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FUNDAMENTALS OF NMR SPECTROSCOPY IN LIQUIDS

1.1 INTRODUCTION TO NMR SPECTROSCOPY

NMR is a spectroscopic technique that relies on the magnetic properties of the atomic nucleus. When placed in a strong magnetic field, certain nuclei resonate at a characteristic frequency in the radio frequency range of the electromagnetic spectrum. Slight variations in this resonant frequency give us detailed information about the molecular structure in which the atom resides.

1.1.1 The Classical Model

Many atoms (e.g., ^{1}H, ^{13}C, ^{15}N, ^{31}P) behave as if the positively charged nucleus was spinning on an axis (Fig. 1.1). The spinning charge, like an electric current, creates a tiny magnetic field. When placed in a strong external magnetic field, the magnetic nucleus tries to align with it like a compass needle in the earth's magnetic field. Because the nucleus is spinning and has angular momentum, the torque exerted by the external field results in a circular motion called precession, just like a spinning top in the earth's gravitational field. The rate of this precession is proportional to the external magnetic field strength and to the strength of the nuclear magnet:

$$\nu_{\mathrm{o}} = \gamma B_{\mathrm{o}}/2\pi$$

where ν_{o} is the precession rate (the "Larmor frequency") in hertz, γ is the strength of the nuclear magnet (the "magnetogyric ratio"), and B_{o} is the strength of the external magnetic field. This resonant frequency is in the radio frequency range for strong magnetic fields

NMR Spectroscopy Explained: Simplified Theory, Applications and Examples for Organic Chemistry and Structural Biology, by Neil E Jacobsen

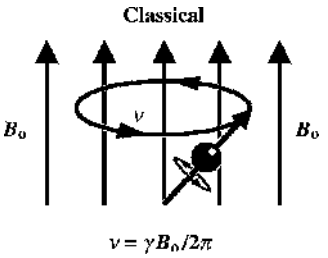


Figure 1.1

and can be measured by applying a radio frequency signal to the sample and varying the frequency until absorbance of energy is detected.

1.1.2 The Quantum Model

This classical view of magnetic resonance, in which the nucleus is treated as a macroscopic object like a billiard ball, is insufficient to explain all aspects of the NMR phenomenon. We must also consider the quantum mechanical picture of the nucleus in a magnetic field. For the most useful nuclei, which are called "spin ½" nuclei, there are two quantum states that can be visualized as having the spin axis pointing "up" or "down" (Fig. 1.2). In the absence of an external magnetic field, these two states have the same energy and at thermal equilibrium exactly one half of a large population of nuclei will be in the "up" state and one half will be in the "down" state. In a magnetic field, however, the "up" state, which is aligned with the magnetic field, is lower in energy than the "down" state, which is opposed to the magnetic field. Because this is a quantum phenomenon, there are no possible states in between. This energy separation or "gap" between the two quantum states is proportional to the strength of the external magnetic field, and increases as the field strength is increased. In a large population of nuclei in thermal equilibrium, slightly more than half will reside in the "up" (lower energy) state and slightly less than half will reside in the "down" (higher energy) state. As in all forms of spectroscopy, it is possible for a nucleus in the lower energy state to absorb a photon of electromagnetic energy and be promoted to the higher energy state. The energy of the photon must exactly match the energy "gap" (ΔE) between

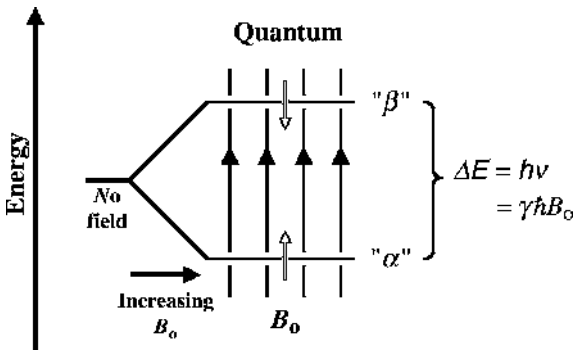


Figure 1.2

the two states, and this energy corresponds to a specific frequency of electromagnetic radiation:

$$\Delta E = h\nu_o = h\gamma B_o/2\pi$$

where h is Planck's constant. The resonant frequency, ν_o, is in the radio frequency range, identical to the precession frequency (the Larmor frequency) predicted by the classical model.

1.1.3 Useful Nuclei for NMR

The resonant frequencies of some important nuclei are shown below for the magnetic field strength of a typical NMR spectrometer (Varian Gemini-200):

Nucleus	Abundance (%)	Sensitivity	Frequency (MHz)
^{1}H	100	1.0	200
^{13}C	1.1	0.016	50
^{15}N	0.37	0.001	20
^{19}F	100	0.83	188
^{31}P	100	0.066	81
^{57}Fe	2.2	3.4×10^{-5}	6.5

The spectrometer is a radio receiver, and we change the frequency to "tune in" each nucleus at its characteristic frequency, just like the stations on your car radio. Because the resonant frequency is proportional to the external magnetic field strength, all of the resonant frequencies above would be increased by the same factor with a stronger magnetic field. The relative sensitivity is a direct result of the strength of the nuclear magnet, and the effective sensitivity is further reduced for those nuclei that occur at low natural abundance. For example, ^{13}C at natural abundance is 5700 times less sensitive ($1/(0.011 \times 0.016)$) than ^{1}H when both factors are taken into consideration.

1.1.4 The Chemical Shift

The resonant frequency is not only a characteristic of the type of nucleus but also varies slightly depending on the position of that atom within a molecule (the "chemical environment"). This occurs because the bonding electrons create their own small magnetic field that modifies the external magnetic field in the vicinity of the nucleus. This subtle variation, on the order of one part in a million, is called the chemical shift and provides detailed information about the structure of molecules. Different atoms within a molecule can be identified by their chemical shift, based on molecular symmetry and the predictable effects of nearby electronegative atoms and unsaturated groups.

The chemical shift is measured in parts per million (ppm) and is designated by the Greek letter delta (δ). The resonant frequency for a particular nucleus at a specific position within a molecule is then equal to the fundamental resonant frequency of that isotope (e.g., 50.000 MHz for ^{13}C) times a factor that is slightly greater than 1.0 due to the chemical shift:

$$\text{Resonant frequency} = \nu(1.0 + \delta \times 10^{-6})$$

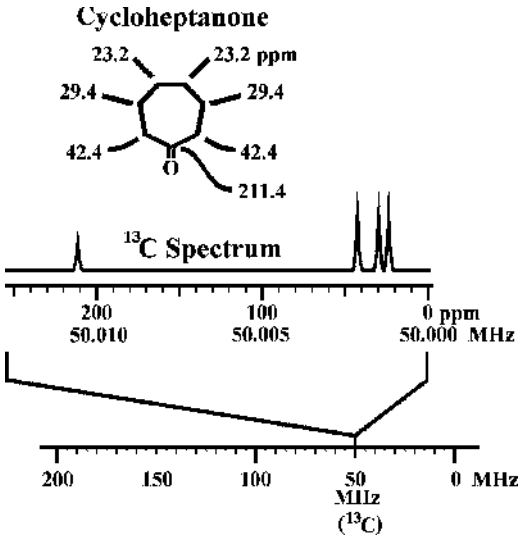


Figure 1.3

For example, a ^{13}C nucleus at the C-4 position of cycloheptanone (δ23.3 ppm) resonates at a frequency of

$$50.000\,\text{MHz}\,(1.0 + \mathbf{23.2} \times 10^{-6}) = 50.000(1.0000232) = 50{,}001{,}160\,\text{Hz}$$

A graph of the resonant frequencies over a very narrow range of frequencies centered on the fundamental resonant frequency of the nucleus of interest (e.g., ^{13}C at 50.000 MHz) is called a *spectrum*, and each peak in the spectrum represents a unique chemical environment within the molecule being studied. For example, cycloheptanone has four peaks due to the four unique carbon positions in the molecule (Fig. 1.3). Note that symmetry in a molecule can make the number of unique positions less than the total number of carbons.

1.1.5 Spin–Spin Splitting

Another valuable piece of information about molecular structure is obtained from the phenomenon of spin–spin splitting. Consider two protons (^1H_aC–C^1H_b) with different chemical shifts on two adjacent carbon atoms in an organic molecule. The magnetic nucleus of H_b can be either aligned with ("up") or against ("down") the magnetic field of the spectrometer (Fig. 1.4). From the point of view of H_a, the H_b nucleus magnetic field perturbs the external magnetic field, adding a slight amount to it or subtracting a slight amount from it, depending on the orientation of the H_b nucleus ("up" or "down"). Because the resonant frequency is always proportional to the magnetic field *experienced* by the nucleus, this changes the H_a frequency so that it now resonates at one of two frequencies very close together. Because roughly 50% of the H_b nuclei are in the "up" state and roughly 50% are in the "down" state, the H_a resonance is "split" by H_b into a pair of resonance peaks of equal intensity (a "doublet") with a separation of J Hz, where J is called the coupling constant. The relationship is mutual so that H_b experiences the same splitting effect (separation of J Hz) from H_a. This effect is transmitted through bonds and operates only when the two nuclei are very close (three bonds or less) in the bonding network. If there is more than one "neighbor"

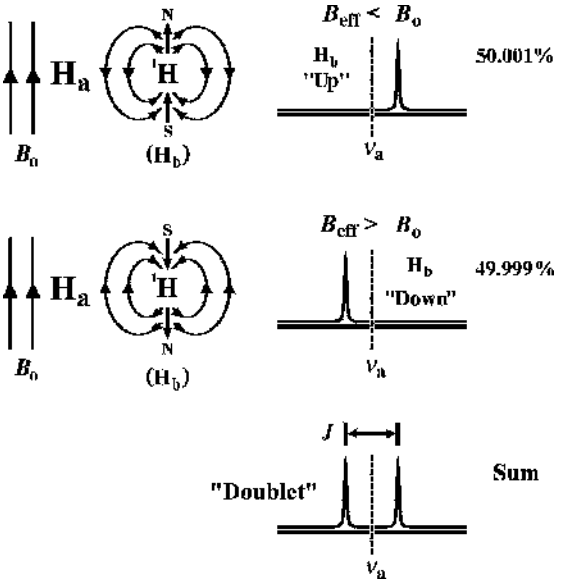


Figure 1.4

proton, more complicated splitting occurs so that the number of peaks is equal to one more than the number of neighboring protons doing the splitting. For example, if there are two neighboring protons ($H_aC–CH^b{}_2$), there are four possibilities for the H_b protons, just like the possible outcomes of flipping two coins: both "up," the first "up" and the second "down," the first "down" and the second "up," and both "down." If one is "up" and one "down" the effects cancel each other and the H_a proton absorbs at its normal chemical shift position (ν_a). If both H_b spins are "up," the H_a resonance is shifted to the right by J Hz. If both are "down," the H_a resonance occurs J Hz to the left of ν_a. Because there are two ways it can happen, the central resonance at ν_a is twice as intense as the outer resonances, giving a "triplet" pattern with intensity ratio 1 : 2 : 1 (Fig. 1.5). Similar arguments for larger numbers of neighboring spins lead to the general case of n neighboring spins, which split the H_a resonance peak into $n + 1$ peaks with an intensity ratio determined by *Pascal's triangle*. This triangle of numbers is created by adding each adjacent pair of numbers to get the value below it in the triangle:

singlet	1	(no neighbors)
doublet	1 1	(one neighbor)
triplet	1 2 1	(two neighbors)
quartet	1 3 3 1	(three neighbors)
quintet	1 4 6 4 1	(four neighbors)
sextet	1 5 10 10 5 1	(five neighbors)
septet	1 6 15 20 15 6 1	(six neighbors)

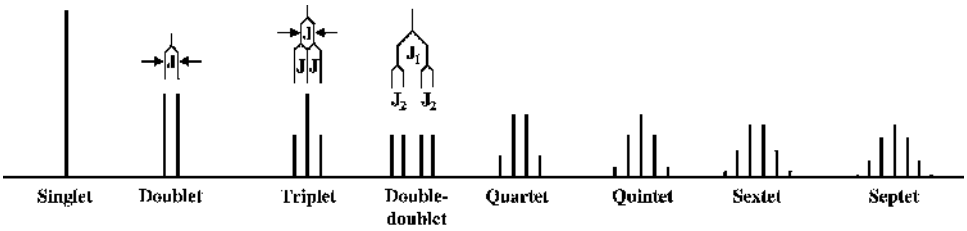


Figure 1.5

The strength of the spin–spin splitting interaction, measured by the peak separation ("*J* value") in units of hertz, depends in a predictable way on the dihedral angle defined by H_a–C–C–H_b, so that information can be obtained about the stereochemistry and conformation of molecules in solution. Because of this dependence on the geometry of the interceding bonds, it is possible to have couplings for two neighbors with different values of the coupling constant, *J*. This gives rise to a splitting pattern with four peaks of equal intensity: a double doublet (Fig. 1.5).

1.1.6 The NOE

A third type of information available from NMR comes from the nuclear Overhauser enhancement or NOE. This is a direct through-space interaction of two nuclei. Irradiation of one nucleus with a weak radio frequency signal at its resonant frequency will equalize the populations in its two energy levels. This perturbation of population levels disturbs the populations of nearby nuclei so as to enhance the intensity of absorbance at the resonant frequency of the nearby nuclei. This effect depends only on the distance between the two nuclei, even if they are far apart in the bonding network, and varies in intensity as the inverse sixth power of the distance. Generally the NOE can only be detected between protons (^{1}H nuclei) that are separated by 5 Å or less in distance. These measured distances are used to determine accurate three-dimensional structures of proteins and nucleic acids.

1.1.7 Pulsed Fourier Transform (FT) NMR

Early NMR spectrometers recorded a spectrum by slowly changing the frequency of a radio frequency signal fed into a coil near the sample. During this gradual "sweep" of frequencies the absorption of energy by the sample was recorded by a pen in a chart recorder. When the frequency passed through a resonant frequency for a particular nucleus in the sample, the pen went up and recorded a "peak" in the spectrum. This type of spectrometer, now obsolete, is called "continuous wave" or CW. Modern NMR spectrometers operate in the "pulsed Fourier-transform" (FT) mode, permitting the entire spectrum to be recorded in 2–3 s rather than the slow (5 min) frequency sweep. The collection of nuclei (sample) is given a strong radio frequency pulse that aligns the nuclei so that they precess in unison, each pointing in the same direction at the same time. The individual magnetic fields of the nuclei add together to give a measurable rotating magnetic field that induces an electrical voltage in a coil placed next to the sample. Over a period of a second or two the individual nuclei get out of synch and the macroscopic signal dies down. This "echo" of the pulse, observed in the coil, is called the free induction decay (FID), and it contains all of the resonant frequencies of the sample nuclei combined in one cacophonous reply. These data are digitised, and a

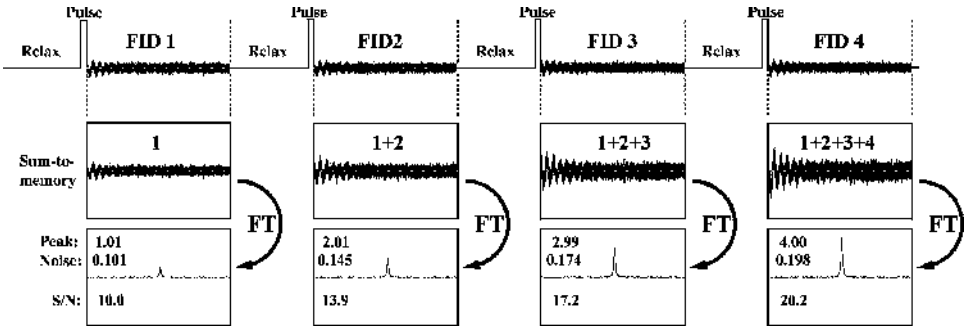


Figure 1.6

computer performs a Fast Fourier Transform to convert it from an FID signal as a function of time (time domain) to a plot of intensity as a function of frequency (frequency domain). The "spectrum" has one peak for each resonant frequency in the sample. The real advantage of the pulsed-FT method is that, because the data is recorded so rapidly, the process of pulse excitation and recording the FID can be repeated many times, each time adding the FID data to a sum stored in the computer (Fig. 1.6). The signal intensity increases in direct proportion to the number of repeats or "transients" (1.01, 2.01, 2.99, 4.00), but the random noise tends to cancel because it can be either negative or positive, resulting in a noise level proportional to the square root of the number of transients (0.101, 0.145, 0.174, 0.198). Thus the signal-to-noise ratio increases with the square root of the number of transients (10.0, 13.9, 17.2, 20.2). This signal-averaging process results in a vastly improved sensitivity compared to the old frequency sweep method.

The pulsed Fourier transform process is analogous to playing a chord on the piano and recording the signal from the decaying sound coming out of a microphone (Fig. 1.7). The chord consists of three separate notes: the "C" note is the lowest frequency, the "G" note is the highest frequency, and the "E" note is in the middle. Each of these pure frequencies gives a decaying pure sine wave in the microphone, and the combined signal of three frequencies is a complex decaying signal. This time domain signal ("FID") contains all three of the frequencies of the piano chord. Fourier transform will then convert the data to a "spectrum"—a graph of signal intensity as a function of frequency, revealing the three frequencies of the chord as well as their relative intensities. The Fourier transform allows us to record all of the signals simultaneously and then "sort out" the individual frequencies later.

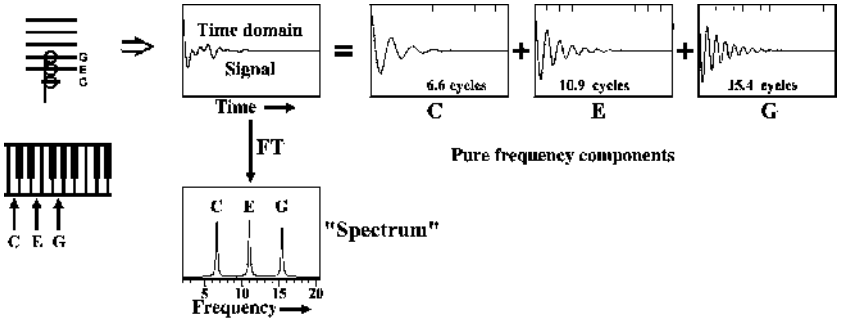


Figure 1.7

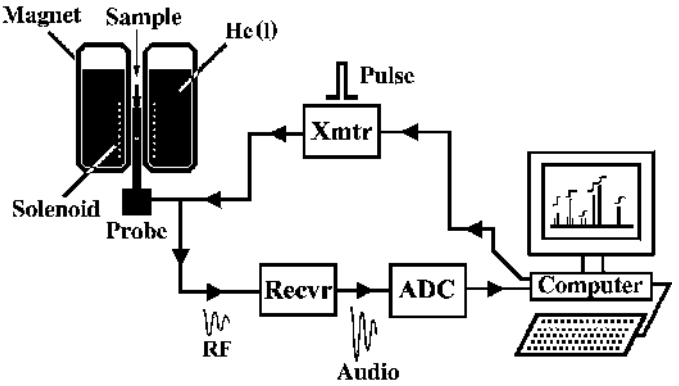


Figure 1.8

1.1.8 NMR Hardware

An NMR spectrometer consists of a superconducting magnet, a probe, a radio transmitter, a radio receiver, an analog-to-digital converter (ADC), and a computer (Fig. 1.8). The magnet consists of a closed loop ("solenoid") of superconducting Nb/Ti alloy wire immersed in a bath of liquid helium (bp 4 K). A large current flows effortlessly around the loop, creating a strong continuous magnetic field with no external power supply. The helium can ("dewar") is insulated with a vacuum jacket and further cooled by an outer dewar of liquid nitrogen (bp 77 K). The probe is basically a coil of wire positioned around the sample that alternately transmits and receives radio frequency signals. The computer directs the transmitter to send a high-power and very short duration pulse of radio frequency to the probe coil. Immediately after the pulse, the weak signal (FID) received by the probe coil is amplified, converted to an audio frequency signal, and sampled at regular intervals of time by the ADC to produce a digital FID signal, which is really just a list of numbers. The computer determines the timing and intensity of pulses output by the transmitter and receives and processes the digital information supplied by the ADC. After the computer performs the Fourier transform, the resulting spectrum can be displayed on the computer monitor and plotted on paper with a digital plotter. The cost of an NMR instrument is on the order of $120,000–$5,000,000, depending on the strength of the magnetic field (200–900 MHz proton frequency).

1.1.9 Overview of ^{1}H and ^{13}C Chemical Shifts

A general understanding of the trends of chemical shifts is essential for the interpretation of NMR spectra. The chemical shifts of ^{1}H and ^{13}C signals are affected by the proximity of electronegative atoms (O, N, Cl, etc.) in the bonding network and by the proximity to unsaturated groups (C=C, C=O, aromatic) directly through space. Electronegative groups shift resonances to the left (higher resonant frequency or "downfield"), whereas unsaturated groups shift to the left (downfield) when the affected nucleus is in the plane of the unsaturation, but have the opposite effect (shift to the right or "upfield") in regions above and below this plane. Although the range of chemical shifts in parts per million is much larger for ^{13}C than for ^{1}H (0–220 ppm *vs.* 0–13 ppm), there is a rough correlation between the shift of a proton and the shift of the carbon it is attached to (Fig. 1.9). For a "hydrocarbon" environment with no electronegative atoms or unsaturated groups nearby, the shift is

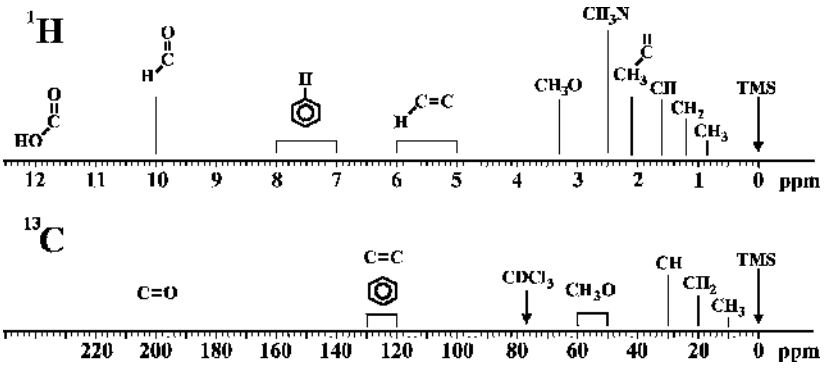


Figure 1.9

near the upfield (right) edge of the range, with a small downfield shift for each substitution: CH > CH_2 > CH_3 (^{1}H: 1.6, 1.2, 0.8; ^{13}C: 30, 20, 10 ppm). Oxygen has a stronger downfield-shifting effect than nitrogen due to its greater electronegativity: 3–4 ppm (^{1}H) and 50–85 (^{13}C) for CH–O. As with the hydrocarbon environment, the same downfield shifts are seen for increasing substitution: C_q–O (quaternary) > CH–O > CH_2O > CH_3O (^{13}C around 85, 75, 65, and 55 ppm, respectively). Proximity to an unsaturated group usually is downfield shifting because the affected atom is normally in the plane of the unsaturation: CH_3 attached to C=O moves downfield to 30 (^{13}C) and 2.1 ppm (^{1}H), whereas in HC=C (closer to the unsaturation) ^{13}C moves to 120–130 ppm and ^{1}H to 5–6 ppm. The combination of unsaturation *and* electronegativity is seen in H–C=O: 190 ppm ^{13}C and 10 ppm ^{1}H. There are some departures from this correlation of ^{1}H and ^{13}C shifts. Aromatic protons typically fall in the 7–8 ppm range rather than the 5–6 ppm range for olefinic (HC=C for an isolated C=C bond) protons, whereas ^{13}C shifts are about the same for aromatic or olefinic carbons. Because carbon has more than one bond, it is sensitive to distortion of its bond angles by the steric environment around it, with steric crowding usually leading to downfield shifts. Hydrogen has no such effect because it has only one bond, but it is more sensitive than carbon to the through-space effect of unsaturations. For example, converting an alcohol (CH–OH) to an ester (CH–OC(O)R) shifts the ^{1}H of the CH group downfield by 0.5 to 1 ppm, but has little effect on the ^{13}C shift.

1.1.10 Equivalence in NMR

Nuclei can be equivalent (have the same chemical shift) by symmetry within a molecule (e.g., the two methyl carbons in acetone, CH_3COCH_3), or by rapid rotation around single bonds (e.g., the three methyl protons in acetic acid, CH_3CO_2H). The intensity (integrated peak area or *integral*) of ^{1}H signals is directly proportional to the number of equivalent nuclei represented by that peak. For example, a CH_3 peak in a molecule would have three times the integrated peak area of a CH peak in the same molecule.

1.1.11 Proton Spectrum Example

The first step in learning to interpret NMR spectra is to learn how to predict them from a known chemical structure. An example of a ^{1}H (proton) NMR spectrum is shown for

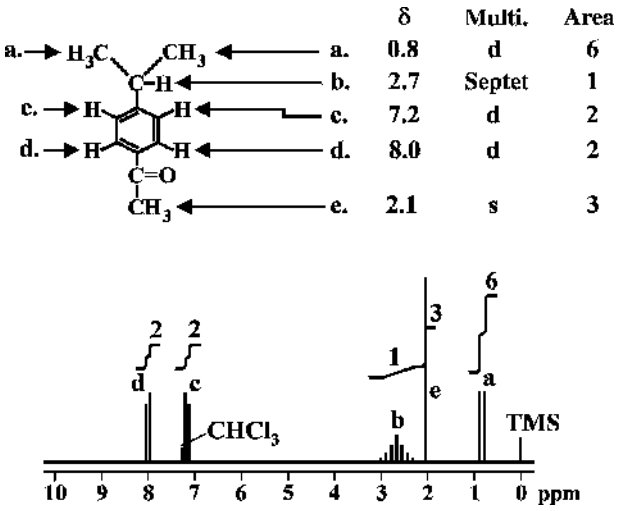


Figure 1.10

4-isopropylacetophenone (Fig. 1.10). The two isopropyl methyl groups are equivalent by symmetry, and each methyl group has three protons made equivalent by rapid rotation about the C–C bond. This makes all six H_a protons equivalent. Because they are far from any electronegative atom, these protons have a chemical shift typical of an isolated CH_3 group: 0.8 ppm (see Fig. 1.9). The absorbance is split into two peaks (a doublet) by the single neighboring H_b proton. The six H_a protons do not split each other because they are equivalent. The integrated area of the doublet is 6.0 because there are six H_a protons in the molecule. The H_b proton is split by all six of the H_a protons, so its absorbance shows up as a septet (seven peaks with intensity ratio 1:6:15:20:15:6:1). Its integrated area is 1.0, and its chemical shift is downfield of an isolated CH_2 (1.2 ppm) because of its proximity to the unsaturated aromatic ring (close to the plane of the aromatic ring so the effect is a downfield shift). The H_e methyl group protons are all equivalent due to rapid rotation of the CH_3 group, and their chemical shift is typical for a methyl group adjacent to the unsaturated C=O group (2.1 ppm). There are no neighboring protons (the H_d proton is five bonds away from it, and the maximum distance for splitting is three bonds) so the absorbance appears as a single peak ("singlet") with an integrated area of 3.0. The H_c and H_d protons on the aromatic ring appear at a chemical shift typical for protons bound directly to an aromatic ring, with the H_d protons shifted further downfield by proximity to the unsaturated C=O group. Each pair of aromatic protons is equivalent due to the symmetry of the aromatic ring. The H_c absorbance is split into a doublet by the neighboring H_d proton (note that from the point of view of either of the H_c protons, only one of the H_d protons is close enough to cause splitting), and the H_d absorbance is split in the same way. Note that the *J* value (separation of split peaks) is the same for the H_c and H_d doublets, but slightly different for the H_a–H_b splitting. In this way we know, for example, that H_a is not split by either H_c or H_d.

1.1.12 Carbon Spectrum Example

The ^{13}C spectrum of the same compound is diagramed in Figure 1.11. Several differences can be seen in comparison with the ^{1}H spectrum. First, there is no spin–spin splitting due

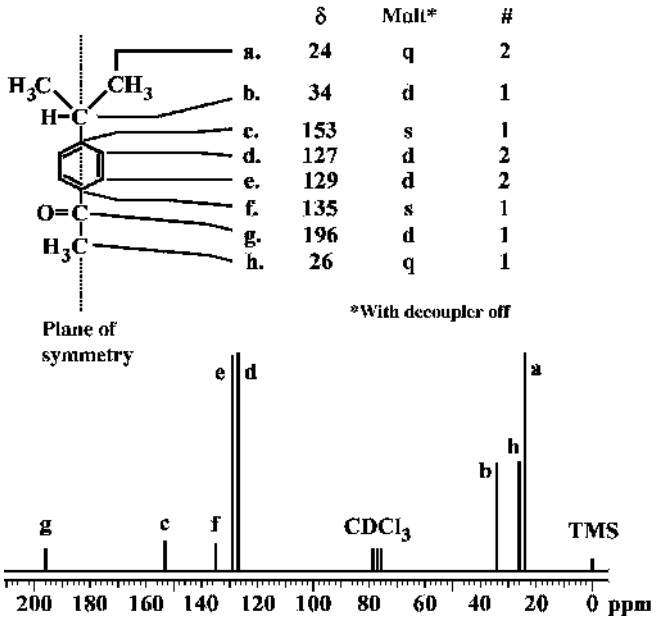


Figure 1.11

to adjacent carbons. This is because of the low natural abundance of ^{13}C, which is only 1.1%. Thus the probability of a ^{13}C occurring next to another ^{13}C is very low, and splitting is not observed because ^{12}C has no magnetic properties. Second, there is no spin–spin splitting due to the protons attached to each carbon. This is prevented intentionally by a process called *decoupling*, in which all the protons in the molecule are simultaneously irradiated with continuous low-power radio frequency energy at the proton resonance frequency. This causes each proton to flip rapidly between the upper and lower (disaligned and aligned) energy states, so that the ^{13}C nucleus sees only the average of the two states and appears as a singlet, regardless of the number of attached protons. The lack of any spin–spin splitting in decoupled ^{13}C spectra means that each carbon always appears as a singlet. The multiplicity (s, d, t, q) indicated for each carbon in the diagram is observed only with the decoupler turned off and is not shown in the spectrum. Third, the peaks are not integrated because the peak area does not indicate the number of carbon atoms accurately. This is because ^{13}C nuclei *relax* more slowly than protons, so that unless a very long relaxation delay between repetitive pulses is used, the population difference between the two energy states of ^{13}C is not reestablished before the next pulse arrives. Quaternary carbons, which have no attached protons, relax particularly slowly and thus show up with very low intensity.

The molecular symmetry, indicated by a dotted line (Fig. 1.11) where the mirror plane intersects the plane of the paper, makes the two isopropyl methyl carbons C_a equivalent. Their chemical shift is a bit downfield of an isolated methyl group due to the steric crowding of the isopropyl group. Unlike protons, ^{13}C nuclei are sensitive to the degree of substitution or branching in the immediate vicinity, generally being shifted downfield by increased branching. C_b is shifted further downfield because of direct substitution (it is attached to three other carbons) and proximity to the aromatic ring. C_h is in a relatively uncrowded

environment, but is shifted downfield by proximity to the unsaturated and electronegative carbonyl group. With the decoupler turned off, CH_3 carbons appear as quartets because of the three neighboring protons. The aromatic CH carbons C_d and C_e are in nearly identical environments typical of aromatic carbons, and each resonance peak represents two carbons due to molecular symmetry. With the decoupler turned off, these peaks turn into doublets due to the presence of a single attached proton. The two quaternary aromatic carbons C_c and C_f are shifted further downfield by greater direct substitution (they are attached to three other carbons) and by steric crowding (greater remote substitution) in the case of C_c and proximity to a carbonyl group in the case of C_f. The chemical shift of the carbonyl carbon C_g is typical for a ketone. All three of the quaternary carbons C_c, C_f, and C_g have low peak intensities due to slow relaxation (reestablishment of population difference) in the absence of directly attached protons.

1.2 EXAMPLES: NMR SPECTROSCOPY OF OLIGOSACCHARIDES AND TERPENOIDS

A few real-world examples will illustrate the use of ^1H and ^{13}C chemical shifts and *J* couplings, as well as introduce some advanced methods we will use later. Two typical classes of complex organic molecules will be introduced here to familiarize the reader with the elements of structural organic chemistry that are important in NMR and how they translate into NMR spectra. Terpenoids are typical of natural products; they are relatively nonpolar (water insoluble) molecules with a considerable amount of "hydrocarbon" part and only a few functional groups—olefin, alcohol, ketone—in a rigid structure. Oligosaccharides are polar (water soluble) molecules in which every carbon is functionalized with oxygen—alcohol, ketone, or aldehyde oxidation states—and relatively rigid rings are connected with flexible linkages. In both cases, rigid cyclohexane-chair ring structures are ideal for NMR because they allow us to use *J*-coupling values to determine stereochemical relationships of protons (*cis* and *trans*). The molecules introduced here will be used throughout the book to illustrate the results of the NMR experiments.

1.2.1 Oligosaccharides

A typical monosaccharide (single carbohydrate building block) is a five or six carbon molecule with one of the carbons in the aldehyde or ketone oxidation state (the "anomeric" carbon) and the rest in the alcohol oxidation state (CH(OH) or CH_2OH). Thus the anomeric carbon is unique within the molecule because it has two bonds to oxygen whereas all of the other carbons have only one bond to oxygen. Normally the open-chain monosaccharide will form a five- or six-membered ring as a result of the addition of one of the alcohol groups (usually the second to last in the chain) to the ketone or aldehyde, changing the C=O double bond to an OH group.

The six-membered ring of glucose prefers the chair conformation shown in Figure 1.12, with nearly all of the OH groups arranged in the equatorial positions (sticking out and roughly in the plane of the ring) with the less bulky H atoms in the axial positions (pointing up or down, above or below the plane of the ring). This limits the dihedral angles between neighboring protons (vicinal or three-bond relationships) to three categories: axial–axial (*trans*): 180° dihedral angle, large *J* coupling (~10 Hz); axial-equatorial (*cis*): 60° dihedral angle, small *J* (~4 Hz); and equatorial–equatorial (*trans*): 60° dihedral angle, small *J* (~4 Hz).

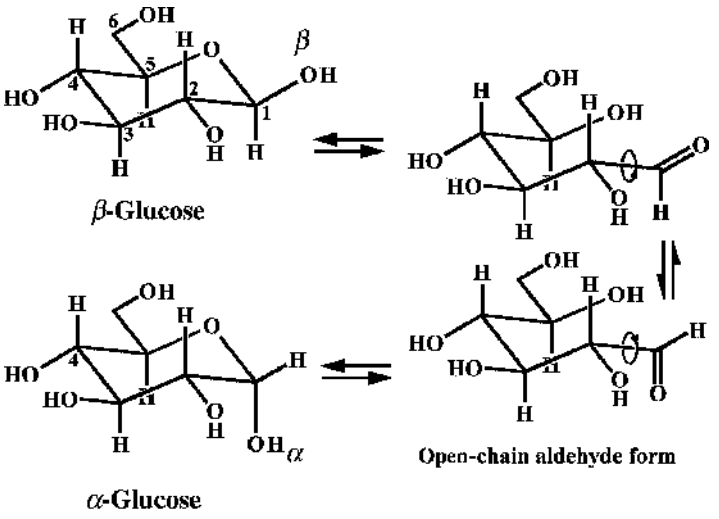


Figure 1.12

The third category is rare in carbohydrates because the bulky OH groups prefer the equatorial position, pushing the H into the axial position.

In this ring form, the anomeric carbon (C1) of an aldehyde sugar (aldose) has one bond to the oxygen of the ring and another to an OH group external to the ring. Also external to the ring is the CH_2OH group of the last carbon in the chain. The anomeric OH group can either be *cis* or *trans* to the external CH_2OH group, depending on which side of the aldehyde or ketone group the OH group is added to. If it is *cis*, we call this isomer the β-anomer, and if it is *trans* we call it the α-anomer. When a crystalline monosaccharide is dissolved in water, these two ring forms rapidly form an equilibrium mixture of α and β anomers with very little of the open-ring aldehyde existing in solution (Fig. 1.12).

It is possible to link a monosaccharide to an alcohol at the anomeric carbon, so that instead of an OH group the anomeric carbon is connected to an OR group (e.g., OCH_3) that is external to the ring. This is called a "glycoside," and the anomeric carbon is now a full acetal or ketal. The ring can no longer freely open into the open-chain aldehyde or ketone, so there is no equilibration of α and β forms. Thus a β-glycoside (OR group *cis* to the CH_2OH group) will remain locked in the β form when dissolved in water. If the alcohol used to form the glycoside is the alcohol of another monosaccharide, we have formed a disaccharide with the two monosaccharides connected by a glycosidic linkage (Figure 1.13). Usually the alcohol comes from one of the alcohol carbons of the second sugar, but it is also possible to form a glycosidic linkage to the *anomeric* carbon of the second sugar. In this case we have a linkage C–O–C from one anomeric carbon to another, and both monosaccharides are "locked" with no possibility of opening to the aldehyde or ketone form.

1.2.2 NMR of Carbohydrates: Chemical Shifts

NMR chemical shifts give us information about the proximity of electronegative atoms (e.g., oxygen) and unsaturated groups (double bonds and aromatic rings). In this discussion we will ignore the protons attached directly to oxygen (OH) because they provide little

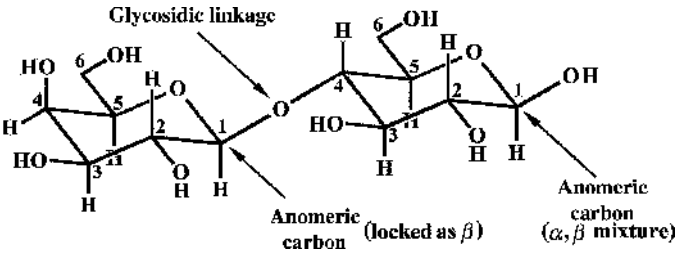


Figure 1.13

chemical information in NMR and are exchanged for deuterium by the solvent if we use deuterated water (D_2O). In the case of carbohydrates, nearly all of the protons attached to carbon are in a similar environment: one oxygen attached to the carbon (C<u>H</u>OH or C<u>H</u>$_2$OH). These protons all have similar chemical shifts, in the range of 3.3–4.1 ppm, so there is often a great deal of overlap of these signals in the ^{1}H NMR of carbohydrates, even at the highest magnetic fields achievable. For this reason carbohydrate NMR (and NMR of nucleic acids RNA and DNA, which have a sugar-phosphate backbone) has been limited to relatively small molecules because the complexity of overlapping signals is limiting. The anomeric proton, however, is in a unique position because the carbon it is attached to has *two* bonds to oxygen. This additional inductive pull of electron density away from the hydrogen atom leads to a further downfield shift of the NMR signal, so that anomeric protons resonate in a distinct region at 5–6 ppm. A similar effect is seen for anomeric carbons, which have ^{13}C chemical shifts in the range of 90–110 ppm, whereas their neighbors with only *one* bond to oxygen resonate in the normal alcohol region of 60–80 ppm. Because each monosaccharide unit in a complex carbohydrate has only one anomeric carbon, we can count up the number of monosaccharide building blocks by simply counting the number of NMR signals in this anomeric region. Thus the analysis of carbohydrate NMR spectra is greatly simplified if we focus on the anomeric region of the ^{1}H or ^{13}C spectrum. The "alcohol" (nonanomeric) carbons of a sugar (H–C–O or H_2C–O) are sensitive to steric crowding, so that the CH_2OH carbons appear at higher field (60–70 ppm) than the more crowded CHOH carbons (70–80 ppm). This steric effect is also seen at the alcohol side of a glycosidic linkage (–O–CH–O–<u>C</u>H–C): this carbon is shifted downfield by as much as 10 ppm from the rest of the "alcohol" carbons (HO–CH–C) that are not involved in glycosidic linkages.

1.2.3 ^{1}H NMR: Coupling Constants

In the proton NMR spectrum, each signal is "split" into a multiple peak pattern by the influence of its "neighbors," the protons attached to the next carbon in the chain. These protons are three bonds away from the proton being considered and are sometimes called "vicinal" protons. For example, the anomeric proton in a cyclic aldose has only one neighbor: the proton on the next carbon in the chain (carbon 2). Note that because of rapid exchange processes or deuterium replacement in D_2O, we seldom see splitting by the OH protons. Because it has only one neighbor, the anomeric proton will always appear as a *doublet* in the NMR spectrum. Also, because of its unique chemical shift position (5–6 ppm) and relatively rare occurrence (only one anomeric position per monosaccharide unit), the anomeric proton signal is usually not overlapped so we can see its splitting pattern clearly. The distance

(J, in frequency units of Hz) between the two component peaks of the doublet is a measure of the intensity of the splitting (or J coupling) interaction. For vicinal ("next-door neighbor") protons the value of J depends on the dihedral angle of the C–C bond between them. This angle is fixed in six-membered ring (pyranose) sugars because the ring adopts a stable *chair* conformation. For many common sugars (glucose, galactose, etc.) all or nearly all of the bulky groups on the ring (OH or CH_2OH) can be oriented in the less crowded equatorial position in one of the two chair forms. Thus the sugar ring is effectively "locked" in this one chair form and we can talk about each proton on the ring as being in an axial or equatorial orientation. This is important for NMR because two neighboring (vicinal) protons that are both in axial positions ("*trans*-diaxial" relationship) have a dihedral angle at the maximum value of 180°, and this leads to the maximum value of the coupling constant J (about 10 Hz separation of the two peaks of the doublet). This does not make intuitive sense because in this arrangement the two protons are as far apart as possible; however, it is the parallel alignment of the two C–H bonds that leads to the strong coupling because the J-coupling (splitting) interaction is transmitted through bonds and not through space. Two vicinal protons in a locked chair with an axial–equatorial or an equatorial–equatorial relationship will have a much smaller coupling constant (much narrower pair of peaks in the doublet) in the range of 4 Hz. Thus we can use NMR coupling constants to determine the stereochemistry of sugars.

Here is how we can use this in the analysis of carbohydrate ^1H NMR spectra: most naturally occurring sugars have an equatorial OH at the 2 position (numbering starts with the anomeric position as number 1), so the proton at carbon 2 is axial in a six-membered ring sugar. In addition, the CH_2OH group is also equatorial in most pyranose sugars. So if the anomeric proton is axial, we should see it in the ^1H NMR spectrum as a doublet with a large coupling (10 Hz), because the H_1–H_2 relationship is axial–axial. If the anomeric proton is axial, then the anomeric OH or OR substituent is equatorial and the sugar is in the β configuration (anomeric OH or OR *cis* to the CH_2OH group at C_5). If we see an anomeric proton with a small (4 Hz) coupling, then the anomeric proton is equatorial, the OH or OR group is axial, and we have an α sugar (anomeric OH or OR *trans* to the CH_2OH group at C_5). This reasoning works *only* if we are dealing with an aldopyranose (six-membered ring sugar based on an open-chain aldehyde) with an equatorial OH at C_2; fortunately, nature seems to favor this situation.

1.2.4 Reducing Sugars

If the anomeric carbon of a sugar in the ring form bears an OH substituent instead of OC (glycosidic linkage), it will have the possibility of opening to the open-chain aldehyde or ketone form and reclosing in either the α or the β configuration. This is called a "reducing sugar" because the open-chain aldehyde form is accessible and can be oxidized to the carboxylic acid. The two isomers (α and β) are in equilibrium and we usually see about a 2:1 ratio of β to α forms. The equilibration is slow on the NMR timescale (milliseconds) and so we see two distinct NMR peaks for the two isomers. The anomeric proton for the major β form will be a doublet with a large coupling constant (10 Hz) and for the minor form a doublet at a different chemical shift with a small coupling constant (4 Hz). The ratio of integrals for these two peaks will be about 2:1 (0.67:0.33 for normalized integrals). This pattern is a dead giveaway that you have a free (reducing) aldopyranose sugar. This monosaccharide could still be linked to other sugars by formation of a glycosidic linkage with one of the nonanomeric OH groups.

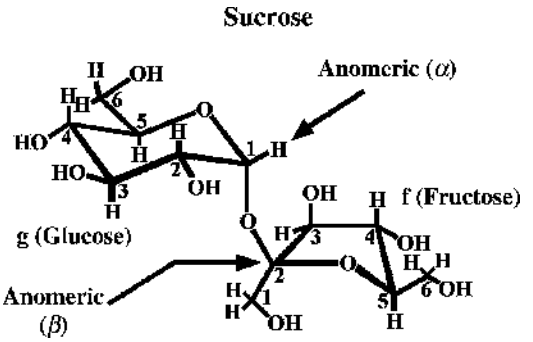


Figure 1.14

1.2.5 Keto Sugars

A ketose or keto sugar is a sugar based on a ketone rather than aldehyde functional group for its anomeric carbon. In this case the anomeric carbon is not C_1 and there is no proton attached to the anomeric carbon (i.e., it is a *quaternary* carbon). The most common naturally occurring ketose is fructose, a 6 carbon sugar with the anomeric (ketone) carbon at position 2 in the chain. It forms a five-membered ring hemiketal (furanose) with the C_1 and C_6 CH_2OH groups external to the ring. For a keto sugar you will not see an anomeric proton signal in the ^1H NMR because the anomeric carbon has no hydrogen bonded to it. The only evidence will be the quaternary carbon in the ^{13}C spectrum that appears at the typical chemical shift (90–110 ppm) for an anomeric carbon (two bonds to oxygen). Furanose (five-membered ring) sugars pose another problem for NMR analysis: five-membered rings are generally flexible and do not adopt a stable chair-type conformation. For this reason we cannot speak of "axial" and "equatorial" protons or substituents in a furanose, so that stereochemical analysis by ^1H NMR is very difficult.

1.2.6 Sucrose

A classic example of a keto sugar occurs in sucrose, a disaccharide formed from glucose in a six-membered ring linked to fructose in a five-membered ring, with the glycosidic linkage between the anomeric carbon of glucose (α configuration) and the anomeric carbon of fructose (β configuration) (Fig. 1.14). In the ^1H spectrum of sucrose (Fig. 1.15) we see the "alcohol" CH protons in the chemical shift range 3.4–4.2 ppm and the glucose anomeric proton at about 5.4 ppm. Fructose has no anomeric proton signal because the anomeric carbon is quaternary (keto sugar). The gl (glucose position 1) proton signal occurs as a doublet (coupled only to g2) with a small coupling constant (3.8 Hz) indicating that it is in the equatorial position (equatorial–axial coupling). This confirms that the glucose configuration is α because the glycosidic oxygen is pointing "down," opposite to the g6 CH_2OH group. There is a double doublet at 3.5 ppm that can be broken down into two couplings: a doublet coupling of 10.0 Hz is further split by another doublet coupling of 3.8 Hz. The 3.8 Hz coupling matches the H–g1 doublet (also 3.8 Hz), so we can assign this peak to H–g2. Because the other coupling (to H–g2's other neighbor H–g3) is large, we know that H–g3 is axial and we confirm that H–g2 is also axial, further confirming that H–g1 is equatorial. There are three triplets with large coupling constants (3.4, 3.7, and

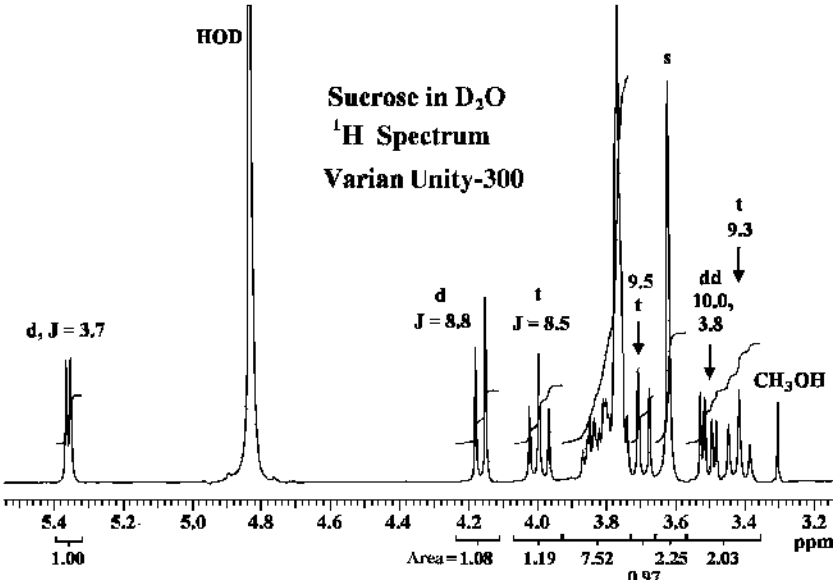


Figure 1.15

4.0 ppm), and it is likely that they represent axial protons in a cyclohexane chair structure with an axial proton on each side. Because all of the OH groups and the CH_2OH group are in equatorial positions in the glucose portion, the nonanomeric H's are in axial positions, and we expect triplets with large couplings ($\sim$10 Hz) for H–g3 and H–g4 because both are in axial positions with one neighbor on each side in an axial position. These two large (axial–axial) couplings, if identical, would lead to a triplet pattern. Because we see three such triplets in the ^1H spectrum, each one with normalized integral area 1, one of them must belong to the fructose part. Only H–f4 can be a triplet because it is the only fructose position with a single neighbor on each side. The doublet at 4.2 ppm ($J = 8.8$) can be assigned to H–f3 because it is next to the quaternary (anomeric) carbon C–f2 and therefore has only one coupling partner: H-f4. Note that this is the only doublet besides H-g1, which can be assigned because of its chemical shift in the anomeric region. Of the three resolved triplets, careful examination of the coupling constants reveals that one has a slightly smaller J value (8.5 Hz) that closely matches the H–f3 doublet splitting. Thus we can assign this triplet at 4.0 ppm to H–f4. A sharp singlet at 3.6 ppm (integral area 2) corresponds to the only CH_2 group (H-f1) that is isolated from coupling by the quaternary carbon (C–f2). Because this is a chiral molecule, the two protons of CH_2–f1 *could* have different chemical shifts, leading to a pair of doublets, but in this case they coincidentally have the same chemical shift and give a singlet. Two protons of the same carbon atom (CH_2) are called "geminal" (twins), and if they have the same chemical shift in a chiral molecule they are called "degenerate." The overlapped group of signals between 3.75 and 3.9 ppm integrates to six protons and must contain the glucose CH_2OH (H–g6), the other fructose CH_2OH (H–f6), and the more complex H–g5 and H–f5 signals (each with one coupling partner at position 4 and two at position 6). Thus the only ambiguity remains the two resolved (not overlapped) triplet signals at 3.4 and 3.7 ppm that correspond to H–g3 and H–g4. To solve this puzzle, we will

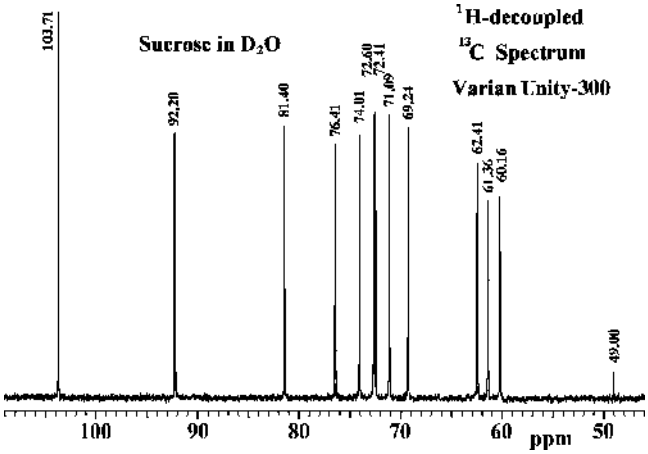


Figure 1.16

need more information from more advanced NMR experiments such as two-dimensional NMR.

The ^{13}C spectrum of sucrose is shown in Figure 1.16. Because it is proton decoupled, we see only one peak for each unique carbon in the molecule: 12 peaks for the $C_{12}H_{22}O_{12}$ molecule of sucrose. We see two peaks in the anomeric (90–110 ppm) region, and we can assign the more substituted C–f2 (two bonds to carbon) to the more downfield of the two at 103.7 ppm. The less substituted C–g1 (one bond to carbon) appears at 92.2 ppm, about 10 ppm upfield of C–f2. This is a rule of thumb: about 10 ppm downfield shift each time an H is replaced with a C in the four bonds to a carbon atom. We see a tight group of three peaks at 60–63 ppm; these are the three CH_2OH groups C–g6, C–f1 and C–f6. The remaining peaks are more spread out over the range 69–82 ppm; these are the nonanomeric "alcohol" or H–C–O carbons that constitute the majority of sugar positions. Again we see the roughly 10 ppm downfield shift due to substitution of an H with a C on the carbon atom of interest: CH_2OH to C–CH–OH. How can we be sure that the CH_2 and CH carbons are so neatly divided into chemical shift regions? More advanced one-dimensional ^{13}C experiments called APT and DEPT allow us to determine the precise number of hydrogens attached to each carbon in the spectrum. To specifically assign the carbons within these three categories will require two-dimensional experiments.

1.2.6.1 Two-Dimensional Experiments A full NMR analysis of a carbohydrate, in which each ^{1}H and ^{13}C peak in the spectrum is assigned to a particular position in the molecule, requires the use of two-dimensional (2D) NMR. In a 2D spectrum, there are two chemical shift scales (horizontal and vertical) and a "spot" appears in the graph at the intersection of two chemical shifts when two nuclei (^{1}H or ^{13}C) in the molecule are close to each other in the structure. For example, one type of 2D spectrum called an HSQC spectrum presents the ^{1}H chemical shift scale on the horizontal (x) axis and the ^{13}C chemical shift scale on the vertical (y) axis. If proton H_a is directly bonded to carbon C_a, there will be a spot at the intersection of the ^{1}H chemical shift of H_a (horizontal axis) and the ^{13}C chemical shift of C_a (vertical axis). Because the peaks are spread out into two dimensions, the chances of overlap of peaks are much less and we can count up the number of anomeric and

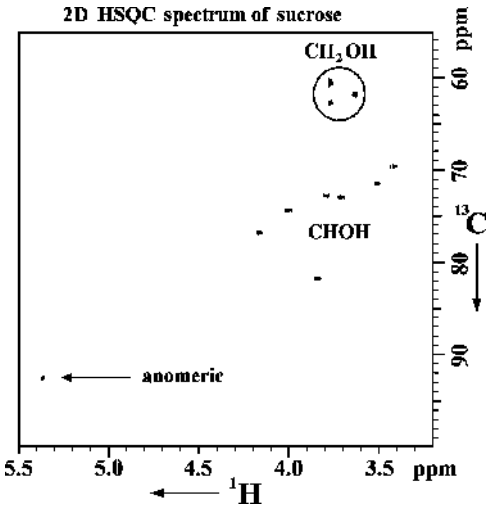


Figure 1.17

nonanomeric peaks very quickly. The HSQC spectrum of sucrose is shown in Figure 1.17. There are 11 "spots" representing the 11 carbons that have at least one hydrogen attached. Quaternary carbons do not show up in the spectrum because the H has to be directly bonded to the C to generate a "spot." Note that the crosspeaks ("spots") fall roughly on a diagonal line extending from the lower left to the upper right. This is because there is a rough correlation between ^{1}H chemical shifts and ^{13}C chemical shifts: the same things that lead to downfield or upfield shifts of protons also affect the carbon they are attached to in the same way. We can also see that the small "triangle" of CH_2OH peaks at the top is shifted "up" from the other nonaromatic peaks, due to the reduced steric crowding of the less-substituted CH_2 (methylene) carbon compared to CH (methine) carbons. The ^{1}H chemical shifts fall in the range of 3.5–4.2 ppm regardless of the degree of substitution.

A variation of this experiment, called HMBC (MB stands for multiple bond), shows spots only when the carbon and the proton are separated by two or three bonds in the structure. For example, for a monosaccharide we would see a spot at the chemical shift of the anomeric proton (H-1, horizontal axis) and the chemical shift of the C-3 carbon (vertical axis). Working together with data from the HSQC and HMBC 2D spectra, we can "walk" through the bonding structure of a carbohydrate, even "jumping" across the glycosidic linkages and establishing the points of connection of each monosaccharide unit.

Figure 1.18 shows a portion of the ^{1}H spectrum of the trisaccharide D-raffinose in D_2O. From just this portion we can conclude that, most probably, one of the sugars is a keto sugar and the other two are aldoses locked in the α configuration. The presence of two anomeric protons, each with a small doublet coupling (3.6 Hz) indicates that two of the sugars have the anomeric proton in the equatorial orientation. This assumes that we have the common pyranose arrangement with H-2 axial and the CH_2OH group equatorial. The exact 1:1 ratio of integrals and the absence of major and minor (β and α) anomeric peaks prove that these anomeric centers are locked in a glycosidic linkage. The absence of a third proton in the anomeric region means that the third sugar is most likely a keto sugar, with a quaternary anomeric carbon.

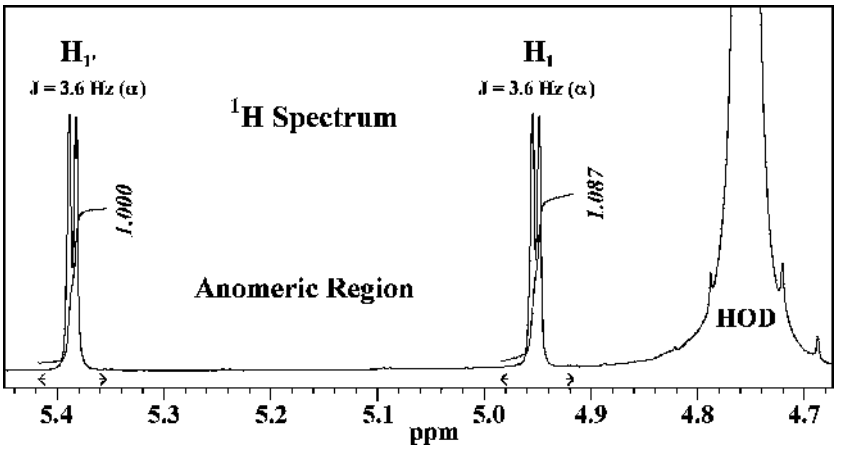


Figure 1.18

1.2.7 Terpenoids

A vast variety of plant and animal natural products are based on a repeating 5-carbon unit called isoprene: C–C(–C)–C–C. The end of the chain nearest the branch can be called the "head" and the other end is the "tail." Two isoprene units connected together make up a "monoterpene" or 10 carbon natural product (e.g., menthol, Fig. 1.19). Six isoprene units make a "triterpene" with 30 carbons. Cholesterol loses three of these in the biosynthetic process to give a 27 carbon "steroid" with four rings (Fig. 1.20). The *trans* ring junctures and the planar olefin "lock" the cyclohexane chairs into a single rigid conformation with well-defined axial and equatorial positions, just as we saw for the glucose ring in sucrose. Another triterpene skeleton that retains all 30 carbons is shown in Figure 1.21; the D and E rings are also locked in cyclohexane chair conformations.

1.2.8 Menthol

Menthol (Fig. 1.19) is a monoterpene natural product obtained from peppermint oil. Typical of terpenoids, menthol is only slightly soluble in water and is soluble in most organic solvents. The *trans* arrangement of the methyl and isopropyl substituents on the cyclohexane

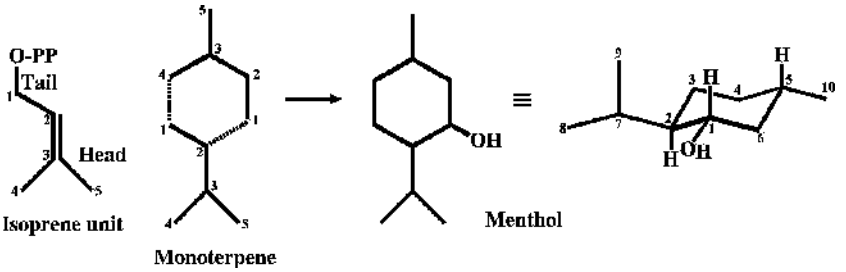


Figure 1.19

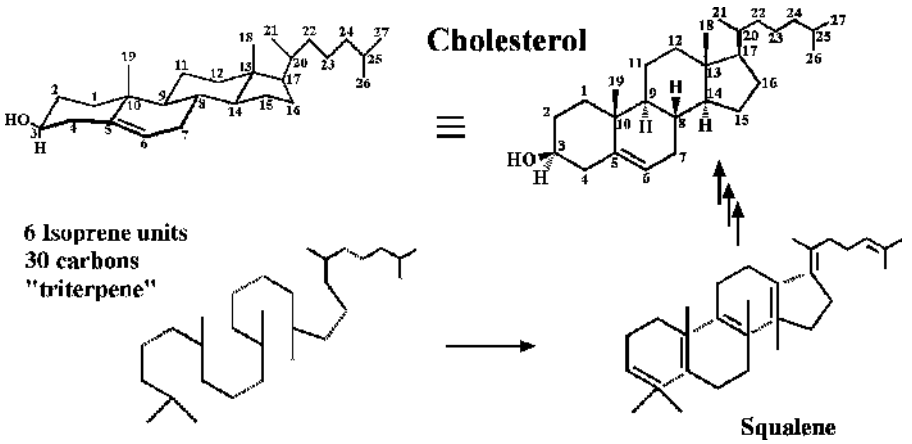


Figure 1.20

ring lock the ring in a single chair conformation with all of the substituents in the equatorial position.

The 250 MHz ^{1}H spectrum of menthol is shown in Figure 1.22. We see that even at 250 MHz a number of single proton signals are resolved (i.e., not overlapped with any other signals): "h," "l," "m," and "n." Integral values (normalized to one for the smallest resolved peaks) add up to 19.88 or 20 protons, consistent with the molecular formula $C_{10}H_{20}O$. The tall, sharp peaks at the right-hand side ("a," "b," and "c") represent the methyl groups, which usually give the most intense peaks because there are three equivalent protons. The most downfield signal ("*n*") corresponds to the proton closest to the single functional group, the H–C–OH proton. The OH proton chemical shift depends on concentration because of hydrogen bonding with the OH oxygen of other menthol molecules in solution—looking at different samples it can be identified as the singlet peak at 1.55 ppm. It is a singlet because *J*-coupling interactions are averaged to zero by exchange: a particular OH proton on one menthol molecule jumps to another menthol molecule rapidly so it is constantly exposed to different H–C–OH protons at position 1, some in the α state and some in the β state, so it sees only a blur and appears as a singlet instead of a doublet. The H-1 proton at 3.37 ppm appears at a chemical shift typical for "alcohol" protons: protons attached to an sp^3 hybridized (i.e., tetrahedral) carbon with a single bond to oxygen (3–4 ppm). Its coupling

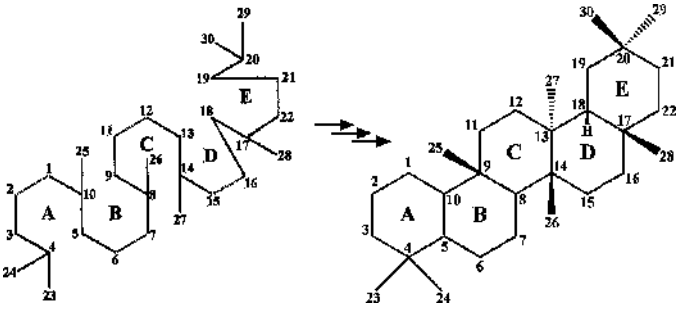


Figure 1.21

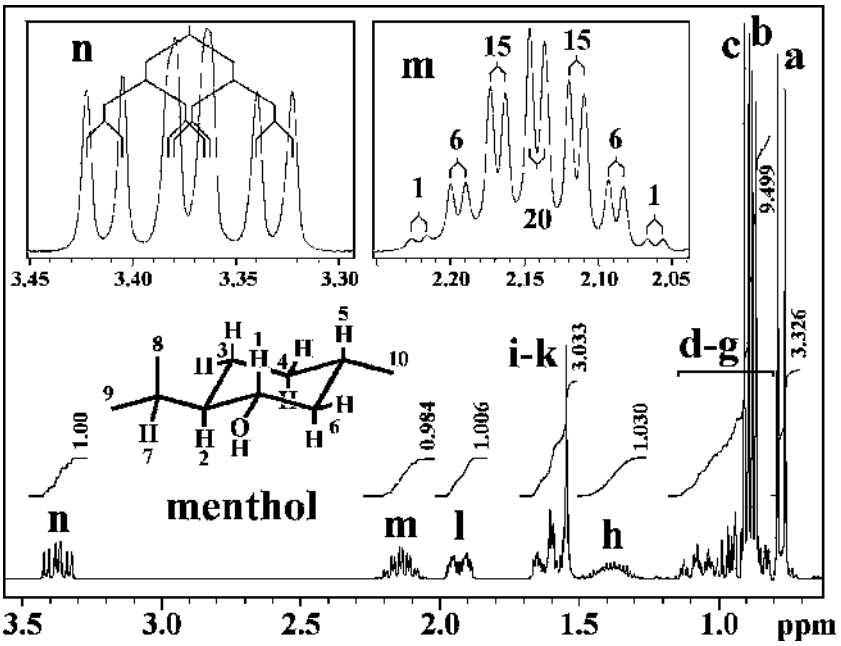


Figure 1.22

pattern (inset, Fig. 1.22) shows two nearly equal large couplings ($J = 9.9$ and 10.9 Hz) due to the axial–axial relationships to H–2 and H–6_{ax}. Because these two couplings are not equal, the double-triplet (1:1:2:2:1:1) pattern is distorted, widening the two center peaks and making them shorter (less than twice the height of the four outer peaks). This is an example of an unresolved splitting: we should be seeing eight peaks, but we see only six because the separation of the third and fourth peaks (and of the fifth and sixth) is comparable to the peak width. This separation is about 1.0 Hz (10.9–9.9) and the peak width (measured at half-height) of the outer peaks is 1.3 Hz. Later on we will see how resolution enhancement can be used to make the peaks sharper and at least begin to see the separation of this multiplet into eight peaks. The third coupling of the double-doublet-doublet (ddd) is 4.3 Hz, due to the interaction with H-6_{eq}. This coupling is axial–equatorial (*gauche* relationship), so it is smaller, in the middle range of observed couplings.

The peak at 2.14 ppm is a double septet, with an intensity ratio 1:1:6:6:15:15:20: 20:15:15:6:6:1:1 and J couplings of 7.0 Hz for the septet and 2.6 Hz for the doublet. The only proton with six coupling partners is the CH proton of the isopropyl group, H-7. A J coupling near 7.0 Hz is typical of a vicinal coupling with free rotation (of the methyl group) averaging the dihedral angle effects. The additional coupling of 2.6 Hz is due to its interaction with H-2. The outer peaks of the septet are only one twentieth of the intensity of the center peaks, so unless you have very good signal-to-noise you might miss these peaks and mistake it for a quintet. The intensity ratio for this "quintet" is 1:2.5:3.3:2.5:1, instead of the expected 1:4:6:4:1. The remaining resolved single-proton peaks ("l" and "h") cannot be assigned without advanced experiments. The strong, sharp peaks at the right-hand side of the spectrum correspond to the methyl groups. All three methyl groups are attached to CH carbons ("methine" carbons) so they will appear as doublets. One doublet ("a") is separate

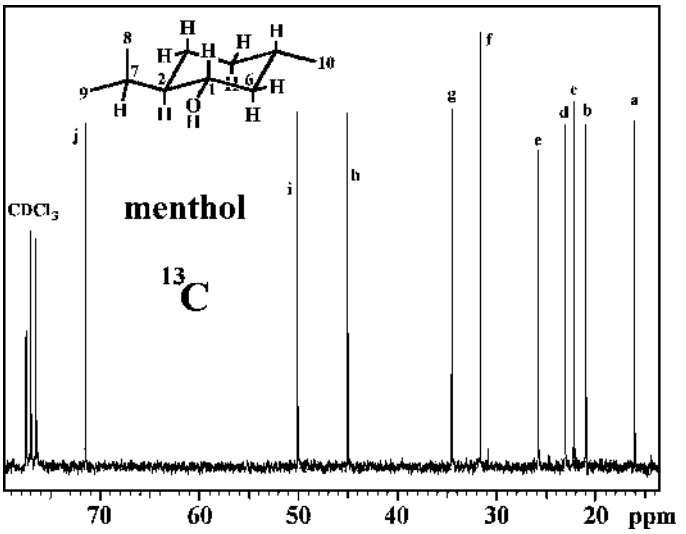


Figure 1.23

from the other two ("b" and "c"), but we cannot make the assumption that it represents the "lone" methyl group H-10. Because this is a chiral molecule, the isopropyl group can have distinct environments and widely different chemical shifts for the two methyls. The *J* couplings for these three doublets are all around 7.0 Hz due to free rotation of the C–C bond, although one is slightly lower (6.6 Hz) and this corresponds to the CH–C$\underline{H}_3$ group, C-10. Chemical shifts for the methyl groups are a bit less than 1 ppm, typical for methyl groups in a saturated hydrocarbon environment, far from any functional group. The same is true for the four proton signals buried in the overlapped region between 0.75 and 1.15 ppm: they are shifted downfield of the methyl groups slightly because of the higher degree of substitution (CH_2 and CH), but they are not close to any functional group.

The ^{1}H-decoupled ^{13}C spectrum of menthol (Fig. 1.23) has ten peaks in addition to the three solvent peaks. All we can say about it is that the most downfield peak ("*j*") corresponds to the carbon with the alcohol oxygen: C-1. We can see a bit of a gap between this peak and the rest of the peaks, and we expect singly oxygenated sp^3 carbons in the range 50–90 ppm, with methine carbon (CHOH) typically in the range 70–80 ppm. Every time we replace an H with C we add about 10 ppm to the chemical shift, so compared to CH_3O (50–60 ppm) we can add about 20 ppm to get the range of CHOH. The rest of the carbons can only be assigned if we can assign the attached protons and then correlate the ^{13}C shifts with the ^{1}H shifts by a 2D spectrum such as HSQC.

1.2.9 Cholesterol

Cholesterol (Fig. 1.20) is a steroid, the same rigid five-ring backbone used for the mammalian sex hormones. There are only two functional groups: an olefin (C-5, C-6) and an alcohol (C-3). The bulk of the molecule can be described as saturated hydrocarbon. There are five methyl groups: two are attached to quaternary carbons so they should appear as singlets; and three are attached to CH carbons so they should appear as doublets. Most of the protons in the A, B, and C rings can be described as "axial" or "equatorial" due to the rigid,

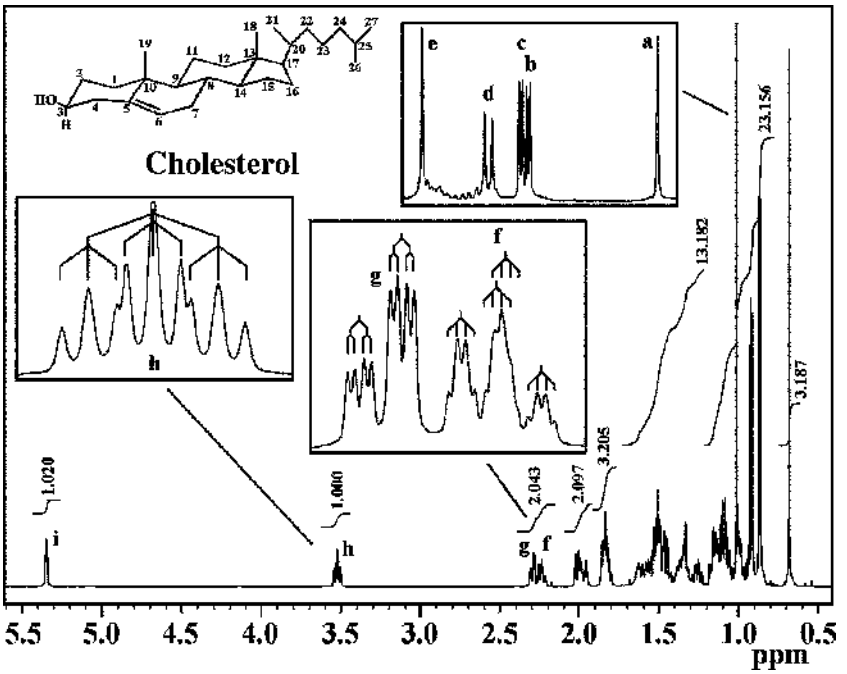


Figure 1.24

locked cyclohexane ring structure. The 600 MHz ^{1}H spectrum is shown in Figure 1.24. The total integration adds up to 48.89 protons, a bit high for the molecular formula $C_{27}H_{46}O$ but consistent with the fact that the resolved peaks in the upfield part of the spectrum integrate several percent above the expected integer values. The olefin functional group (C-5 and C-6) has a single proton, H-6, which we expect in the region 5–6 ppm. Thus the one-proton signal at 5.35 ppm (peak "i") can be assigned to H-6 (only the resolved peaks are identified with letters). The other functional group is an alcohol, and we expect the H–C–OH proton at 3–4 ppm; we can assign the one-proton signal at 3.52 ppm (peak "h") to H-3. The splitting pattern of H-3 can be described as a triplet of triplets, with a small triplet coupling of 4.6 Hz and a large triplet coupling of 11.2 Hz (Fig. 1.24, proton h inset). Because the OH group is equatorial, H-3 is axial and is split by its two equatorial neighbors, H-2_{eq} and H-4_{eq}. Because both the relationships are axial–equatorial (*gauche*), the couplings are identical and in the medium range (4.6 Hz). H-3 is also split by its two axial neighbors, H-2_{ax} and H-4_{ax}. Each of these relationships is axial–axial (*anti*), so the couplings are identical and large (11.2 Hz). Taken together, we get a large triplet (1:2:1 intensity ratio, $J = 11.2$), with each of the three arms split into a smaller triplet (1:2:1 ratio, $J = 4.6$). These coupling relationships are shown in the partial structure in Figure 1.25.

Moving from left to right, the next resolved peak is a two-proton multiplet at 2.20–2.32 ppm (peaks "g" and "f"). The most likely assignment for these peaks would be H-4_{ax} and H-4_{eq}, since C-4 lies between the two functional groups and we expect the minor downfield-shifting effects of both groups to add together, pulling the H-4 resonances out of the "pack" of saturated hydrocarbon peaks (0.6–1.7 ppm). We cannot be absolutely sure of this assignment until we see two-dimensional data, but this is a reasonable guess. Looking at the fine structure of these two peaks (inset, Fig. 1.24) and ignoring the smaller couplings, we

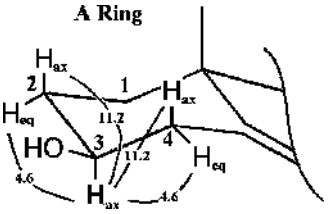


Figure 1.25

see a triplet on the right (peak "g") and a doublet on the left (peak "f"). These two peaks are "leaning" toward each other, with the outer peaks reduced in intensity and the inner peaks increased relative to a "standard" doublet (1:1) or triplet (1:2:1). This distortion of peak intensities is a common feature when the chemical shift difference (in Hz) is relatively small compared to the J coupling between the two protons. In this case, the chemical shift difference is 0.053 ppm or 32 Hz and the large geminal ($^2J_{HH}$) coupling is 13.0 Hz, leading to a large distortion of peak intensities. The basic doublet and triplet patterns are further split by smaller couplings: each side of the doublet is split into a double doublet ($J = 5.0$ and 2.1 Hz) and each of the three peaks of the triplet on the right is split into a quartet ($J = 2.8$ Hz). Ignoring the "small" couplings, we can ask how many large couplings each proton experiences and in this way count the number of geminal and axial–axial relationships. The "doublet" peak ("*g*") has only the geminal ($^2J_{HH}$) coupling, which is always large for saturated (sp^3 hybridized) carbons. So it must be the equatorial proton, H-4_{eq}. The "triplet" peak ("f") has the geminal coupling and one axial-axial coupling, so it must be the axial proton, H-4_{ax}, which has an axial–axial coupling to H-3. The smaller couplings can be explained as follows: H-4_{eq} has one equatorial–axial coupling (5.0 Hz) to H-3_{ax} and one "*W*" coupling ($^4J_{HH}$) to H-2_{ax} (2.1 Hz). A "*W*" coupling occurs in a series of saturated carbons when the H–C–C–C–H network is rigidly aligned in a plane in the form of a "*W*." H-4_{ax} has small long-range couplings to H-6, H-7_{ax}, and H-7_{eq}, all around 2.8 Hz. These long-range couplings will be discussed later, but you can think of the C=C double bond as a kind of "conductor" for J couplings that allows these small interactions to occur over four or five bonds as long as the double bond is in the path: H–C–C=C–H ("allylic coupling") and H–C–C=C–C–H ("bis-allylic" coupling). In each case, if you remove the C=C from the path, you have a close bonding relationship of two bonds ("geminal") or three bonds ("vicinal").

The five methyl groups of cholesterol give rise to tall, sharp peaks in the upfield region of the ^{1}H spectrum (inset, Fig. 1.24, peaks a–e). We can see two singlet methyl signals ("a" and "e") that correspond to the "angular" methyls attached to the quaternary carbons at the A-B and C-D ring junctures (C-18 and C-19). Later on we will use an NOE experiment to assign these two peaks specifically, taking advantage of the proximity of CH_3-19 to the H-4_{ax} proton. There are also three doublet methyl signals ("b," "c," and "d") that correspond to the three methyl groups in the side chain attached to CH carbons: C-21, C-26, and C-27. Specific assignments for these signals will require two-dimensional experiments such as HSQC and HMBC.

The 125 MHz ^{1}H-decoupled ^{13}C spectrum of cholesterol is shown in Figure 1.26. Because the ^{13}C nuclear magnet is only about one fourth as strong as the ^{1}H nuclear magnet, the ^{13}C resonant frequency is always about one fourth of the ^{1}H frequency in the same magnetic field. Thus on a "500 MHz" NMR spectrometer (i.e., an 11.74 T B_o field in which ^{1}H resonates at 500 MHz) the ^{13}C frequency is about 125 MHz. The $CDCl_3$ peaks (a 1:1:1

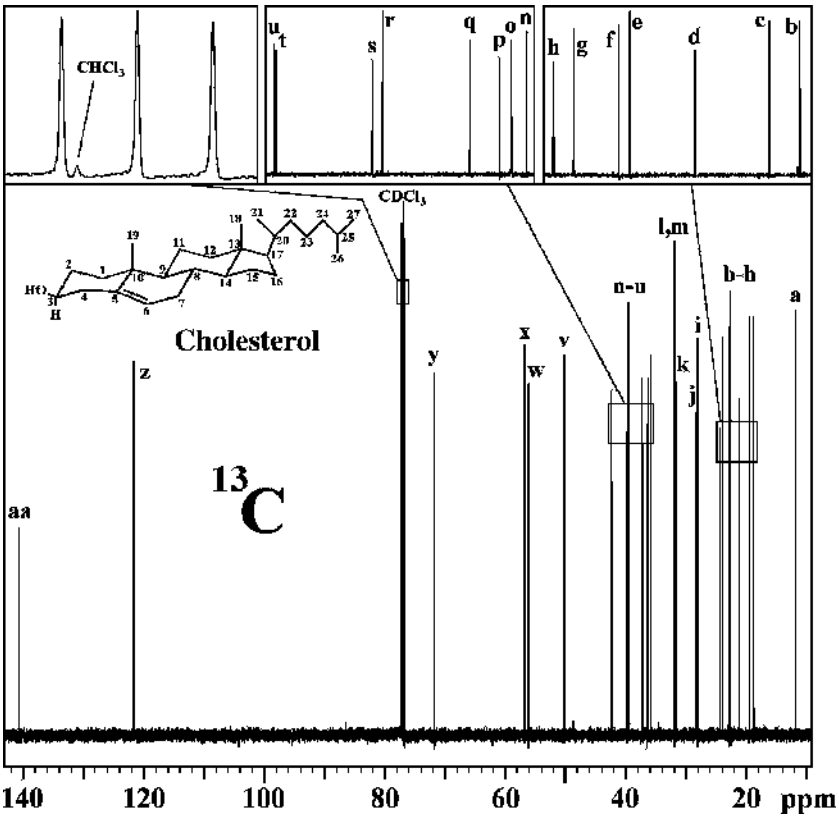


Figure 1.26

triplet at 77.0 ppm) appear at the center of the spectrum. Note that there is a small peak due to $CHCl_3$ at 77.21 ppm (upper left inset, Fig. 1.26). This may be residual $CHCl_3$ in the $CDCl_3$ (0.2%) or $CHCl_3$ residue in the solid cholesterol sample. Such a small amount of $CHCl_3$ is visible in the spectrum due to the effects of relaxation and decoupling. Because the ^1H nuclear magnet is about seven times stronger than the ^2H nuclear magnet, the ^{13}C in $CHCl_3$ relaxes faster than the ^{13}C in $CDCl_3$ and thus gives a stronger NMR peak. In addition, due to ^1H decoupling there is only one peak for $CHCl_3$, and this makes for a taller peak than these for $CDCl_3$, whose ^{13}C intensity is divided into three peaks. Note also that there is a deuterium isotope effect on the ^{13}C chemical shift: $CHCl_3$ appears 0.21 ppm downfield of $CDCl_3$.

In addition to these solvent peaks, we can count 26 peaks in the spectrum. Because there are 27 carbons in the cholesterol molecule (three are lost in the biosythesis from a triterpene precursor), there must be one peak that accounts for two carbons. The tallest peak (labeled "l, m") in fact corresponds to two different carbons with nearly identical chemical shifts. The most downfield peaks ("aa" and "z") are in the olefin/aromatic region of the ^{13}C spectrum (120–140 ppm), so they must correspond to C-5 and C-6. Peak "aa" is less intense ("shorter") than all of the other peaks because of slow relaxation: it must be a quaternary carbon. We will see that the proximity of protons is the primary means of relaxation of ^{13}C nuclei, so carbons lacking a proton relax much more slowly and give less intense peaks, especially if

the relaxation delay is short (in this case the recycle delay was only 1.74 s (1.04 s acquisition time and 0.7 s relaxation delay). So we can assign peak "aa" (140.75) to C-5 and peak "z" (121.69) to C-6. Note also that the more substituted carbon, C-5 (three bonds to carbon) is shifted downfield relative to C-6 (two bonds to carbon) due to the steric crowding effect.

Peak "y" (71.78 ppm) is in the "alcohol" region (C–O) in the range expected for methine carbon (CH–O), so it can be assigned to C-3. The next three carbons (peaks "x," "w," and "v," 50–57 ppm) could be methoxy (CH_3O) groups, but because we have accounted for all the functional groups of cholesterol they must be either close to these functional groups (inductive effect) or shifted downfield due to steric crowding. The inductive effect (electron withdrawing and donating groups) is most important for ^1H chemical shifts, so let us consider the steric effects. The most sterically crowded carbons in the cholesterol structure are the methine (CH) groups next to an sp^3-hybridized quaternary carbon: C-9, C-14, and C-17. These three carbons account for this group of downfield-shifted peaks. The rest of the ^{13}C peaks (a–u) lie in the region of saturated hydrocarbon (sp^3 carbon with no functional groups) and cannot be assigned without more advanced experiments such as DEPT and 2D HSQC/HMBC.

1.3 TYPICAL VALUES OF CHEMICAL SHIFTS AND COUPLING CONSTANTS

1.3.1 Typical Values of ^1H Chemical Shifts

The chemical shift scale can be roughly divided into regions that correspond to specific chemical environments (olefinic, aromatic, etc.). Knowing these regions gives you a useful first guess as to the interpretation of a resonance, but you must keep in mind that more than one functional group might contribute in an additive fashion to the chemical shift. For example, we can estimate the chemical shift of a CH_2 group situated between an olefin and a carbonyl group (C=C–CH_2–C=O) as follows: A CH_3 group next to an olefin or carbonyl resonates at 2.1 ppm (see below under "b"). This represents a downfield shift of 1.25 ppm from a "hydrocarbon" CH_3 group (0.85 ppm, under "a" below). Thus we can estimate the shift for this CH_2 as follows:

1.2	CH_2 in hydrocarbon environment
+2.5	effect of neighboring C=C or C=O (+1.25 ppm) times 2
3.7	total: predicted chemical shift of C=C–CH_2–C=O

If we saw a resonance at 3.7 ppm, our first guess would be a proton on a singly oxygenated carbon, -CH_2–O- (part "d" below), but it is dangerous to get "locked into" that idea because the possibility exists of smaller effects adding together, as shown in the example above.

(a) "*Hydrocarbon*": attached to an sp^3-hybridized carbon and many bonds away from any unsaturation or electronegative atom. The same differences between methyl, methylene, and methine are observed in all other environments.
   1. CH_3 0.85,
   2. CH_2 1.2,
   3. CH 1.8.

(b) α to a carbonyl, olefin, or aromatic group: H–C–C=O or H–C–C=C: 2.1.

(c) *Next to a nitrogen*: H–C–N (attached to an sp^3-hybridized carbon with one single bond to nitrogen): 2.6.

(d) *Next to an oxygen*: H–C–O (attached to an sp^3-hybridized carbon with one single bond to oxygen):
   1. alcohol or ether (H–C–OH or H–C–O–C): 3.3
   2. ester (H–C–O–CO–R): 3.8.

(e) "*Olefinic*": H–C=C: 5–6 ppm (where C=C is not part of an aromatic ring). Resonance effects can shift out of this range: up to 1 ppm upfield for electron-donating groups (e.g., H–C=C–O–) and 1 ppm downfield for electron-withdrawing groups (e.g., H–C=C–C=O). This is a result of increased or decreased electron density at the carbon bearing the proton in resonance structures such as $H–C^-–C=O^+–$ (electron donation: vinyl ether) and $H–C^+–C=C–O^-$ (electron withdrawal: α,β-unsaturated ketone).

(f) "*Anomeric*": H–C(–O)–O (attached to an sp^3-hybridized carbon that has two single bonds to oxygen): 5–6 ppm.

(g) "*Aromatic*": attached to carbon of a benzene, furan, pyrrole, pyridine, indole, naphthalene, and so on, ring: generic 7–8 ppm. The effect of substituents due to resonance effects (strongest at *ortho* position):
   1. electron-rich carbon (e.g., *ortho* or *para* to O or N of phenol, aniline, phenolic ether, or in an electron-rich heteroaromatic: pyrrole, furan): 6–7 ppm;
   2. electron-poor carbon (e.g., *ortho* or *para* to C=O or NO_2, or in the two or four position of pyridine): 8–9 ppm.

(h) *Aldehyde*: H–C=O: 10 ppm.

(i) Carboxylic acid: HO–C(=O) or *phenolic*: HO–C(aromatic): 12–14 ppm.

Note that there are other types of protons not listed here that can fall into the same chemical shift ranges listed above. The above categories are simply the most common ones. Also, through-space ("anisotropic") effects of unsaturated groups (C=C, C=O, and aromatic rings) can change chemical shifts from the above categories in ways that depend on conformation.

1.3.2 Typical Values of $^1H–^1H$ Coupling Constants (J)

A superscipt preceding the letter J refers to the number of bonds between the two nuclei: ^3J means three-bonds or vicinal (H–C–C–H) and ^2J means two bonds or geminal (H–C–H). Sometimes a subscript is used to clarify which types of nuclei are coupled: J_{HH} means proton-to-proton coupling.

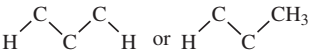
(a) $^3\boldsymbol{J}_{HH}$ (vicinal):
   1. In freely rotating alkyl groups (e.g., $CH_3–CH_2$-): 7.0 Hz
   2. In benzene rings: $^3J_{HH} = 7.5$, $^4J_{HH} = 1.5$, $^5J_{HH} = 0.7$ Hz
   3. In a pyridine ring: $J_{2,3} = 5.5$, $J_{3,4} = 7.6$, $J_{3,5} = 1.6$, $J_{2,5} = 0.9$, $J_{2,6} = 0.4$ Hz
   4. In a furan (pyrrole) ring: $J_{2,3} = 1.8$ (2.6), $J_{3,4} = 3.4$ (3.5), $J_{2,4} = 0.9$ (1.3), $J_{2,5} = 1.5$ (2.1) Hz
   5. In a chair cyclohexane ring: $J_{1,2} = 12$ (ax-ax), 3 Hz (eq–ax or eq–eq)
   6. In a chair six-membered ring sugar, $J_{1,2}$(eq–ax) = 4, $J_{1,2}$(ax–ax) = 9 Hz.
   7. In an isolated olefin C_1H-C_2H=C_3H-C_4H: $J_{2,3}$ = 8–12(cis), 14–17(trans), $J_{1,2} = 7$ Hz
   8. In a cyclopropane, 7–13(cis), 4–9 Hz (trans)

(b) $^2J_{HH}$ (geminal):

1. In a terminal olefin C=CH_2, $J_{1,1}$ = 0–2 Hz
2. On a saturated (sp^3) carbon: 12–15 Hz (12.5 in a cyclohexane chair)

(c) Long-range ($^4J_{HH}$ and $^5J_{HH}$):

1. Isolated olefin C_1H–C_2H=C_3H–C_4H: $J_{1,3}$ and $J_{2,4}$ ("allylic") 0–3; $J_{1,4}$ ("bis-allylic") 1–2 Hz
2. "*W*" coupling (saturated chain in rigid planar *W* conformation):



1–4 Hz (2.5 in a cyclohexane chair: $J_{1,3}$ eq–eq)

1.3.3 Typical Values of ^{13}C Chemical Shifts

^{13}C chemical shifts are more sensitive to steric crowding effects and less sensitive to through-space effects of double bonds than ^1H chemical shifts. Increasing the substitution of a carbon (CH_3 to CH_2 to CH to C) leads to downfield shifts of about 10 ppm in each step.

(a) *Carbonyl* (C=O) shifts are far downfield (155–210 ppm) and the peaks are generally weak due to slow relaxation of quaternary carbons (except aldehydes, which are not quaternary). Ketones and aldehydes: 200–210 (isolated), 190–200 (α,β unsaturated), Carboxylic acids, esters, amides: 170–180, Urethanes (NC(O)O): 150–160.

(b) *Aromatic* carbons are typically 120–130 ppm for unsubstituted positions (i.e., CH) and 136–150 at the position of alkyl substitution (weak quaternary peak). Strong electron-withdrawing groups (O, N, NO_2, F) can shift the substituted (*ipso*) carbon to 150–160. Substituents that can donate to the ring by resonance (O, N) shift the *ortho* carbons and, to a lesser extent, the *para* carbon upfield to 110–120. Likewise, substituents that are electron-withdrawing by resonance (CO, CN) shift the *ortho* and *para* carbons downfield to 130–140. *Meta* carbons are unaffected because resonance structures cannot place + or − charges at these positions. Nitro (NO_2) is unusual in that it shifts the *ortho* carbon upfield about 5 ppm and the *para* carbon downfield about 6 ppm. At the point of attachment of the substituent ("*ipso*" carbon) the range is 130–140 for "neutral" substituents and farther downfield (150–160) for electron-withdrawing substituents (e.g., O).

(c) *Nitrile* (CN with triple bond): 110–120.

(d) *Olefinic* carbons (isolated C=C) fall in the same range as aromatic CH: 120–130. Substitution pulls this value downfield: a quaternary olefinic carbon resonates in the range of 140 ppm. They can also be shifted by resonance effects when electron withdrawing or donating groups are attached, just like in aromatic systems. For example, a quaternary β carbon of an α, β-unsaturated ketone resonates in the 170–180 ppm range, making it easy to confuse with an ester carbonyl carbon. This is due to the resonance structure: $-C_\beta{}^+-C_\alpha=C-O^-$.

(e) *Anomeric* carbons of sugars (O–C–O) and in acetals and ketals: 90–110.

(f) *Singly oxygenated* carbons (C–O single bond): 50–85. CH_2OH carbon is in the upfield range (60–70) and quaternary carbons in the downfield range (75–85). A methoxy group (CH_3O) is even farther upfield: 50–60.

(g) Carbons with a single bond to *nitrogen*: 50–70.

(h) *Saturated* carbons with no nearby electronegative atoms or double bonds: 10–50, with CH_3 on the upfield side and quaternary carbons on the downfield side. Strained rings (cyclobutane, cyclopropane) show significant upfield shifts.

1.4 FUNDAMENTAL CONCEPTS OF NMR SPECTROSCOPY

1.4.1 Spin

The atomic nucleus can be viewed as a positively charged sphere that is spinning on its axis. This spin is an inherent property of the nucleus, and because charge is being moved it creates a small magnetic field aligned with the axis of spinning. Thus we can consider the nucleus as a tiny, permanent bar magnet. Because different isotopes of a given atom (e.g., ^{12}C, ^{13}C, ^{14}C) have different numbers of neutrons in the nucleus, they have different magnetic properties. For this reason we only talk about specific isotopes in NMR: ^{1}H, ^{19}F, ^{11}B, and so on, and our attention is focused on the nucleus of these isotopes.

The nucleus of each isotope has the following intrinsic properties:

1. Magnetogyric ratio, γ. This is essentially the strength of the nuclear magnet. Different nuclei have different magnet strengths; for example, the ^{13}C nuclear magnet is only one-fourth as strong as the ^{1}H nuclear magnet, and the ^{15}N nuclear magnet has only one-tenth of the strength of the ^{1}H magnet. The γ is the same for every nucleus of a given type (e.g., ^{19}F), regardless of its position within a molecule.
2. "Spin." This determines the number of quantum states available for the nucleus.

spin-0	no magnetic properties	
spin-½	2 states:	1/2,−1/2
spin-1	3 states:	1, 0, −1
spin-3/2	4 states:	3/2, 1/2,−1/2, 3/2
etc.		

For example, a spin-½ nucleus can be viewed as having two quantum states: one with the spin axis at a 45° angle to the external magnetic field and one with the spin axis at a 135° angle to the external field. A spin-1 nucleus can be viewed as having three possible states: 45°, 90°, and 135°. In this book we will be concerned primarily with spin-½ nuclei.

Here are some examples showing the composition of the nucleus (p = protons, n = neutrons):

Spin-0	spin-½	spin > 1/2
^{12}C $(6p + 6n)$	^{1}H $(1p + 0n)$	^{2}H $(1p + 1n)$
^{16}O $(8p + 8n)$	^{3}H $(1p + 2n)$	^{14}N $(7p + 7n)$
^{18}O $(8p + 10n)$	^{13}C $(6p + 7n)$	^{17}O $(8p + 9n)$
	^{15}N $(7p + 8n)$	
	^{19}F $(9p + 10n)$	
	^{29}Si $(14p + 15n)$	
	^{31}P $(15p + 16n)$	

Note that there is a pattern: Nuclei with an even number of protons and neutrons (even–even) have spin zero; "odd–even" and "even–odd" nuclei tend to be spin-½; and "odd-odd" nuclei tend to have a spin greater than 1/2. This is just a rule of thumb (e.g., ^{17}O violates the "rule"). Nuclei with spin greater than 1/2 are more difficult to observe than spin-½ nuclei because they have a "nuclear quadrupole moment" that makes their NMR peaks very broad. For this reason, most NMR work is focused on the spin-½ nuclei. Because NMR is usually done in deuterated solvents (D_2O, CD_3OD, etc.), we will have to occasionally consider the effects of a spin-1 (three quantum states) nucleus.

1.4.2 Precession

When we place a spin-½ nucleus in a strong external magnetic field, the nucleus wants to align itself with the magnetic field, just like a compass needle moves to align with the earth's magnetic field. But because the nucleus is spinning (i.e., it has an intrinsic property of angular momentum), it cannot simply change its angle with the magnetic field from 45° to 0°. The torque it experiences from the external magnetic field instead causes the spin axis to "wobble" or precess around the magnetic field direction. This is analogous to a spinning top or gyroscope, which responds to the torque produced by the earth's gravitational field by describing a circle with its spin axis. The precession rate of the nucleus in a magnetic field is the *resonant frequency* referred to in the name "nuclear magnetic resonance." The precession rate is in the range of radio frequency, tens or hundreds of megahertz, or millions of rotations per second. In this classical model the torque exerted on the nucleus is proportional to both the laboratory magnetic field strength, B_o, and to the strength of the nuclear magnet, γ. The rate of precession is proportional to the torque, so we have:

$$\omega_o = 2\pi\nu_o = \gamma B_o$$

It cannot be emphasized too much that the resonant frequency in NMR is proportional to the magnetogyric ratio, γ, and to the laboratory magnetic field strength, B_o. This relationship forms the basis of nearly every phenomenon observed in NMR. There are two ways to measure the precession rate: the angular velocity, ω_o, in units of radians per second and the frequency, ν_o, in units of cycles per second or hertz. In this book we will use frequencies in hertz. This frequency is sometimes called the Larmor frequency, and the zero subscript refers to this fundamental frequency, which results from the laboratory magnetic field interacting with the nucleus' magnetic field.

As an example, consider a proton (^1H nucleus) in a 7.05 T laboratory magnetic field:

$$\gamma_H = 2.675 \times 10^8\,T^{-1}\,rad\,s^{-1}$$

$$B_o = 7.05\,T$$

$$\nu_o = \gamma B_o/2\pi = 3.001 \times 10^8\,Hz = 300.1\,MHz$$

Such a magnet would be called a "300 MHz" magnet because the ^1H nucleus precesses at a rate of 300 MHz in this magnet. NMR magnets are almost never described in tesla but rather by their ^1H resonance frequency. This can be confusing because if you are observing ^{13}C nuclei on a 500-MHz NMR instrument, you are operating at a resonant frequency of 125 MHz, not 500 MHz. Because the resonant frequency for a given magnet (NMR magnets

have a fixed magnetic field strength) is proportional to the magnetogyric ratio, γ, of the nucleus being observed, the NMR frequencies for different nuclei will always be in the same ratio: the ratio determined by their relative γ values.

	$B_o = 7.05$ T	$B_o = 11.74$ T	γ/γ_H(%)	γ_H/γ	Abund. (%)
^{1}H	300.0 MHz	500.0 MHz	100.0	1.000	99.98
^{2}H	46.05	76.75	15.35	6.515	0.015
^{13}C	75.43	125.72	25.14	3.977	1.11
^{15}N	30.40	50.66	10.13	9.870	0.37
^{19}F	282.23	470.39	94.08	1.063	100
^{31}P	121.44	202.40	40.48	2.470	100

Proton (^{1}H) is the king of the nuclei (radioactive tritium, ^{3}H, is actually 6.7% stronger) and all other nuclei can be viewed in terms of their magnet strength (γ) relative to proton. ^{19}F is a bit weaker than proton (94%) and ^{31}P is about 40% of the proton frequency. Proton is about four times stronger than ^{13}C, seven times stronger than ^{2}H, and 10 times stronger than ^{15}N. Of all these spin-½ nuclei, three have very low natural abundance: 1.11% for ^{13}C, 0.37% for ^{15}N, and 0.015% for ^{2}H. This makes them difficult to observe because the signal strength in NMR is proportional to the number of NMR-active nuclei in the sample: for ^{13}C, only one in every 100 carbon atoms is participating in the NMR experiment. However, we will see that there are advantages to having a "dilute" nucleus—one that is "sprinkled" lightly over the collection of molecules in the sample. We can improve on nature by isotopically labeling or enriching the sample either by synthesis from labeled starting materials or by biosynthesis on labeled growth media. Many compounds can be purchased with nearly 100% abundance of ^{13}C, ^{2}H, or ^{15}N either at one site in the molecule or at all sites ("uniformly labeled"). This can be very costly, but the benefits often justify the cost. For example, with uniform ^{13}C labelling, the ^{13}C signal can be increased by a factor of 100, reducing the experiment time by a factor of 10,000. It should be noted that all three of these isotopes are stable, that is, they are not radioactive.

1.4.3 Chemical Shift

Because at any given field strength each nucleus has a characteristic resonant frequency, we can "tune" the radio dial to any nucleus we are interested in observing. We can think of the various NMR-active nuclei in the sample as "radio stations" that we can tune into very accurately, just as stations come into tune in a very narrow range of frequencies on an FM radio. Having chosen a "station" to listen to, what can we learn by observing a particular type of nucleus? The resonant frequency is always, always, always proportional to the magnetic field:

$$\nu_o = \gamma B_o / 2\pi$$

but the exact magnetic field *experienced by the nucleus* may be slightly different than the external magnetic field. The nucleus is located at the center of a cloud of electrons, and we know that electrons are easily pulled away or pushed toward an atom, changing the electron density around that nucleus. Furthermore, electron clouds can begin to circulate under the influence of the laboratory field, creating their own magnetic fields, which subtract from or

add to the external field. So the nucleus "feels" a slightly different field, depending on its position within a molecule (its "chemical environment"):

$$B_{\text{eff}} = B_{\text{o}}(1-\sigma) \quad \nu_{\text{o}} = \gamma B_{\text{eff}} = \gamma B_{\text{o}}(1-\sigma)$$

Where σ is a "shielding constant" in units of parts per million, which reflects the extent to which the electron cloud around the nucleus "shields" it from the external magnetic field. These differences, which we call "chemical shifts" are really tiny: for a ^{1}H nucleus the "spread" of resonant frequencies around the fundamental frequency is only about 10 ppm. That means that on a 500 MHz NMR instrument, the protons in a molecule might have a range of resonant frequencies between 499.9975 and 500.0025 MHz (0.0025 MHz is 5 ppm of 500 MHz), depending on their location within the molecule. Thus we tune in to a "station" (499.9975–500.0025 MHz) and study the tiny variations (chemical shifts) in resonant frequency to learn something about the chemical structure of the molecule. In this way, physics (and radio electronics) comes to the aid of chemistry in helping us determine a molecule's structure. An NMR spectrum is just a graph of intensity versus frequency for the narrow range of frequencies corresponding to the particular nucleus we are interested in. Each "peak" in this graph corresponds to a particular environment within the molecule, such as a particular hydrogen atom position in an organic structure. When each position in a molecule has a different chemical shift, we can "talk" to these atoms individually in NMR experiments, looking around at the local environment from the point of view of one atom in the structure at a time.

1.4.4 The Energy Diagram

If we consider the energy of a nucleus as it interacts with the external magnetic field, we see that there are two energy levels for a spin-½ nucleus. The "aligned" state (or α state) has the nuclear magnet aligned with the laboratory field, giving it a lower energy (more stable) state (Fig. 1.2). The "disaligned" state (or β state) is aligned opposite to the external field, resulting in a higher energy. The energy "gap" between these two levels is:

$$\Delta E = h\nu_{\text{o}} = h\gamma B_{\text{o}}/2\pi$$

where h is Planck's constant and ν_{o} is the Larmor ("resonant") frequency. This relationship between the energy gap between two quantum states and the frequency of electromagnetic radiation ("photons"), which can excite a particle from the lower energy level to the higher one, is fundamental to all forms of spectroscopy. The Larmor frequency, ν_{o}, is the same as the rate of precession of the spinning nucleus in the classical model (Fig. 1.1). Note that the size of the energy gap is proportional to the strength of the nuclear magnet (γ) and also to the strength of the laboratory magnetic field (B_{o}). Much effort and expense is put into getting the largest possible energy gap, as we design and build bigger and stronger superconducting magnets for NMR. We will see that a larger energy gap results in a more sensitive NMR experiment and better separation of the resonant frequencies of like nuclei in different chemical environments.

1.4.5 Populations

In an NMR sample there are a very large number of identical spins, a number approaching Avogadro's number. Even though there may be different types of spins (^{1}H, ^{13}C, ^{15}N, etc.)

within a molecule and different environments (H_1, H_2, H_3, etc.) within a molecule for each type of spin, we can view each molecule in a sample of a pure compound as identical and experiencing the same magnetic field. This is because the magnetic field has a very high degree of spatial homogeneity (on the order of parts per billion variation in B_o) and each molecule is tumbling very rapidly and has no preferred orientation in the magnetic field. Let us focus on one type of nucleus (^{1}H) and one position within the molecule (H_2). If there are N molecules in the sample (e.g., for a 1 mM sample, $N = 3 \times 10^{17}$), then we can talk about the N ^{1}H nuclei at position H_2 in the molecule: each one will be either aligned with the B_o field (lower energy or α state) or disaligned with the B_o field (higher energy or β state). At thermal equilibrium, there will be a tendency for the spins to prefer the lower energy state, but because the energy difference ($\Delta E = h\gamma B_o/2\pi$, where h is Planck's constant) is small compared to the average energy available at room temperature (kT), the populations are very nearly equal in the α and β states. The population of the more stable α state is $N/2 + \delta$, and the population of the less stable β state is $N/2 - \delta$, where δ is a very small number roughly equal to $N\Delta E/4kT$.

For example, at 7.05 T magnetic field (a 300 MHz NMR instrument) and 25 °C, the population difference for protons is 0.00064% of the number of nuclei N. This equilibrium population difference is a constant throughout the NMR experiment and, as we perturb the equilibrium, the spins will always try to return to this equilibrium population distribution. Because the measureable signal from a nucleus in the β state is exactly cancelled by the signal from a nucleus in the α state, it is this population difference that is the only material we have to work with and to detect in the NMR experiment. Because the difference is so small, the sensitivity of NMR is in many orders of magnitude lower than all other analytical techniques; so low, in fact, that NMR is not considered a branch of "analytical chemistry" but rather a tool used by organic chemists and biologists.

1.4.6 Net Magnetization at Equilibrium

At thermal equilibrium, the Boltzmann distribution determines the populations in various energy levels. For any two quantum states, the ratio of populations between the higher energy state and the lower energy state at equilibrium will always be:

$$P_\beta / P_\alpha = \mathrm{e}^{-\Delta E/kT}$$

where k is the microscopic gas constant, T is the absolute temperature in kelvin (K), and ΔE is the difference in energy between the two states—the "energy gap." We can think of kT as the average amount of total energy that a molecule has—analogous to the amount of money the average person is carrying in his or her pocket. ΔE is analogous to the price difference between a hamburger and a cheeseburger. If the amount of money the average person has (kT) is very small and the price difference (ΔE) is large, then nearly everyone will take the hamburger. But if the average person is carrying around a lot of money and the price difference is very small, there will be only a very slight preference for the hamburger. Just how big is kT compared to the energy difference in NMR? At 25 °C (298 K), kT is equal to 2478 J/mol. For a proton (^{1}H) in a 7.05 T magnetic field (ν_o = 300 MHz), the energy gap is:

$$\Delta E = h\nu_o = \hbar(\gamma B_o) = 0.0315\,\mathrm{J/mol}$$

So the energy gap is very, very small compared to the average energy that a molecule has at room temperature. Another way of saying this is that $\Delta E/kT$ is a number much, much less than 1. The exponential function can be simplified by approximation if the argument is a very small number compared to 1:

$$e^{-x} \sim 1 - x, \quad \text{if } x \ll 1$$

We can now simplify the Boltzmann equation:

$$P_\beta / P_\alpha = e^{-\Delta E/kT} \sim 1 - \Delta E/kT$$

The population difference, $P_\alpha - P_\beta$, is the most interesting thing for us because the magnetism of every "up" nuclear magnet cancels the magnetism of every "down" nuclear magnet, and it is only the difference in population that results in a "net magnetization" of the sample.

$$P_\beta / P_\alpha = 1 - \Delta E/kT;\ 1 - P_\beta / P_\alpha = \Delta E/kT;\ P_\alpha / P_\alpha - P_\beta / P_\alpha = \Delta E/kT$$

$$(P_\alpha - P_\beta)/P_\alpha = \Delta E/kT;\ P_\alpha - P_\beta = P_\alpha \Delta E/kT = N\Delta E/2kT$$

The last equality is obtained by substituting $N/2$ for P_α because both P_α and P_β are very close to half the total number of spins in the sample. Finally, substituting $\hbar\gamma B_o$ for ΔE we obtain:

$$P_\alpha - P_\beta = N\hbar\gamma B_o/2kT$$

Thus the population difference is proportional to the total number of spins in the sample and to the strength of the nuclear magnet (γ) and inversely proportional to the absolute temperature (T). If we add together all of the nuclear magnets, each spin in the β state cancels one in the α state and we end up with only $P_\alpha - P_\beta$ spins in the α state, aligned with the magnetic field. These add together to give a *net magnetization*, which is equal to the net number of spins pointing "up" times the magnet strength of each individual spin, γ. The magnitude of this net magnetization is called M_o,

$$M_o = \gamma (P_\alpha - P_\beta) = N\hbar\gamma^2 B_o/2kT$$

The net magnetization of the sample at equilibrium is proportional to the amount of sample (N), the square of the nuclear magnet strength (γ^2), and the field strength (B_o), and inversely proportional to the absolute temperature (T).

1.4.7 Absorption of Radio Frequency Energy

In order to measure the resonant frequency of each nucleus within a molecule, we need to have some way of getting the nuclei to absorb or emit RF energy. If we subject the sample to an oscillating magnetic field provided by a coil (the equivalent of a radio transmitter's antenna), a spin in the lower energy state can be "bumped" into the higher energy state if the radio frequency is exactly equal to the Larmor frequency, ν_o. Formally, one spin jumps up to the higher energy level and one "photon" of electromagnetic radiation (energy $h\nu_o$) is

absorbed. Unfortunately, there is another process that is equally likely, called "stimulated emission," in which one photon ($h\nu_o$) is absorbed by a spin in the upper (β) energy state, kicking it down to the lower state with the emission of two photons. So as long as our RF energy is applied at the resonant frequency, spins are jumping up (absorption of one photon) and down (emission of one photon) constantly. The rate of these processes is proportional to the population of spins in each of the two states: absorption occurs at a rate proportional to the number of spins in the sample that are in the lower energy state, and emission occurs at a rate proportional to the number of spins in the upper energy state.

In order to understand the net behavior of this system, we have to think about the populations (number of spins in the sample) in each of the two states. At thermal equilibrium, there will be a slight preference for the lower energy state according to the Boltzmann distribution. For now we will only think about this preference qualitatively; it turns out to be very small indeed at room temperature—a population difference of about 1 in 10^6 spins. But as long as there are more spins in the lower energy state, we will see a net absorption of RF energy when we turn on an RF energy source at the Larmor frequency. As there is a net migration of spins from the lower energy state to the upper energy state (absorption exceeds emission), we will quickly see the two populations become equal:

$$\begin{array}{lcll} N/2-\delta & & N/2 & (\beta) \\ & \rightarrow & & \\ N/2+\delta & & N/2 & (\alpha) \end{array}$$

where N is the total number of identical spins in the sample and δ is a very small fraction of this number. With the equal populations, the rate of absorption equals the rate of emission and we no longer have any net absorption of RF energy. This condition is called *saturation*. If there were no other way for the spins to drop down to the lower energy state, this would be the end of the NMR experiment: a quick burst of absorption and then nothing. But there is a pathway to reestablish the Boltzmann distribution: spins can drop down from the higher energy state to the lower energy state with the energy appearing as thermal energy (molecular motion) instead of in the form of a photon. This process is called *relaxation* and is an extremely important phenomenon that will be discussed in detail. If our source of RF energy is weak enough, we can reach a steady state in which the absorption of RF energy is exactly equal to the rate of relaxation. The amount of energy absorbed is very small, and the heating of the sample resulting from relaxation is not even noticeable.

1.4.8 A Continuous Wave Spectrometer

So now we have a way to construct a simple NMR spectrometer: We have a weak source of RF energy (a transmitter) and we gradually decrease the frequency, with the magnetic field strength (B_o) remaining constant. A detector in the transmitter circuit monitors the amount of RF energy absorbed, and this signal is applied to a pen, which moves up and down. The pen moves from left to right across the paper as the frequency is gradually decreased, and when we reach the Larmor frequency (ν_o), there is a net absorption of energy and the pen moves up. As we pass through the Larmor frequency, the resonance condition is no longer met and absorption stops, so the pen moves back down. The spins never reach the saturated state because the RF energy level is very low, and after passing through the resonance condition they quickly reestablish the equilibrium energy difference through the process of relaxation. The result is an NMR spectrum: a graph of absorption of RF energy (vertical axis) versus

frequency (horizontal axis). The range of frequencies "scanned" by the spectrometer is very narrow—for example, from 500.0025 MHz down to 499.9975 MHz, and the position of the absorption peak on the spectrum (its "chemical shift") tells us something about the chemical environment of the spin within the molecule. This technique is called "continuous wave" (CW) NMR because the radio frequency energy is applied continuously as the frequency is gradually varied. The first commercial NMR spectrometers (e.g., the Varian T-60 operating at 60 MHz) were all continuous wave. In the earliest CW instruments, the radio frequency was held constant and the field (B_o) was gradually changed ("swept"). This gave the same result because the absorption of RF energy led to a peak when the field reached a value that satisfied the resonance condition ($\nu_o = \gamma B_o/2\pi$). The left-hand side of the spectrum was called "low field" and the right-hand side was called "high field." The chemical shift scale was in ppm units of τ ($\tau = 10 - \delta$), which increased from left to right. To this day we use the terms "downfield" and "upfield" to refer to the left-hand and right-hand side of the spectrum, respectively, and the frequency scale runs from right-hand to left-hand side, contrary to all other graphical scales. This is because a higher frequency in the frequency-swept spectrum corresponds to a lower field ("downfield") in the old field-swept instruments.

1.4.9 Pulsed Fourier transform NMR

All modern spectrometers now use a "pulsed Fourier transform" method, which is much faster and allows repeating the experiment many times and summing the resulting data to increase sensitivity. A very brief pulse of high-power radio frequency energy is used to excite all of the nuclei in the sample of a given type (e.g., ^{1}H). Immediately after the pulse is over, the nuclei are organized in such a way that their precessing magnets sum together to form a net magnetization of the sample, which rotates at the Larmor frequency. The coil that was used to transmit RF is now used as a receiver, and a signal is observed at the precise Larmor frequency, ν_o. This signal, which oscillates in time at the Larmor frequency, is recorded by a computer and a mathematical calculation called the Fourier transform converts it to a spectrum, a graph of intensity versus frequency. Essentially the Fourier transform measures the frequency of oscillation of the signal. If there are a number of slightly different Larmor frequencies, corresponding to different positions within a molecule, their signals add together to give the recorded signal, and the Fourier transform can sort out all the signals into a spectrum with many peaks at different frequencies. The whole experiment (pulse followed by recording the "echo" signal) takes only a few seconds and can be repeated as many times as desired, summing the data to get a stronger signal.

1.4.10 Sensitivity of the NMR Experiment

Although techniques like mass spectrometry require only nanograms (10^{-9} gram) of sample, NMR requires milligrams (10^{-3} gram) of a typical organic molecule. This insensitivity stems primarily from the fact that only the *difference* in population at thermal equilibrium is active in the experiment. That means that only approximately one spin in 10^6 is actually detected. We saw this in the CW experiment, where absorption of RF energy is almost completely cancelled by stimulated emission. Another important aspect is the relative sensitivity of different nuclei: because of the inherent differences between different nuclei in the strength of the nuclear magnet (γ), the signal strength received can be very much weaker than a proton signal. There are three ways in which γ affects the sensitivity of the experiment ("the three gammas"):

1. The *population difference* at thermal equilibrium is proportional to the energy gap, which is in turn proportional to γB_o, and inversely proportional to absolute temperature. This population difference is the only thing we can observe by NMR.
2. As the nuclear magnet precesses, it induces a signal in the receiver coil. The amplitude of this signal is proportional to the *strength of the rotating magnet*, which is the magnetogyric ratio γ.
3. The *rate at which the nuclear magnet precesses* (ν_o) is also proportional to γB_o. As with any electrical generator, if you turn the crank faster you get a higher voltage out of the generator.

Factors 1 and 2 taken together give the net magnetization at equilibrium, M_o, so we can also think of a large magnet of strength M_o rotating in the *x*-*y* plane when we consider the final factor, the rate of rotation (3). Either way we can say that the amplitude of the NMR signal (sensitivity) for a spin-½ nucleus is proportional to:

$$[N \times \gamma B_o/T] \times [\gamma] \times [\gamma B_o] = N\gamma^3 B_o^2/T$$

where N is the number of identical spins in the sample. This tells us that sensitivity depends on the third power of γ as well as the square of B_o. So it is worth a lot of money to build larger and more powerful magnets, and we will pay a big price in sensitivity to study nuclei with relatively small γ. Consider some of the most useful nuclei for organic chemistry and biological research:

	$B_o = 7.05$ T	$B_o = 11.74$ T	γ/γ_H(%)	$\gamma^3/{\gamma_H}^3$	Abundance (%)
^{1}H	300.0 MHz	500.0 MHz	1.000	1.000	99.98
^{13}C	75.43	125.72	0.2514	0.0159	1.11
^{15}N	30.40	50.66	0.1013	0.00104	0.37

Using our rule of thumb that ^{13}C has a γ value four times smaller than ^{1}H and that ^{15}N has a γ value 10 times smaller than ^{1}H, we can see that the FID signal will be $4^3 = 64$ times less with ^{13}C and $10^3 = 1000$ times less with ^{15}N when compared to ^{1}H with the same number of identical nuclei (N) in the sample. But even at the same sample concentration we do not have the same number of nuclei because for ^{13}C only about one in 100 carbon atoms is ^{13}C and for ^{15}N only about one in 300 nitrogen atoms is ^{15}N. Accounting for this smaller value of N, the signal strength (sensitivity) is 5670 times less than ^{1}H for ^{13}C and 260,000 times less than ^{1}H for ^{15}N at natural abundance. For this reason, commercial continuous wave NMR spectrometers could only detect ^{1}H. With pulsed Fourier transform NMR it became possible to detect ^{13}C with long experiments (1 h or more) and concentrated samples (30 mg or more of a typical organic molecule). Detection of ^{15}N is still very difficult without isotopic labeling of ^{15}N in the sample. Biological NMR experiments (proteins, nucleic acids, etc.) now typically involve preparation of uniformly ^{13}C and ^{15}N labeled samples by biosynthesis (e.g., protein expression in *E. coli*) on labeled media (e.g., $^{15}NH_4Cl$ and U-^{13}C-glucose). We will see that NMR tricks can also allow us to avoid the disadvantage of the first γ (e.g., the DEPT experiment, Chapter 7), or even to avoid the disadvantage of all three gammas (e.g., ^{1}H-detected two-dimensional experiments, Chapter 11). Without isotopic labeling, however, there is no trick that can overcome the disadvantage of low isotopic abundance.