *¹³C NMR Spectroscopy. A Working Manual with Exercises*. Chur, Switzerland: Harwood.

This book consists of 100 pages of NMR theory and then 75 example problems of ¹³C-NMR spectra. Worked-out solutions to the problems follow. A unique and useful introduction.

Kolodziejski, W., Klinowski, J. 1996. *New NMR Techniques for the Study of Catalysis. NMR Techniques in Catalysis*. A. T. Bell and A. Pines, eds. New York: Marcel Dekker. 361–411.

A concise review of contemporary methods in NMR of samples that are solids. The descriptions are quite clear given the underlying complexity of the nuclear-spin physics.

Liu, C. C. 1996. "Quantitative Analysis of Solids by High-Resolution 1H NMR." *Anal. Chem.* 68:1401–1407.

A method is described where silicone rubber is used as an intensity standard and special pulse sequences and sample spinning allow quantitative ¹H-NMR to be obtained on some solid samples.

Exercises

11.1 A 250-MHz ¹H-NMR spectrum was run, and six peaks with equal integral areas were found. The peaks were at 0, 346, 408, 467, 950, and 1787 Hz downfield relative to TMS. In the sample are tetramethylsilane, acetone, benzene, cyclohexane, *t*-butanol, and dioxane. The hydroxy proton of the alcohol could not be seen.
(a) Assign the six peaks to the six compounds.
(b) If the spectrum were a 500-MHz ¹H-NMR spectrum, at what frequencies relative to TMS would the six resonances be?
(c) If the TMS is assigned a concentration of 10.0, what are the concentrations of the other five components of the mixture?

11.2 A spectrum of toluene (that is, methylbenzene) was run. The spectrum consists of two singlets. The singlet at 7.2 δ has an integral of 72 units. The singlet at 2.35 δ has an integral of 36 graph units. Is the toluene pure?

11.3 Assign spectra I through V in Figure 11.3.1. The possible compounds are
A. 1-chloropropane, $CH_3CH_2CH_2Cl$
B. 1,2-dichloropropane, $CH_3CH(Cl)CH_2Cl$
C. 1,3-dichloropropane, $Cl(CH_2)_3Cl$
D. isopropanol, $(CH_3)_2CHOH$
E. allyl chloride, $H_2C{=}CHCH_2Cl$
F. 1-propanol, $CH_3CH_2CH_2OH$

11.4 Assign spectra I through VI in Figure 11.4.1. The possible compounds are
A. ethylbenzene, $C_6H_5C_2H_5$
B. *p*-cresol, $CH_3C_6H_4OH$
C. *p*-xylene, $CH_3C_6C_4CH_3$
D. benzene, C_6H_6
E. toluene, $C_6H_5CH_3$
F. anisole, $C_6H_5{-}OCH_3$
G. *p*-dimethoxybenzene, $CH_3O{-}C_6H_4{-}OCH_3$

11.5 A new sulfonate drug was being developed that contained the following group in its structure
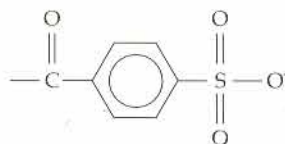
Running a conventional mass spectrum was not possible because the compound degrades when heated, so it was decided to find the molecular mass by NMR. To do this, 10.0 mg of the compound was weighed into an NMR tube. To the tube was added 5.0 mg of anhydrous sodium acetate (f.w. 82.04). Both compounds were dissolved in D_2O. An NMR spectrum was run. The sodium acetate peak at 1.90 δ had an integral area of 82.0 units (average of 3 runs). A singlet at 8.0 δ (no other peaks in the region) had an integral of 30.5 units (average of 3 runs). What is the formula weight of the analyte?

11.6 Two different energy levels exist for a proton pointing along and away from the external field, and the transition between them occurs at 100 MHz at 298 K.
(a) What fraction of the nuclei are in the upper and lower levels? (Hint: Think Boltzmann.)
(b) Do the same calculation for 200 MHz and 500 MHz.
(c) You read that the intensities of the component lines of a multiplet are due to the probabilities that the protons will be pointing away from or along the magnetic field direction. Will there be any observable differences in the relative heights of the components of multiplets in NMR spectra at these different frequencies? Assume that 2% is observable experimentally.

11.7 Figure 11.20 shows the proton NMR spectrum of 2-iodo-1,1,1-trifluoroethane. If you were to obtain the ¹⁹F-NMR of the compound, what kind of multiplet splitting would the fluorines exhibit?

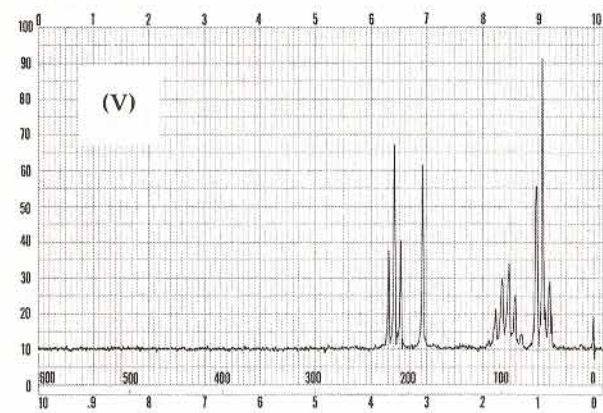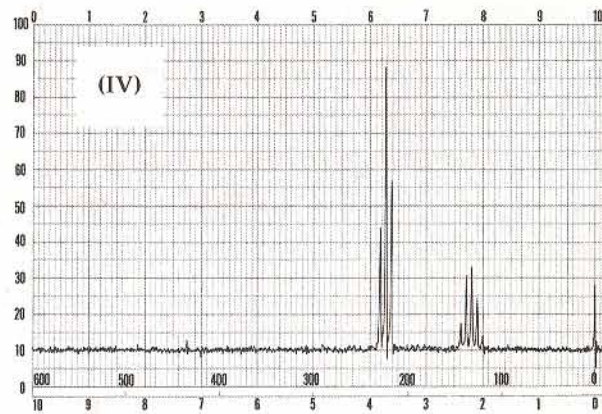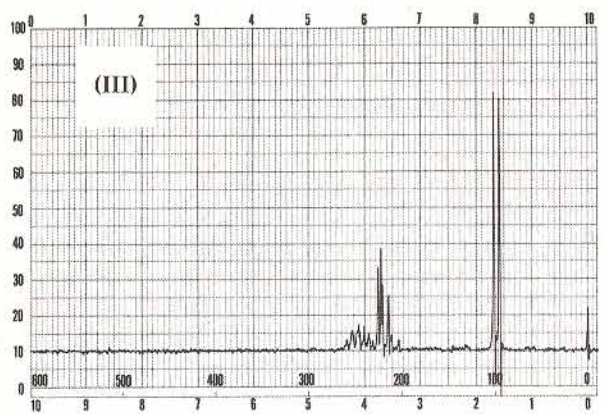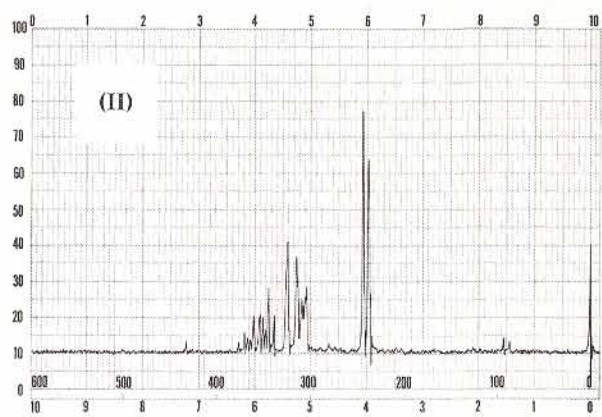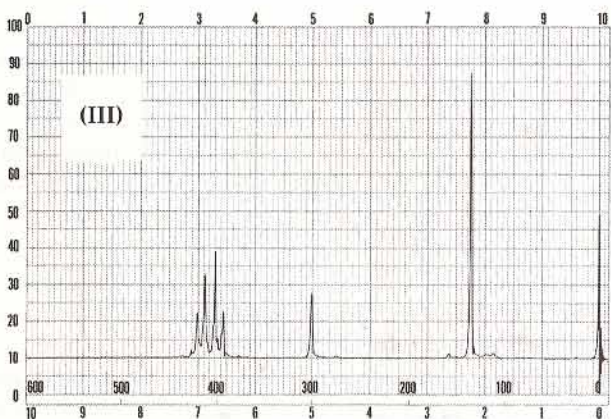**11.8** In Figure 11.9, is upfield to the left or right?
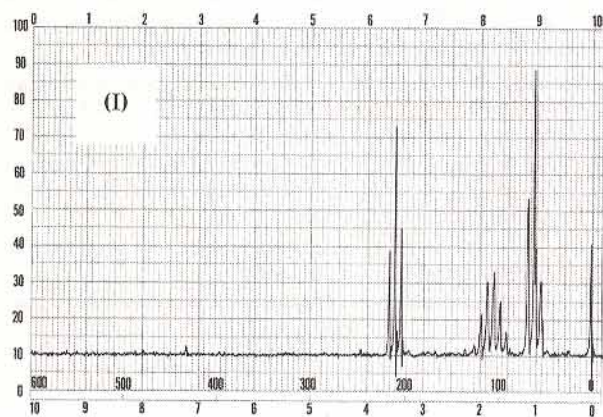
FIGURE 11.3.1 ▲
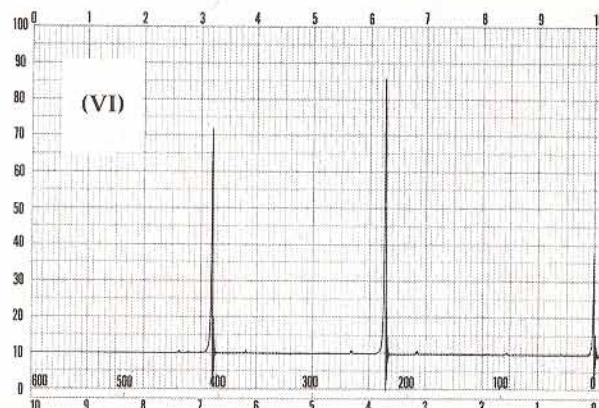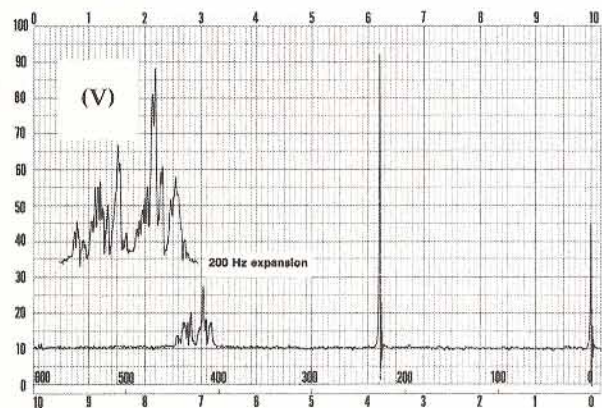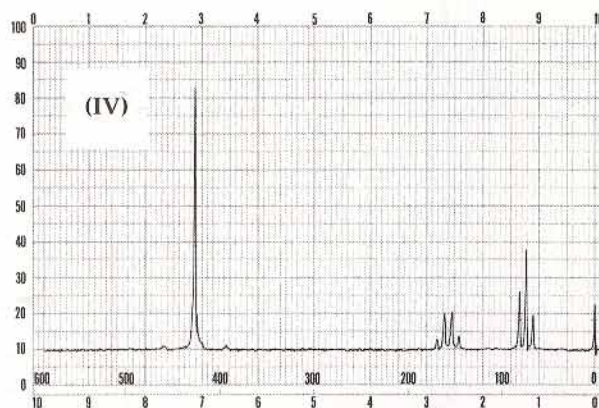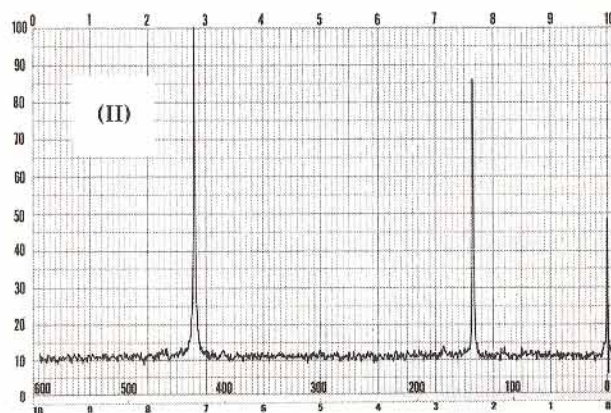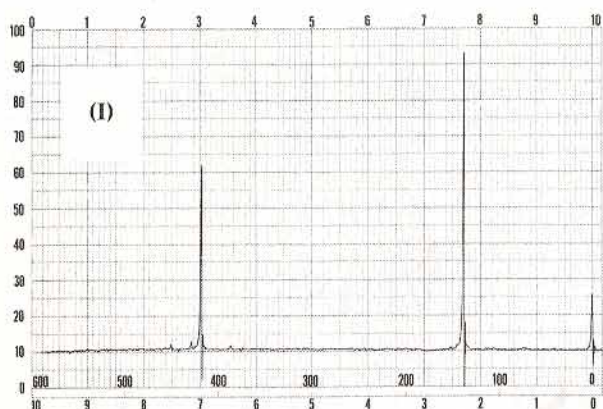
FIGURE 11.4.1 ▲

KENNETH A. RUBINSON

JUDITH F. RUBINSON
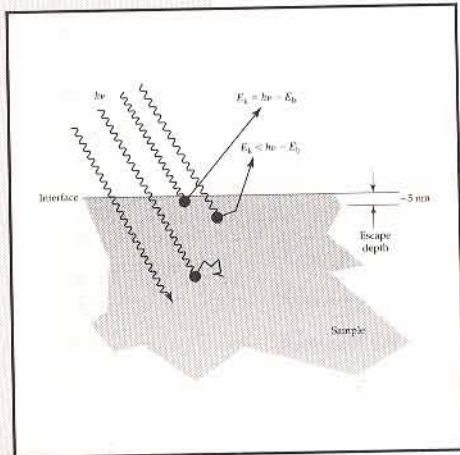
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Cover Art: *The map on the cover shows ozone levels measured worldwide along commercial airline routes (see Case Study 8-1). Pictured is the record of average ozone concentrations at 9–12 km altitude in the months of September, October, and November in 1994, 1995, and 1996. Thouret, V., A. Marenco, P. Nédélec, and C. Grouhel, "Ozone climatologies at 9–12 km altitude as seen by the MOZAIC airborne program between September 1994 and August 1996," J. Geophys. Res. (Atmospheres), vol. 103, No. D19, pages 25,653–25,679, October 20, 1998 (MOZAIC special section). Copyright by the American Geophysical Union.*

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