8 Nuclear magnetic resonance spectroscopy

KEYPOINTS

Principles

Radiation in the radiofrequency region is used to excite atoms, usually protons or carbon-13 atoms, so that their spins switch from being aligned with to being aligned against an applied magnetic field. The range of frequencies required for excitation and the complex splitting patterns produced are very characteristic of the chemical structure of the molecule.

Applications in pharmaceutical analysis

- A powerful technique for the characterisation of the *exact structure* of raw materials and finished products
- Can determine impurities, including enantiomeric impurities, without separation down to *ca* the 10% level
- Can potentially be used for fingerprinting mixtures
- Has good potential for quantitative analysis of drugs in formulations without prior separation.

Strengths

- Provides much more information about molecular structure than any other technique.

Limitations

- A relatively insensitive technique requiring > 5 mg of sample for proton nuclear magnetic resonance (NMR) and > 20 mg for carbon-13 NMR
- Expensive instrumentation requiring a specialist operator although automation is increasingly available for routine methods.

Introduction

The nuclei of certain atoms act as if they are spinning and this gives them the properties of a magnetic vector. Common nuclei with this property are ^{1}H; ^{13}C; ^{15}N; ^{19}F; ^{29}Si and ^{31}P. When such nuclei are placed in a magnetic field they will tend to align with the field (Fig. 8.1).

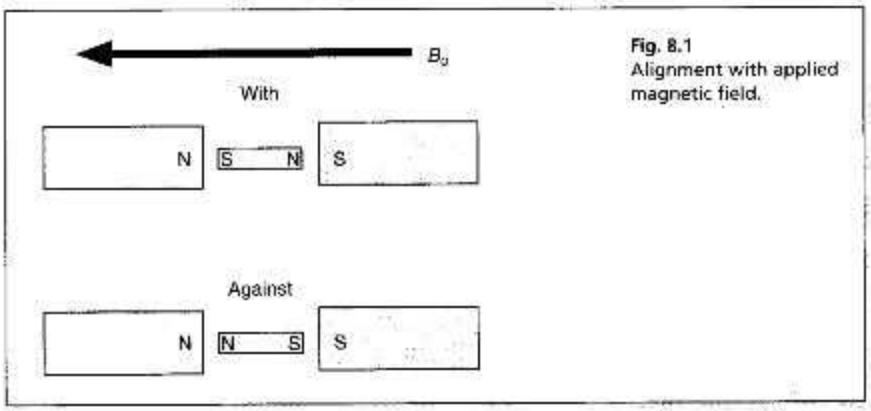


Fig. 8.1
Alignment with applied magnetic field.

The energy difference between the spin being aligned with the field and against the field depends on the strength of the magnetic field applied. The greater the field strength the greater the energy difference ΔE:

$$\Delta E = h\gamma B_0$$

where h is Planks constant, γ is the magnetogyric ratio of a particular nucleus and B_0 is the applied magnetic field.

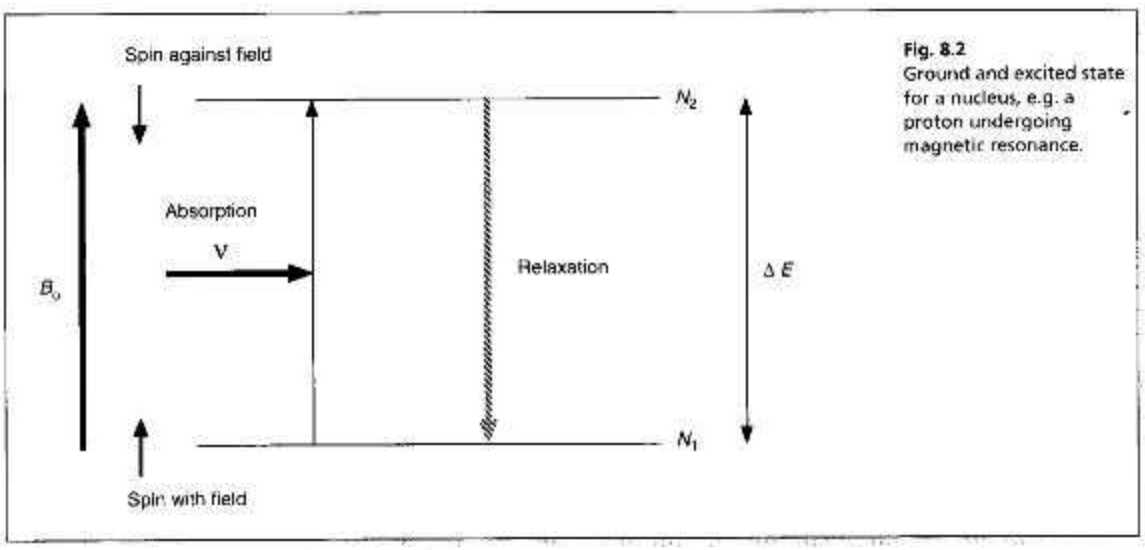


Fig. 8.2
Ground and excited state for a nucleus, e.g. a proton undergoing magnetic resonance.

Figure 8.2 illustrates the absorption of energy to produce alignment against the applied magnetic field. Compared to other spectroscopic techniques, the energy difference between the ground and excited state is not large and thus ΔN, the difference between the number of protons in the low energy (N_1) and high energy states (N_2), is very small. This is because the energy difference between the two states

is low relative to the thermal energy in the environment. This means that NMR is a relatively insensitive technique because the net energy absorption by the population of low energy protons in a sample is low. The wavelength of the radiation used in NMR is of low energy and is in the radiofrequency region. The units of energy used in NMR are in Hertz, which is a unit of frequency (c/λ, where $c = 3 \times 10^{10}$ cm/s and λ is in cm). The stronger the magnetic field applied the greater the radiation frequency in Hertz (the shorter the wavelength) required to cause the spin of a nucleus to align against the field. The values for the strength of the applied magnetic field are in the range 14 000–140 000 Gauss (1.4–14 Tesla). A proton in the ground state will absorb radiation having a frequency of *ca* 60 mHz at 1.4 T and *ca* 600 mHz at 14 T. NMR instruments are described in terms of the frequency at which they cause protons to resonate, thus a 600 mHz instrument is one which causes protons to resonate at a frequency of *ca* 600 mHz. At higher magnetic field strength greater sensitivity is obtained because of the greater difference in the populations of the higher and lower energy states. For a 60 mHz instrument the population difference between the ground and excited state for a proton is *ca* 1 in 100 000, whereas for a 600 mHz instrument the population difference is *ca* 1 in 10 000, i.e. about a 10-fold increase in sensitivity.

Instrumentation

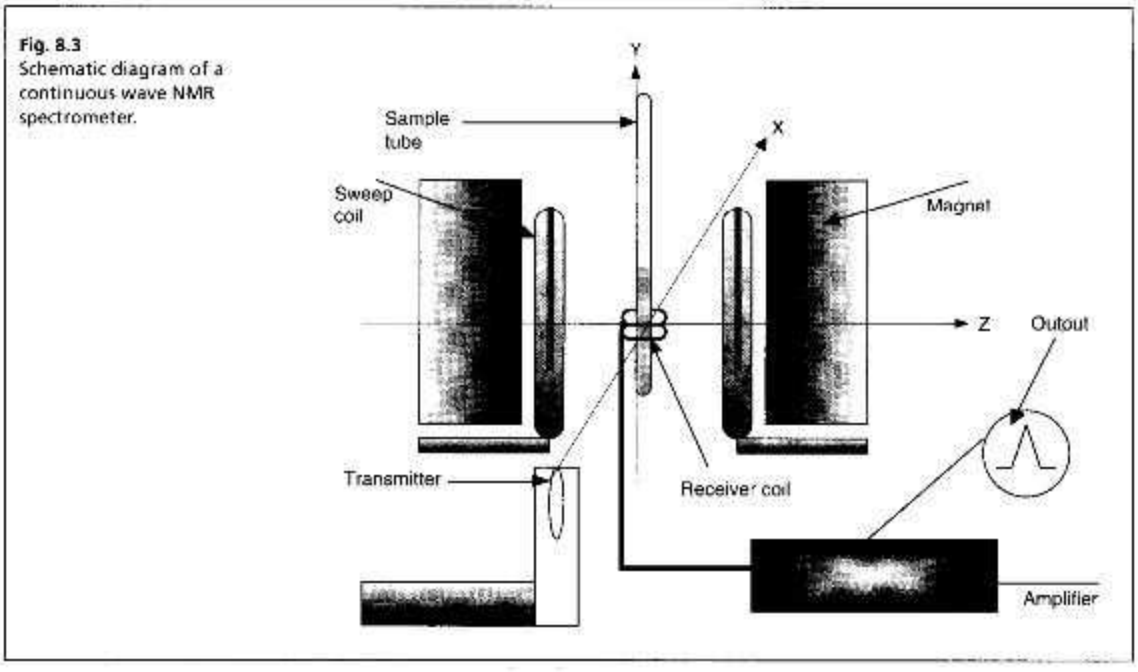


Fig. 8.3
Schematic diagram of a continuous wave NMR spectrometer.

Figure 8.3 gives the basic layout of a continuous wave NMR spectrometer. These intruments were the original type of instrument and have largely been replaced by Fourier transform instruments. However, the principles of operation are broadly similar:

(i) The sample is placed in a narrow glass NMR tube and is spun in the fixed magnetic field at *ca* 30 revolutions/s by means of an air turbine thus ensuring uniformity of the magnetic field across the sample in a horizontal direction. The sample is analysed in solution in a deuterated solvent to ensure there is no

interference from protons in the relatively much larger amount of solvent with the signal from the sample protons.

(ii) The reference point of 0 parts per million (ppm) is determined by the frequency at which the protons in tetramethylsilane (TMS) absorb. Sometimes residual protons in the solvent are used to lock the protons in a spectrum, e.g. the residual proton in deuterated chloroform is at 7.25 ppm relative to TMS.

(iii) In order to obtain a proton spectrum the radiofrequency radiation is swept across a range of *ca* 10 ppm, e.g. 1000 Hz when the magnetic field is recorded on a 100 mHz instrument or 6000 Hz when the spectrum is recorded on a 600 mHz instrument. The receiver coil measures the absorption of radiation as the frequency is swept over the range being examined.

(iv) As well as determining the frequency at which protons in the molecule absorb, the instrument determines the area of each signal which is proportional to the number of protons absorbing radiation, e.g. three protons give an area three times as large as a signal due to one proton in the same molecule.

(v) Modern instruments, rather than being based on a continuous wave, are based on a pulsed wave. In brief, the short powerful pulse used in this type of spectroscopy behaves as a spread of frequencies covering the Hz range of interest, e.g. the range in which protons resonate. Most of the principles of the continuous wave instrument still hold but rather than the absorption of radiation by the sample being observed emission is observed as the excited protons relax back to the ground state following the short high energy pulse of radiation. Thus spectra are accumulated using a high intensity pulse followed by a time delay of a few seconds while the relaxation data of different protons in the molecule are collected. This type of procedure enables a spectrum to be acquired every few seconds as opposed to a few minutes required to collect the data using a frequency sweep on a continuous wave instrument. The data from a number of pulses are accumulated using a computer, undergo mathematical manipulation (Fourier transformation) and are combined to produce a spectrum in which the signal to noise characteristics are much improved compared to a spectrum obtained on a single scan continuous wave instrument.

Proton NMR

Chemical shifts

Proton (^{1}H) NMR is the most commonly used form of NMR because of its sensitivity and the large amount of structural information it yields. The exact absorption or resonance frequency of a proton depends on its environment. For example, a proton attached to carbon atom is affected predominantly by the groups which are separated from the carbon atom to which it is attached by one bond or to a lesser extent two bonds. As discussed earlier, the chemical shift of a proton is determined in relation to the protons of tetramethylsilane, which are arbitrarily assigned a shift of 0 ppm. Shift values for individual protons in a molecule are expressed in ppm and the value of 1 ppm in Hertz depends on the strength of the applied magnetic field which determines the energy required to excite a proton. For example, at a field strength 100 mHz a shift of 1 ppm = 100 Hz. Proton shifts in organic compounds range from slightly below 0 ppm to 14 ppm, i.e. from a ∂ value of slightly less than 0 to a ∂ value of 14.

The chemical shift is determined by the extent to which a proton is deshielded by the groups to which it is attached. The more a proton is shielded by the electron density around it, the lower its ∂ value. If a proton is attached to a system that withdraws electrons from its environment such as an electronegative group or to a group which affects its environment by creating a field opposing the applied

magnetic field, such as occurs in the case of protons attached to an aromatic ring, its ∂ value will increase, i.e. it will resonate at lower field (lower frequency).

Note:

(i) Alkyl protons such as those in CH_3 and CH_2 groups not attached to adjacent electronegative groups resonate between ∂ 0.2–2 ppm.

(ii) Protons on CH_3, CH_2 and CH groups attached to electronegative atoms or groups such as O, N, F, Cl, CN, C=C and C=O resonate between ∂ 2–5.

(iii) Protons attached directly to C=C resonate between ∂ 4–7.

(iv) Protons attached to aromatic rings resonate between ∂ 6–9. Tables 8.1 and 8.2 show ∂ values in ppm for protons attached to some common organic groups.

If the NMR spectrum of methylacetate is examined, (Figure 8.4) it can be seen to yield two signals of the same size at ∂ 2.06 and ∂ 3.67 more or less as predicted for CH_3CO and CH_3OCO groups according to the values in Table 8.1.

Self-test 8.1

Determine the frequency difference between the shifts of the protons of the methyl groups of methyl acetate in Hz at field strengths of 60 mHz, 250 mHz and 400 mHz.

∂ 2.06 ∂ 3.67
CH_3COOCH_3
Methyl acetate

Answers: 96.6 Hz; 402.5 Hz; 644 Hz

Table 8.1 Approximate chemical shift values for non-aromatic protons attached to carbon

Group	∂ ppm	Group	∂ ppm	Group	∂ ppm
CH_3-C	0.9	R-CH_2-C	1.4	CH-C	1.5
CH_3-C-O	1.3	R-CH_2-C-N	1.4	CH-C-O	2.0
CH_3-C=C	1.6	R-CH_2-C-O	1.9	CH-CO-N	2.4
CH_3-CO	2.0	R-CH_2-CO-N	2.2	CH-CO	2.7
CH_3-CO-N	2.0	R-CH_2-C=C	2.3	CH-N	2.8
CH_3-N	2.4	R-CH_2-CO	2.4	CH-Ar	3.3
CH_3-Ar	2.3	R-CH_2-N	2.5	CH-O	3.9
CH_3-O	3.3	R-CH_2-Ar	2.9	CH-N-CO	4.0
$CH_3N^+(R)_3$	3.3	R-CH_2-O	3.6	CH-Cl	4.2
CH_3-O-CO	3.7	R-CH_2-O-CO	4.1	R-CH=C	4.5–6.0

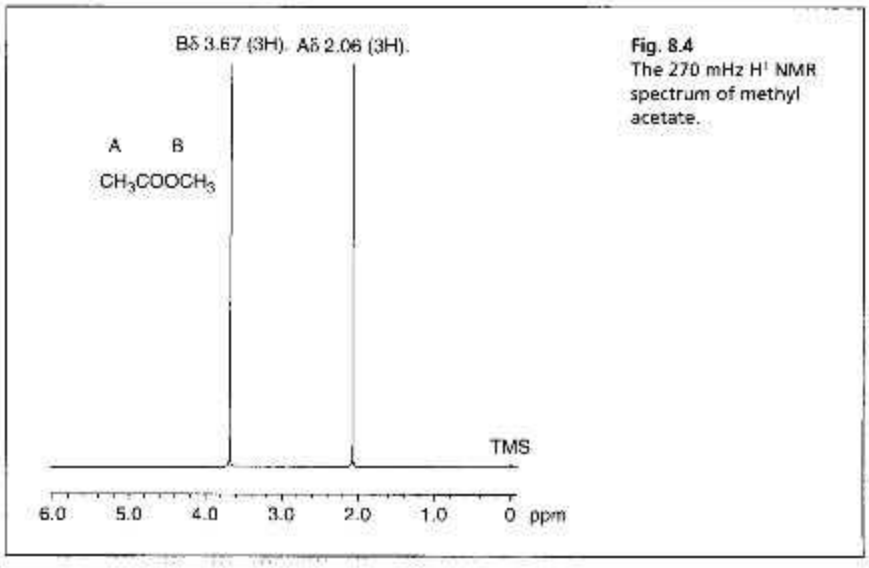
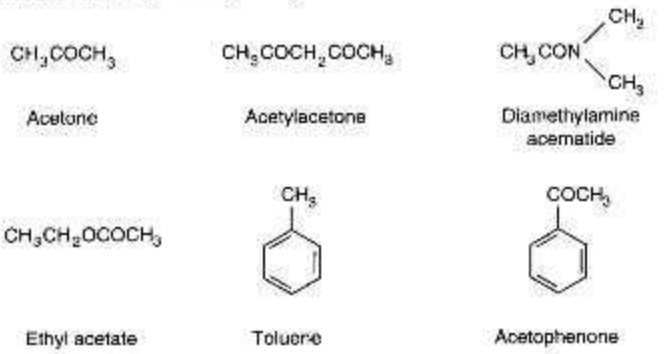


Fig. 8.4
The 270 mHz H^1 NMR spectrum of methyl acetate.

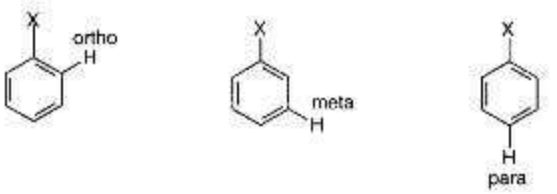
Self-test 8.2

Predict the approximate shifts in ppm of the CH_3 and CH_2 groups in the following molecules and the number of protons producing the signal at each shift:



Answers: acetone δ 2.0 6H; acetylacetone δ 2.0 6H, δ 2.4 2H; dimethylamine acetamide δ 2.0 3H, δ 2.4 6H; ethylacetate δ 1.3 3H, δ 2.0 3H, δ 4.1 2H; toluene δ 2.3 3H; acetophenone δ 2.6 3H

Table 8.2 Chemical shift values for protons attached to an aromatic ring. The effects of the substituents are either added to or subtracted from the chemical shift for benzene at δ = 7.27



Substituent X	H ortho	H meta	H para
NO_2	0.94	0.18	0.39
OH	−0.49	−0.13	−0.20
NH_2	−0.76	−0.25	−0.63
Cl	0.01	−0.06	−0.08
COOH	0.80	0.16	0.25
NH_3^+	0.40	0.20	0.20
CH_3	−0.16	−0.09	−0.17
OR	−0.46	−0.1	−0.46
CH_3-CO-NH	−0.12	−0.07	−0.21
COOR	0.71	0.1	0.21

Calculation example 8.1

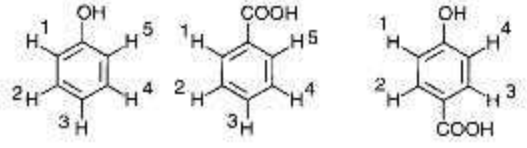
Aniline: In aniline the 1 and 5 and 2 and 4 protons are equivalent:
H-1 and H-5 shift = 7.27 – 0.76 = 6.51 ppm.
H-2 and H-4 shift = 7.27 – 0.25 = 7.02 ppm.
H-3 shift = 7.27 – 0.63 = 6.64 ppm.
Thus the spectrum of aniline would contain:
2H 6.51 ppm; 2H 7.02 ppm and 1H 6.64 ppm.

Nitrobenzene: In nitrobenzene the 1 and 5 and 2 and 4 protons are equivalent.
H-1 and H-5 shift = 7.27 + 0.94 = 8.21 ppm.
H-2 and H-4 shift = 7.27 + 0.18 = 7.45 ppm.
H-3 shift = 7.27 + 0.39 = 7.66 ppm.
Thus the spectrum of nitrobenzene would contain:
2H 8.21 ppm; 2H 7.45 ppm and 1H 7.66 ppm.

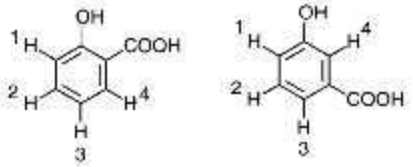
Nitroaniline: In nitroaniline the 1 and 4 and 2 and 3 protons are equivalent.
H-1 and H-4 shift = 7.27 – 0.76 + 0.18 = 6.69 ppm.
H-2 and H-3 shift = 7.27 – 0.25 + 0.94 = 7.96 ppm.
Thus the spectrum of nitroaniline would contain:
2H 6.69 ppm and 2H 7.96 ppm.

Self-test 8.3

Determine the shifts of the protons in the following molecules:



Phenol Benzoic acid *p*-hydroxybenzoic acid



o-hydroxybenzoic acid *m*-hydroxybenzoic acid

Answers: Phenol H1 and H5 δ 6.78, H2, H4 δ 7.14, H3 δ 7.07; benzoic acid H1 and H5 δ 8.07, H2 and H4 δ 7.43, H3 δ 7.52; *p*-hydroxybenzoic acid H1 and H4 δ 6.94, H2 and H3 δ 7.94; *o*-hydroxybenzoic acid H1 δ 6.94, H2 7.39, H3 7.23, H4 δ 7.94; *m*-hydroxybenzoic acid H1 δ 7.03, H2 7.30, H3 7.87, H4 7.58

Spin spin coupling

In some of the molecules considered above we have neglected an additional shift effect which is caused by the spin of the protons on the atoms next to a particular proton. In examining the proton NMR spectrum of ethyl acetate (Fig. 8.5), it can be seen that its spectrum is more complicated than that of methyl acetate and that the signal due to the CH_2 group B in the alcohol part of the ester is now three lines instead of one, the middle line of the three corresponding to the chemical shift

estimated from Table 8.1. The protons of the adjacent CH_2 group C can align their spins in three different ways relative to the CH_3 as seen in Figure 8.6. For alignment 1 there are two equivalent alignments where the effects of the adjacent protons cancel each other out and do not perturb the signal of the methyl group from its predicted shift (*ca* 1.30 ppm). This produces a central line which is twice the height/area of the two lines produced by alignments 2 and 3. The CH_2 group itself is also split by the effect of the adjacent methyl group. In this case statistical analysis of the possible combination of the spins of the adjacent methyl protons indicates that the signal of the CH_2 protons should be a quartet with the lines in the quartet being in the ratio 1:3:3:1; the mid-point between the two central lines gives the predicted chemical shift of 4.1 ppm. The methyl group A appears, as it does in methyl acetate, as a singlet since it is isolated from any adjacent protons. The effect of adjacent protons on the signal for a given group is known as coupling and coupling constants are given in Hz; the range of coupling constants between adjacent protons is 0–20 Hz. When when two protons are close in chemical shift, coupling can cause their signals to overlap. The coupling constant is independent of the applied magnetic field and thus the size of coupling constants in ppm will decrease with increasing field strength although their values in Hz remain the same. The higher the field strength the less likely it is that signals from individual protons will overlap.

The spectrum in Figure 8.5 was obtained on a 270 mHz (1 ppm = 270 Hz) instrument. The shifts in ppm obtained for the three lines in the CH_3 group signal are: 1.235, 1.262 and 1.289. Therefore these lines are evenly spaced 0.027 ppm apart, which is equivalent to 270×0.027 Hz = 7.29 Hz. Figure 8.7 shows the spectrum of ethyl acetate obtained on a 60 mHz (1 ppm = 60 Hz) instrument; in this case the coupling constants are large (*ca* 0.1 ppm) relative to the ppm scale but have a similar coupling constant of *ca* 7 Hz to that observed using the 270 mHz instrument.

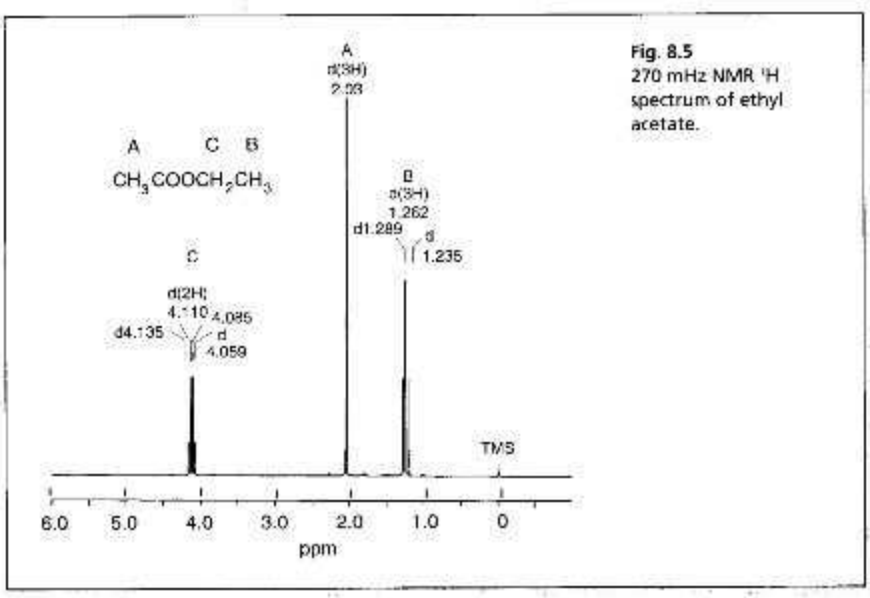


Fig. 8.5
270 mHz NMR ^1H spectrum of ethyl acetate.

As the number of adjacent protons in a molecule increases, the splitting pattern of the protons increases in complexity. Figure 8.8 shows the proton NMR spectrum of propyl acetate. In this case the CH_3 group B is present as a triplet as in ethyl acetate, but the CH_2 group C now has six lines due to the presence of five adjacent protons – three on group A and two on group D. The ratio of the lines in this case is 1:5:10:10:5:1.

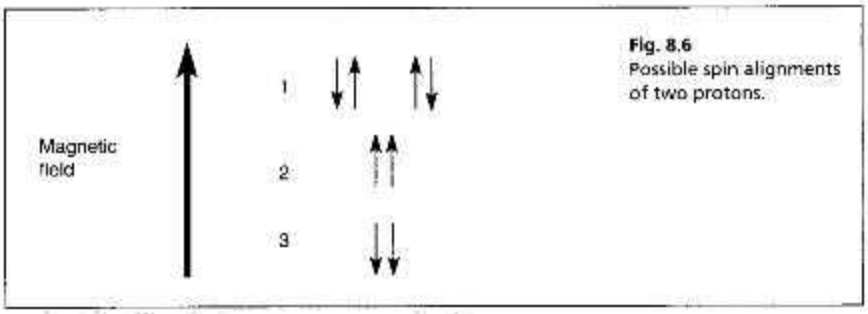


Fig. 8.6
Possible spin alignments of two protons.

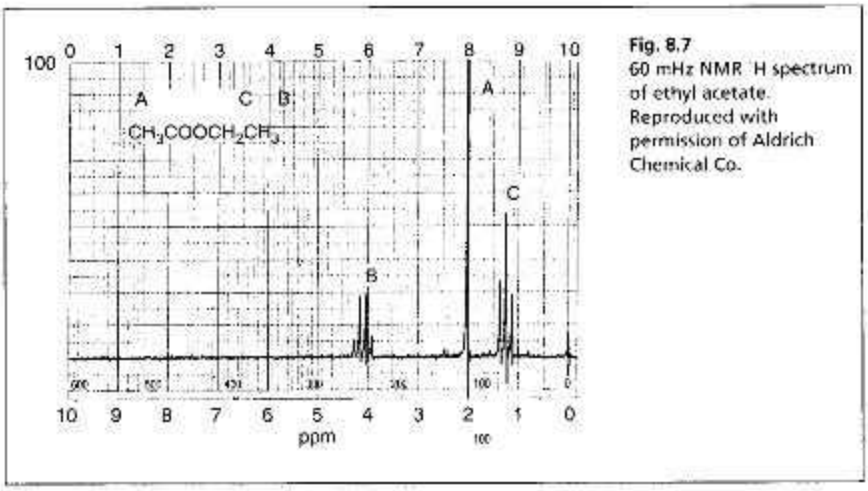


Fig. 8.7
60 mHz NMR ^1H spectrum of ethyl acetate. Reproduced with permission of Aldrich Chemical Co.

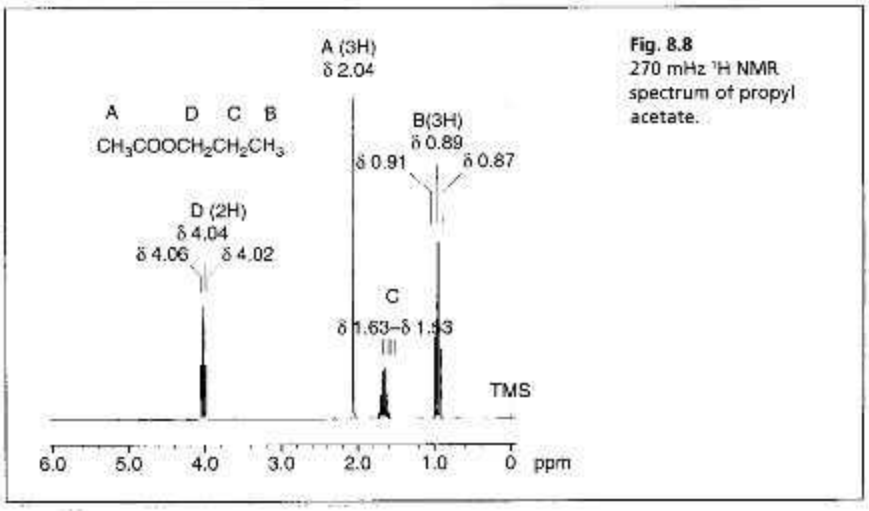


Fig. 8.8
270 mHz ^1H NMR spectrum of propyl acetate.

The number of lines expected and their relative intensities can be obtained from Pascal's triangle shown in Figure 8.9.

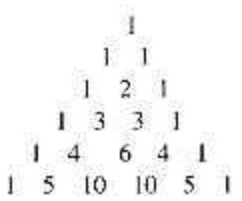


Fig. 8.9 Pascal's triangle indicating the pattern of lines produced by a particular number of adjacent protons having the same coupling constants to a particular signal.

If the protons of groups B and D in propyl acetate did not have similar coupling constants with the protons on group C a more complicated pattern of lines would result as indicated in Figure 8.10. In the case shown in Figure 8.10A, the protons can be viewed as being split into four lines by an adjacent methyl group and each of the four lines are further split into three lines by a CH_2 group giving a total of 12 lines (a quartet of triplets). If the coupling constants to adjacent protons are not widely different the patterns tend to merge into those that would be expected if all the adjacent protons coupled identically as in the case of propyl acetate, where coupling to five adjacent protons on two carbons produces six lines.

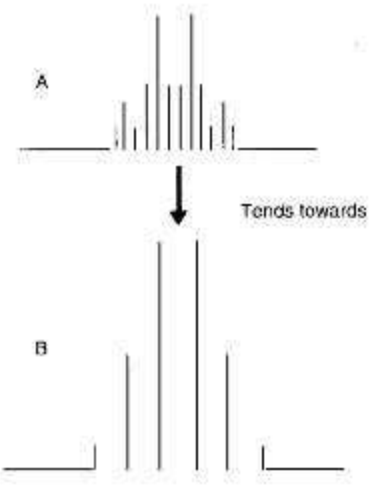


Fig. 8.10 Coupling of CH_2 with adjacent and CH_3 and CH_2 groups where the coupling constants to the adjacent groups are unequal.

As indicated in Figure 8.10 splitting patterns can be complex but the NMR spectra of many drug molecules do not reach this level of complexity. Some classes of molecules such as the steroids or prostaglandins provide examples of spectra where complex splitting patterns occur but the majority of drug molecules contain one or two aromatic rings with varying types of relatively simple side chain. In the case of complex molecules such as steroids, two-dimensional NMR techniques involving proton–proton and proton–carbon correlations have simplified spectral interpretation. The most complex applications of NMR are found in the structure elucidation of natural products.

Application of NMR to structure confirmation in some drug molecules

Proton NMR spectrum of paracetamol

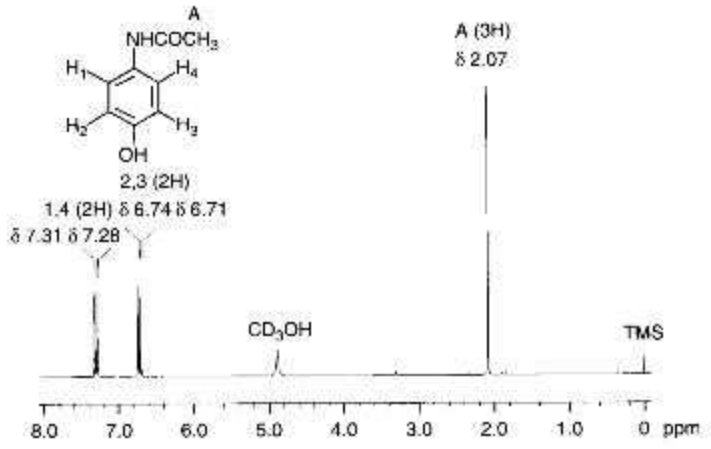


Fig. 8.11
270 mHz ^{1}H NMR spectrum of paracetamol.

The NMR spectrum of paracetamol run in CD_3OD is shown in Figure 8.11. The spectrum shows a signal for an isolated CH_3 group at ∂ 2.07 ppm due to CH_3CONH. The broad signal at ∂ 4.88 is due to the proton in CD_3OH, which forms as a result of exchange of deuterium with the NH and OH protons of paracetamol, this is why the protons attached to these groups are not seen in the spectrum. The other signals in the spectrum are two doublets with mean shifts of ∂ 6.72 ppm and ∂ 7.30, which from the information given in Table 8.2 can be said to be assigned to the equivalent protons 2 and 3 and 1 and 4, respectively. Proton 1 is coupled to proton 2 and proton 4 is coupled to proton 3, thus causing the signals to appear as doublets.

Proton NMR spectrum of aspirin

Figure 8.12 shows the proton NMR spectrum for aspirin run in CD_3OD. The methyl group is isolated and appears at ∂ 2.28, which could be predicted from Table 8.1. There is a broad peak at ∂ 4.91 due to CD_3OH formed by exchange with the –COOH group on aspirin. The aromatic region is more complex than that observed for paracetamol because the four aromatic protons are non-equivalent. The four proton signals have mean shifts of 7.13 ppm, 7.34 ppm, 7.60 ppm and 8.02 ppm and from Table 8.2 it is possible to assign these signals to protons 4, 2, 3 and 1 respectively. H-1 is a doublet due to coupling to H-2; H-2 appears as a triplet due to overlap of two doublets caused by coupling to H-1 and H-3. Similarly, H-3 is a triplet due to coupling equally with H-2 and H-4, and H-4 is a doublet due to coupling with H-3. It is actually possible with a closer view to see additional splitting of all of the aromatic proton signals and this is due to long-range coupling between the protons meta to each other, i.e. H-1 and H3 and H2 and H4, which can occur in aromatic systems and can be up to 3 Hz. Para coupling can also occur but it is only *ca* 1 Hz.

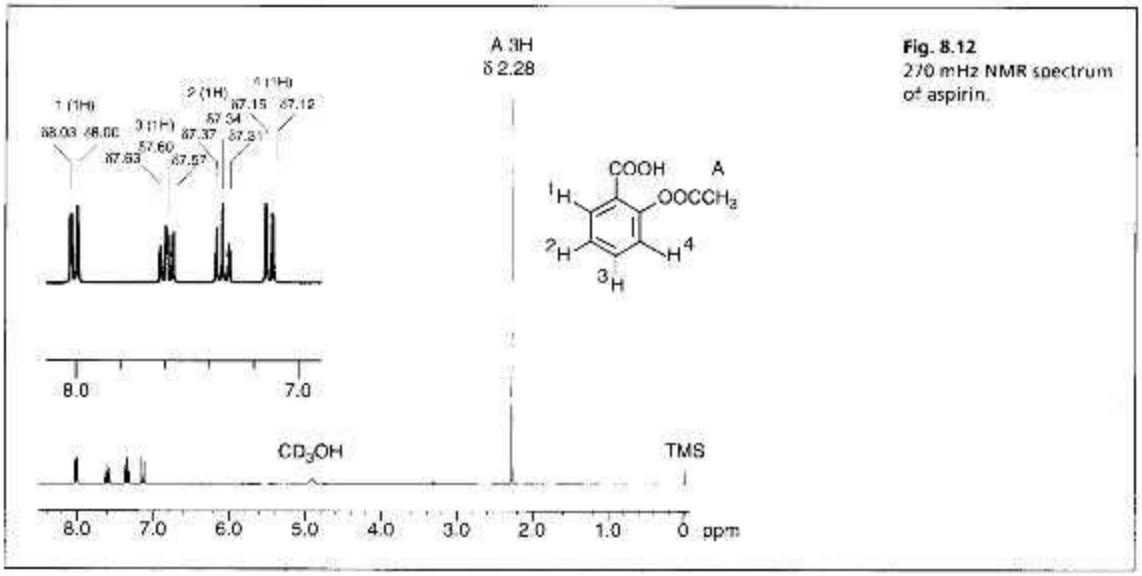


Fig. 8.12
270 mHz NMR spectrum of aspirin.

Proton NMR spectrum of salbutamol: A more complex example

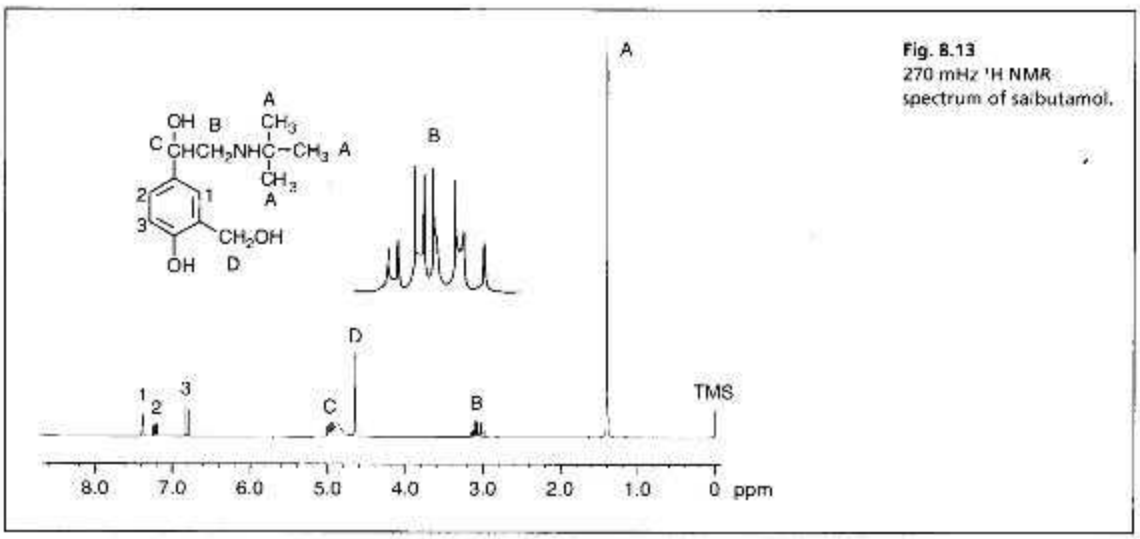


Fig. 8.13
270 mHz ^{1}H NMR spectrum of salbutamol.

Figure 8.13 shows the NMR spectrum of salbutamol obtained in CD_3OD and in this case the spectrum is somewhat more complex. The signal at ∂ 1.40 arises from the t-butyl group A in which the CH_3 groups are all equivalent and have no adjacent protons to which they could couple. The signal at ∂ 4.65 is due to the CH_2 group D, which is also not coupled to any other protons. The protons on the aromatic ring are also readily assigned: the doublet at ∂ 6.80 is due to H-3, which is coupled to H-2. H-2 appears at ∂ 7.22 and is split into a doublet via coupling to H-3 and each peak in

the doublet is split again through meta-coupling to H-1, which appears at ∂ 7.37 and is split into a closely spaced doublet through meta-coupling to H-2. A signal centred at ∂ 4.97, appearing on the shoulder of a broad peak due to CD_3OH, is due to proton C. This proton is attached to a chiral centre and is coupled to the two adjacent protons B1 and B2, which are non-equivalent since they are immediately next to a chiral centre. Thus proton C has two different coupling constants to protons B1 and B2, and appears as a doublet of doublets. The most complex signal in the spectrum is due to protons B1 and B2 and this requires more detailed explanation. These protons produce what is known as an 'AB type signal' where, because the protons are close in chemical shift (less than 30 Hz apart), they give lines which are of unequal sizes as shown in Figure 8.14.

The NMR spectrum is perturbed in an AB system so that the ratio of line intensities composing the doublet instead of being 1:1 are given by:

$$\text{Line ratio} = \frac{\nu_2 - \nu_3}{\nu_1 - \nu_4}$$

where the difference between ν_1 and ν_4 is 30 Hz and the difference between ν_2 and ν_3 is 10 Hz, the line ratio of the outer to inner lines will be 1:3. The smaller the difference in shift in Hz between the inner A and B lines the smaller will be the satellite peaks.

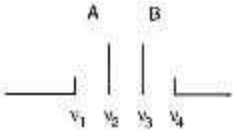
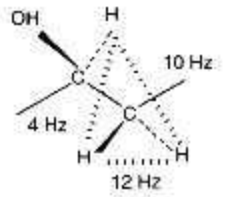


Fig. 8.14
Line intensities in AB systems.

The place to start with the analysis of the signal for the B protons in salbutamol is with signal C, which gives the couplings of the B1 and B2 protons with the proton on position C. From analysis of this signal the two couplings are 4 and 10 Hz. The total width of the B signal is 44 Hz, thus its width in the absence of coupling to the C proton would be 37 Hz (see Fig. 8.15 for clarification). To make the full analysis one has to try some values for the AB coupling that make approximate sense in relation to the final signal. If the coupling of the B protons to each other is 12 Hz, then the pattern when plotted on graph paper (Fig. 8.15) gives more or less the pattern seen for the B protons in salbutamol (leaving 13 Hz from the total signal width for separation of the inner lines). Two other points should be noted: the ratio of the outer to the inner lines is *ca* 1:3 as predicted from the equation shown in Figure 8.14 and the original separation of the B1 and B2 signals is given by the following equation:

$$\delta_{B1} - \delta_{B2} = \sqrt{(\nu_4 \quad \nu_1)\,(\nu_3 - \nu_2)}$$

If the differences between the frequencies are substituted in the equation this gives a difference in frequency of B1 and B2 of 22 Hz, i.e. the germinal coupling (coupling of protons on the same carbon) of the two signals gives shifts of 7.5 Hz in one direction and 4.5 Hz in the other direction instead of the usual even splitting.



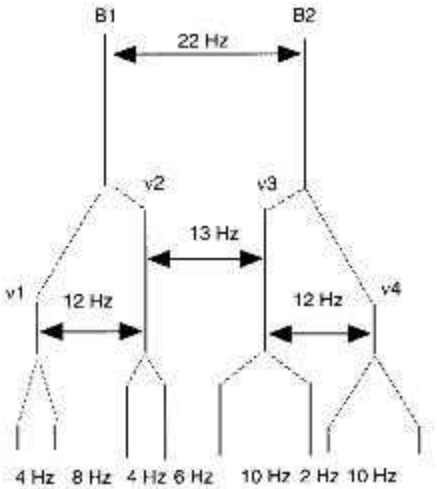
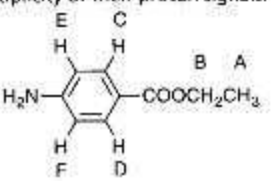


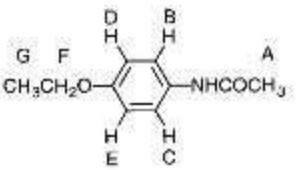
Fig. 8.15
A typical AB system split by a third (X) proton with coupling constants observed for the B1 and B2 protons in salbutamol.

Self-test 8.4

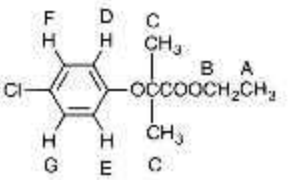
Using the values given in Tables 8.1 and 8.2 predict the approximate NMR spectra of the following drug molecules with respect to chemical shift, the number of protons in each signal and the multiplicity of their proton signals.



Benzocaine



Phenacetin

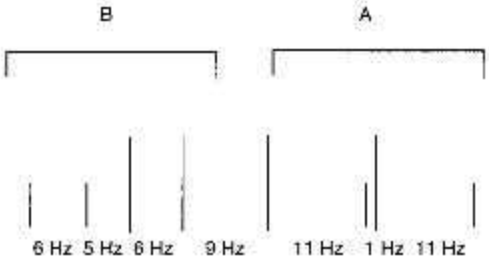


Clofibrate

Answers: Benzocaine: δ 1.3, 3H, triplet (A); δ 4.1, 2H, quartet (B); δ 7.73, 2H, doublet (C,D); δ 6.61, 2H, doublet (E,F).
Phenacetin: δ 1.3, 3H, triplet (G); δ 2.0, 3H, singlet (A); δ 4.1, 2H, quartet (F); δ 6.74, 2H, doublet (D,E); δ 7.05, 2H, doublet (B,C)
Clofibrate: δ 1.3, 3H, triplet (A); δ 1.3, 6H, singlet (C); δ 4.1, 2H, quartet (B); δ 7.18, 2H, doublet (F,G); δ 6.75 2H, doublet (D,E).
Very small couplings might also be observed for aromatic protons para to each other.

Self-test 8.5

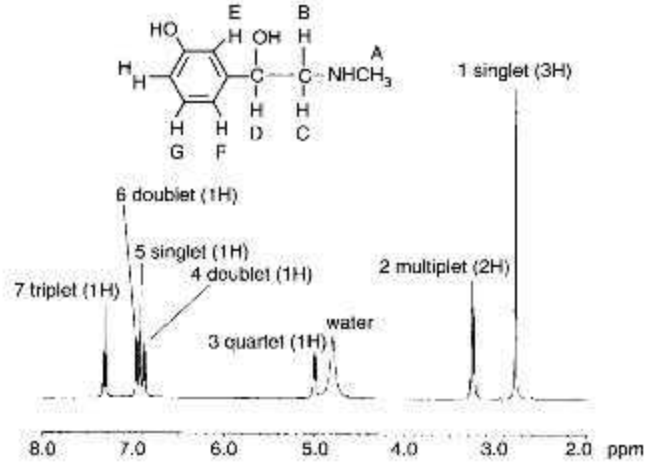
Use graph paper to determine the coupling constants experienced by lines for protons A and B shown below, which are coupled to each other and to a third proton and determine the separation of the A and B signals.



Answer on page 165

Self-test 8.6

Assign the protons in the ^{1}H NMR spectrum of phenylephrine. (Note: protons B and C are non-equivalent since they are next to an asymmetric centre).



Answer: Signal 1A; signal 2B + C compare with salbutamol (in this case the B and C protons are much closer in shift); signal 3D; signal 4H; signal 5E; signal 6F; signal 7G, which has identical couplings to protons H and F

Carbon-13 NMR

Chemical shifts

Nuclei other than ^{1}H give nuclear magnetic resonance spectra. One of the most useful is ^{13}C but since the natural abundance of ^{13}C is only 1.1% of that of ^{12}C, the ^{13}C resonance is relatively weak. ^{13}C resonance occurs at a frequency *ca* 25.1 mHz when proton resonance is occurring *ca* 100 mHz (i.e. at 2.33 Tesla). Thus it is at lower energy than proton resonance and the spread of resonances for ^{13}C is over *ca* 180 ppm, thus there is less likelihood of overlapping lines in ^{13}C NMR. Table 8.3 shows the chemical shifts of some ^{13}C signals. It is possible to calculate these quite precisely[1] and the following table is only an approximate guide. A ^{13}C atom will

couple to any protons attached to it, e.g. a carbon with one proton attached will appear a doublet, to get the most information from the weak carbon spectrum it is better if this coupling is removed. In the salbutamol example the coupling is removed using the J mod technique.

Table 8.3 Typical chemical shifts of ^{13}C atoms

Group	∂ ppm	Group	∂ ppm	Group	∂ ppm
H_3C^{13}-C	5–20	C-H_2C^{13}-N	35–65	$(C)_2C^{13}$-C-N	50–70
H_3C^{13}-C=C	15–30	C-H_2C^{13}-O	55–75	$(C)_2C^{13}$-C-O	70–90
H_3C^{13}-Ar	*ca* 20	$(C)_2HC^{13}$-C	25–55		
H_3C^{13}-COO	*ca* 20	$(C)_2HC^{13}$-CO	40–70	$ArC^{13}H$	115–135
H_3C^{13}-CO	22–32	$(C)_2HC^{13}$-Ar	*ca* 40	ArC^{13}-C	137–147
H_3C^{13}-N	25–40	(C)(O)HC^{13}-Ar	70–80	ArC^{13}-Cl	135
H_3C^{13}-O	45–55	$(C)_2HC^{13}$-N	45–75	$ArC^{13}CO$	137
C-H_2C^{13}-C	16–46	$(C)_2HC^{13}$-O	65–85	ArC^{13}-N	145–155
C-H_2C^{13}-CO	30–50	$(C)_3C^{13}$-C	35–55	ArC^{13}-O	150–160
C-H_2C^{13}-Ar	*ca* 30	$(C)_3C^{13}$-C-CO	45–65	C^{13}=O	170–200
O-H_2C^{13}-Ar	60–70	$(C)_3C^{13}$-C-Ar	45–65		

An example of a ^{13}C spectrum

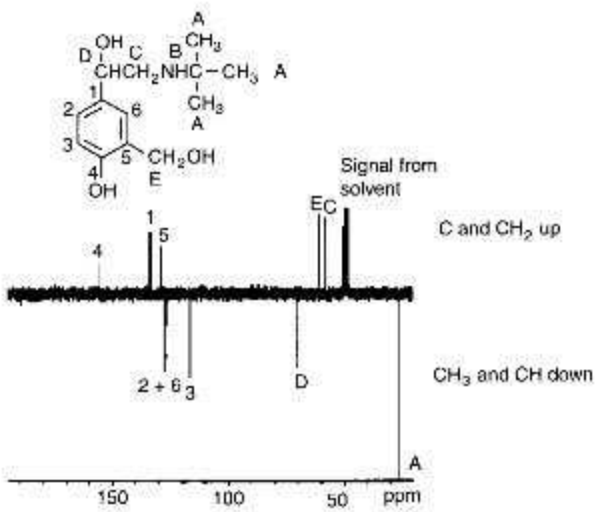


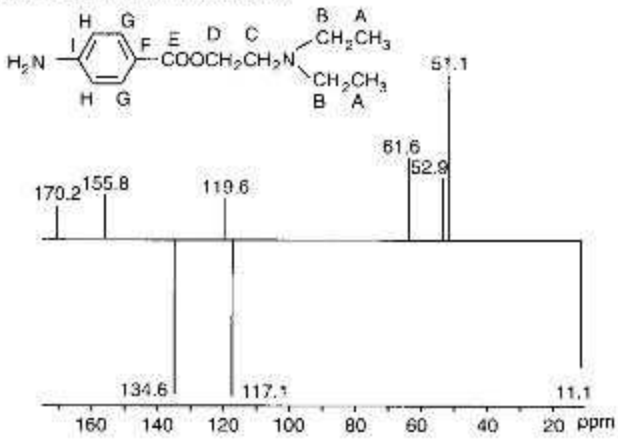
Fig. 8.16
J mod ^{13}C NMR spectrum of salbutamol sulphate.

Figure 8.16 shows the J mod spectrum of salbutamol sulphate. As can be seen the J mod ^{13}C spectrum of salbutamol is much simpler than its proton spectrum. The carbons can be assigned as follows: A ∂ 26 ; C ∂ 58.8; E ∂ 61; D ∂ 70.4; 3 ∂ 116; 2 + 6 ∂ 127.1; 5 ∂ 129.5; 1 ∂ 133 and 4 ∂ 156 (carbons 3 and 5 are shifted upfield through being ortho to an OH group). The signal due to carbon B is missing and this illustrates one of the problems of ^{13}C NMR which is that the relaxation times of the carbon atoms tend to vary more widely than those for protons in ^{1}H NMR and thus their signals may be missed or not fully accumulated. This is particularly true for quaternary carbons and it can be seen in Figure 8.16 that the quaternary carbons 1, 4 and 5 give weaker signals than the other carbons which have protons attached. In the case of quaternary carbon B, its signal has been completely missed. Thus the signals

in ^{13}C are less quantitative than ^{1}H NMR signals. A J mod spectrum is one of the modern equivalents of the ^{13}C spectrum; it allows the number of protons attached to the carbon atoms to be known while at the same time removing the signal broadening due to the coupling between ^{13}C and its attached protons.

Self-test 8.7

Assign the signals in the J mod C-13 spectrum of procaine shown below (C and CH_2 up CH_3 and CH down) obtained on a 400 mHz instrument.



Answer: A 11.1; B 51.1; C 52.9; D 61.6; E 170.2; F 119.6; H 117.1; G 134.6; I 155 = B

Two-dimensional NMR spectra

A simple example

Computer control of NMR instruments has led to great advances in both data acquisition and processing and has given rise to advanced NMR structural elucidation techniques. One of the the first of these was two-dimensional

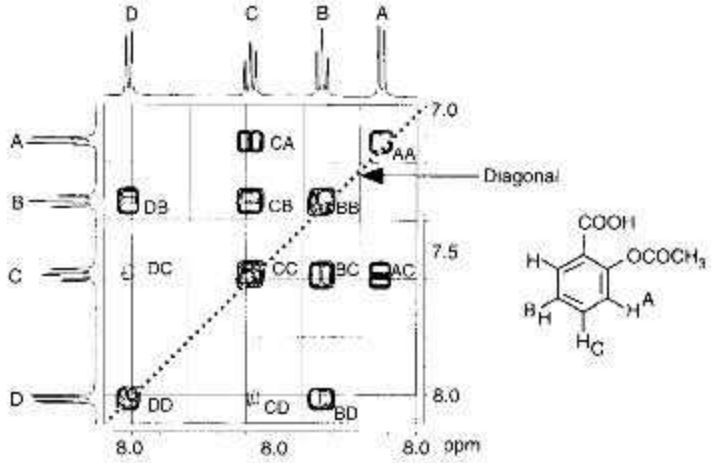


Fig. 8.17
The proton–proton correlation spectrum of the aromatic region of aspirin.

proton–proton correlation or COSEY. This technique enables correlations to be made between protons which are coupled to each other. Taking the simple example of the aromatic proton region of aspirin the correlated spectroscopy COSEY spectrum appears as shown in Figure 8.17, the diagonal gives the correlation of the signals with themselves, i.e. A with A, B with B, etc. On either side of the diagonal identical information is presented, thus only one side of the diagonal is required for spectral interpretation. From the information given in Figure 8.17, it can be seen that A is coupled to C, B is coupled to C and D, and C is weakly coupled to D via long-range meta-coupling. COSEY has simplified interpretation of complex NMR spectra. There are a number of techniques stemming from the basic two-dimensional technique, which for example allow correlation between carbon atoms and the protons attached to them and correlations of carbon atoms with protons one or two bonds removed from them, heteronuclear multiple bond correlation (HMBC).

A more complex example

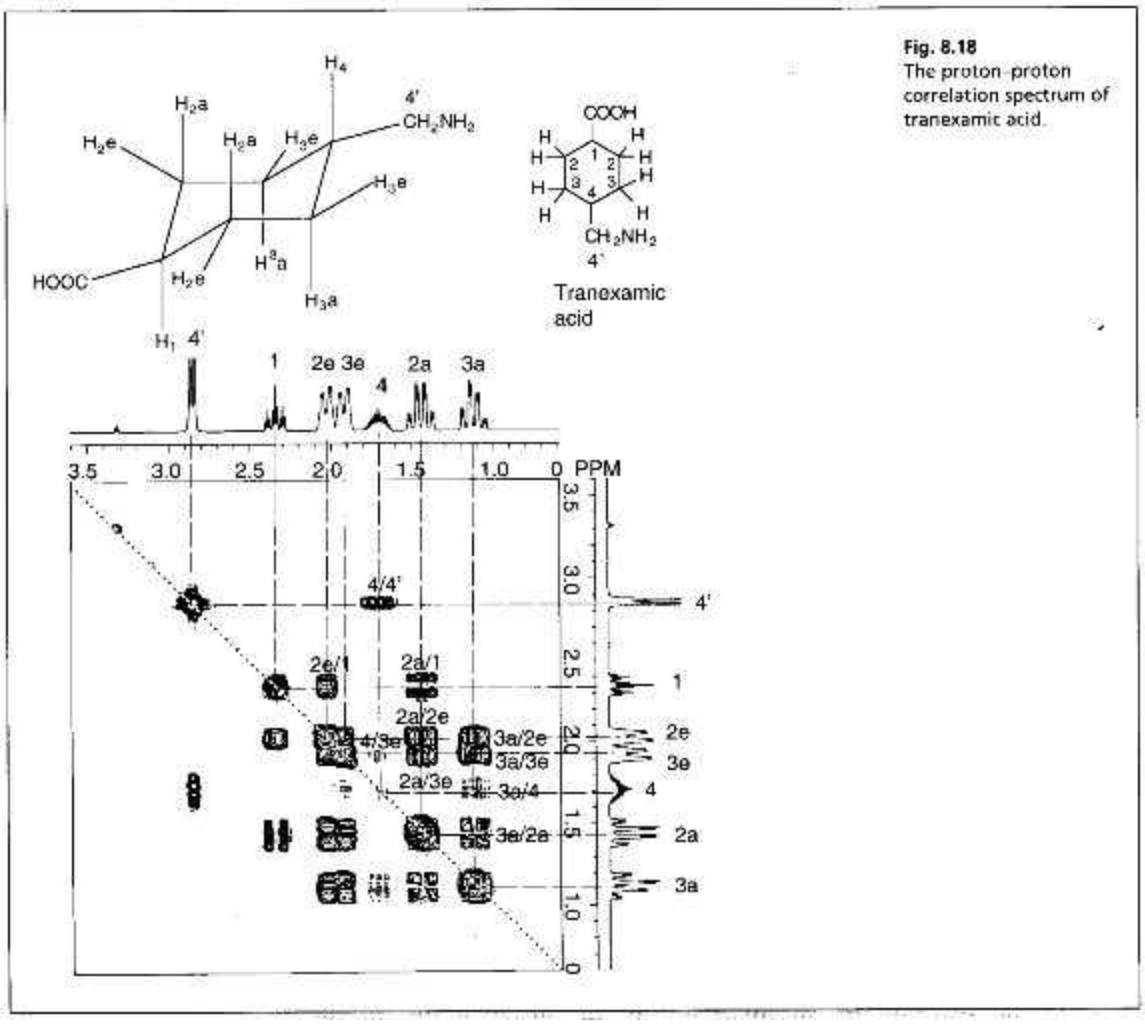


Fig. 8.18
The proton–proton correlation spectrum of tranexamic acid.

The anti-haemorrhagic drug tranexamic acid when drawn in a two-dimensional representation may look as if all four protons on position 2 and all four protons on position 3 are equivalent. However, when the structure is drawn as indicated on the left in Figure 8.18 it is apparent that, because the molecule is forced for steric reasons to remain with the carboxylic acid and methylamine groups more or less in the plane of the paper, the axial (a) protons, which are held above and below the plane of the paper, and the equatorial (e) protons, which are held more or less in the plane of the paper, are no longer in an equivalent environment. This introduces a number of additional couplings between the protons in the molecule leading to an increased complexity of its spectrum. Assignment of the protons in this spectrum is simplified by two-dimensional NMR and as for the aspirin example the correlations can be derived from the signals either side of the diagonal. The place to start in this type of assignment is usually with the simplest signal, which in this case is due to the 4′ protons which only couple to the 4 protons. The 4 protons themselves present the most complex signal since they are separately coupled to the 4′, 3a and 3e protons producing $3 \times 3 \times 3 = 27$ lines which are not all seen because of the overlap of the signals. Two additional factors emerge from examination of the signals due to axial and equatorial protons in Figure 8.18 that are applicable to the interpretation of more complex molecules:

(i) The signals due to 2a and 3a experience three couplings due to coupling to the equatorial protons attached to the same carbon (germinal coupling) and due to coupling (9–13 Hz) to two adjacent axial protons resulting overall in broad signals. The signals due to 2e and 3e protons are narrower since they only experience one large germinal coupling to the axial proton attached to the same carbon. The axial/equatorial and equatorial/equatorial couplings (e.g. 2e/3a and 2e/3e) are small (2–5 Hz) resulting in narrower signals overall.

(ii) Axial protons (2a and 3a) are usually upfield from equatorial protons (2e and 3e) since they are shielded by being close in space to other axial protons.

Application of NMR to quantitative analysis

NMR can be used as a rapid and specific quantitative technique. For example a drug can be rapidly quantified by measuring suitable protons (often isolated methyl protons) against the intense singlet for the methyl groups in t-butanol. The amount of drug present can be calculated using the following formula for the methyl groups in t-butanol used as an internal standard (int. std.):

$$\text{Amt. of drug} = \frac{\text{Area signal for drug protons}}{\text{Area signal for int. std. protons}} \times \text{mass of int. std. added} \times \frac{\text{MW drug}}{\text{MW int.std.}} \times \frac{\text{No. protons from int. std.}}{\text{No. protons from drug}}$$

An advantage of this method of quantitation is that a pure external standard for the drug is not required since the response is purely proportional to the number of protons present and this can be measured against a pure internal standard. Thus the purity of a substance can be determined without a pure standard for it being available. Figure 8.19 shows the spectrum of an extract from tablet powder containing aspirin, paracetamol and codeine with 8 mg of t-butanol added as an internal standard. In the analysis, deuterated methanol containing 8 mg/ml of t-butanol was added to the sample of tablet powder, and the sample was shaken for 5 min, filtered and transferred to an NMR tube.The t-butanol protons gave a signal at ∂ 1.31, the

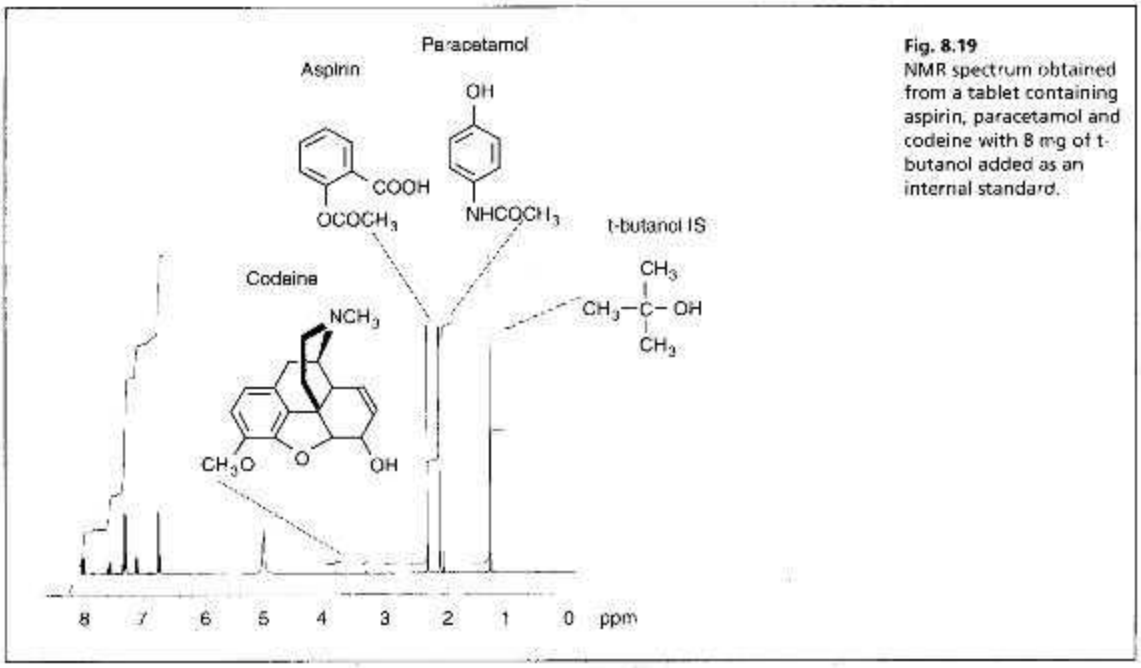


Fig. 8.19
NMR spectrum obtained from a tablet containing aspirin, paracetamol and codeine with 8 mg of t-butanol added as an internal standard.

CH_3CO group in aspirin gave a signal at ∂ 2.35, the CH_3CON group in paracetamol gave a signal at ∂ 2.09 and the CH_3O group in codeine gave a signal at ∂ 3.92. The low amount of codeine present would be likely to make its quantitation inaccurate in the example shown, which was only scanned for a few minutes. Since its signal is close to the baseline, a longer scan would improve the signal:noise ratio giving better quantitative accuracy.

The data obtained from the analysis is as follows:

- Stated content/tablet = aspirin 250 mg, paracetamol 250 mg, codeine phosphate 6.8 mg
- Weight of 1 tablet = 0.6425 g
- Weight of tablet powder taken for analysis = 0.1228 g
- Weight of t butanol internal standard added = 8.0 mg
- Area of internal standard peak = 7.2
- Area of aspirin CH_3 peak = 5.65
- Area of paracetamol CH_3 peak = 6.73
- Codeine phosphate CH_3 peak = 0.115
- MW t-butanol = 74.1
- MW aspirin = 180.2
- MW paracetamol = 151.2
- MW codeine phosphate = 397.4
- Number of protons in t-butyl group = 9
- Number of protons in methyl groups of aspirin, paracetamol and codeine = 3.

Calculation of the paracetamol in the tablets is shown in Example 8.2.

Other specialised applications of NMR

There are a number of other specialised applications of NMR which are valuable in

Calculation example 8.2

Weight of aspirin and paracetamol expected in the tablet powder $= 250 \times \frac{0.1228}{0.6425} = 47.97$ mg

Weight of codeine expected in the tablet powder $= 6.8 \times \frac{0.1228}{0.6425} = 1.300$ mg

Calculation for aspirin

Substituting into the formula given above:

mg of aspirin present in extract $= \frac{5.65}{7.2} \times 8 \times \frac{180.2}{74.1} \times \frac{9}{3} = 45.80$ mg

Percentage of stated content $= \frac{45.8}{47.97} \times 100 = 95.48\%$

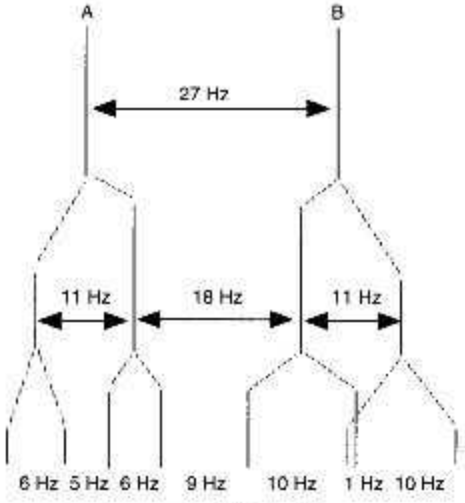
Self-test 8.8

From the above data calculate the percentage of stated content for paracetamol and codeine phosphate.

Answers: paracetamol 95.42%, codeine phosphate 158.5%

pharmaceutical development. Chiral NMR employs chiral shift reagents, e.g. europium tris(d,d-dicamphoylmethanate), which can be used to separate signals from enantiomers in a mixture and thus quantify them. Solid state NMR can be used to examine crystalline structures and characterise polymorphs and crystal hydrates. Biological NMR uses wide bore sample tubes and can be used to examine drugs and their metabolites directly in biological fluids such as urine or cerebrospinal fluid. High-pressure liquid chromatography (HPLC) NMR is currently under development so that impurities or drug metabolites can be chromatographically separated by HPLC and identified by using an NMR spectrometer as a detector.

Answer to Self-test 8.5



References

1. D.H. Williams and I. Fleming. Spectroscopic methods in organic chemistry, 5th Edn. McGraw-Hill Book Co., London (1996).
2. Methods for Structure Elucidation by High-Resolution NMR. G. Batta, K. Kover and C. Szantay, eds. Elsevier, Amsterdam (1997).

Further reading

Basic One- and Two-Dimensional NMR Techniques. H. Frebolin, ed. Wiley Interscience, Chichester (1993).