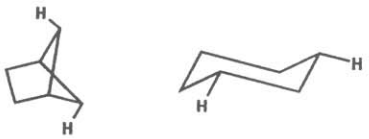


$^4J = +7$ Hz $^4J = +1.1$ Hz

Scheme 9.3-3

appreciable couplings can occur in special cases, where the coupled protons are in a sterically fixed "W" configuration. This is shown in two examples in Scheme 9.3-3, a bicyclohexane and cyclohexane.

Couplings through four bonds can often be observed in unsaturated compounds. The *meta* couplings in aromatic compounds are examples of this. In alkenes the couplings of protons in allylic positions are always detectable and the allylic coupling constant ^4J can be quite large, up to 3 Hz. Where the coupling pathway includes a triple bond, ^4J is typically in the range 1–4 Hz.

C,H coupling constants and chemical structure

Table 9.3-5. Coupling constants ^1J(C,H) in ethane, ethene, benzene, and ethyne

Compound	^1J(C,H)
H_3C-CH_3	124.9
$H_2C=CH_2$	156.4
C_6H_6	158.4
$HC\equiv CH$	249.0

As mentioned in Sec. 9.3.2 and 9.3.4 ^{13}C NMR spectra are routinely recorded with broad-band decoupling. Measurements of C,H coupling constants are time-consuming and are therefore only performed in exceptional cases. Nevertheless, many C,H coupling constants have been determined and their relationships to chemical structure have been studied. The most important couplings are those through *one bond*, ^1J(C,H). Table 9.3-5 lists values for ethane, ethene, benzene, and ethyne. The differences can be quite large: ^1J(C,H) in ethane is 124.9 Hz, whereas the value for ethyne is 249 Hz! However, for ethene and benzene the difference is quite small.

These observations suggest that there may be a relationship between ^1J(C,H) and the hybridization of the carbon atom involved. In fact there exists an empirical correlation between the *s*-fraction (denoted by *s*) and the coupling constant:

$$^1J(\mathrm{C,H}) \approx 500\, s \qquad (9.3\text{-}20)$$

The value of *s* for sp^3 hybridization is 0.25, that for sp^2 is 0.33, and for sp hybridization $s = 0.5$.

Substituents have a considerable influence on C,H coupling constants, as can be seen from the few examples below:

	CH_4	CH_3Cl	CH_2Cl_2	$CHCl_3$
^1J(C,H) =	125	151	178	209Hz

C,H couplings through two or more bonds are at least one order of magnitude smaller. They are used as an aid to structure determination only in special cases.

9.3.5 Special methods for assigning ^{1}H and ^{13}C signals

Introduction

Data collections are a valuable aid to assigning spectral features.

Most NMR spectra are measured to get information about the structure of molecules. This information is contained in the spectral parameters, namely the chemical shifts (δ), the coupling constants (J), and the intensities. In analyzing the spectrum to determine these parameters, the most important step is the assignment of all (or nearly all) the signals to specific nuclei or groups in the molecule. Nevertheless, a complete assignment is not always possible at first, even for an experienced NMR spectroscopist. In many cases more information is needed, and this may come from additional experiments or from data collections. In Sec. 9.3.3 we mentioned the use of empirical correlations to estimate ^{1}H or ^{13}C NMR chemical shifts. These rules are based on the observation that within a particular class of compounds the contribution of a substituent to the chemical shift is nearly constant. It would not be appropriate here to go into details of these methods, or of those based on the effects of solvent and temperature, H-D exchange, or altering the chemical structure of the molecules by derivatization. However, a few common experimental methods that have now become routine, or nearly so, in ^{1}H and ^{13}C NMR spectroscopy will be described briefly in the following sections. These are spin decoupling, the DEPT experiment, and two types of two-dimensional (2D)

experiments. The DEPT and 2D experiments are introduced here with the aim of awakening the reader's interest in learning more about these procedures, and should serve to give a modest impression of the potential of modern NMR spectroscopy. However, the theory and experimental details of these procedures are beyond the scope of this short introduction.

Spin decoupling

Heteronuclear decoupling simplifies the spectrum of one nuclide by eliminating the coupling to another.

Homonuclear decoupling is a useful technique for analyzing proton spectra.

In Sec. 9.3.2, "The indirect spin-spin coupling", it was explained that the interaction of neighboring nuclear dipoles via the indirect spin-spin coupling mechanism causes splitting of the signals, giving characteristic multiplet patterns. These coupling patterns yield information about the structures of molecules. For example, the presence of a quartet and a triplet in the spectrum indicates an ethyl group. In Sec. 9.3.2, "The NMR method and the spectrometer", we learned that ^{13}C NMR spectra are normally recorded with broad-band (BB) decoupling so that indirect spin-spin couplings are removed. This is achieved by irradiating with a high power level at the frequency corresponding to proton transitions, so that the spin orientation of the protons changes rapidly; thus the lifetime in each spin state is shortened, with the result that the coupling is averaged to zero. The signal splitting disappears; multiplets become singlets. This ^{1}H BB decoupling procedure is a *heteronuclear* decoupling experiment because protons are irradiated while ^{13}C resonances are observed. Homonuclear decoupling experiments are also possible; they are very important and are used routinely in ^{1}H NMR spectroscopy to identify signals belonging to mutually coupled protons. As an example we may take acetylsalicylic acid, whose partial spectrum in the aromatic region, which we discussed earlier, is shown in Fig. 9.3-30a. To demonstrate the method, we irradiate at the resonance frequencies of the doublet of doublets centered at $\delta = 7.95$, which we assigned to H-6, the proton in the *ortho* position relative to the carboxy group. Comparing the coupled and the decoupled spectra (see Fig. 9.3-30) we see that the doublet of triplets at $\delta = 7.25$ has simplified, so that one *ortho* coupling is now missing. Therefore we can assign that signal to H-5. However, we also see a simplification of the other doublet of triplets at $\delta = 7.5$ because the *meta* coupling ^{4}J(H-4/H-6) has been eliminated too; consequently this multiplet can be assigned to H-4.

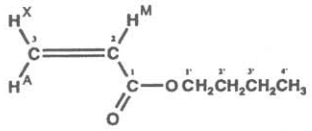
Thus a single decoupling experiment, the irradiation of the resonance frequencies of H-6, led to the simplification of the spectrum and to an assignment of the signals of all the aromatic protons. In other cases more than one decoupling experiment may be necessary to reach a complete assignment.

In our example we *selectively* irradiated the resonances of one proton and identified the signal positions of all coupling partners. In contrast to this, the heteronuclear ^{1}H BB-decoupling method which results in a ^{13}C NMR spectrum consisting of singlets is *nonselective*, because all the ^{1}H resonances are irradiated simultaneously. However, selective heteronuclear decoupling experiments (as opposed to broad-band, BB) are also possible. Such experiments are, of course, not restricted to the combination of the nuclides ^{1}H and 13C; in principle we can decouple any given pair of nuclides, for example $^{1}H/^{19}F$ or $^{13}C/^{31}P$.

The DEPT experiment

Off-resonance decoupling reduces multiplet splittings, thus helping in assignments.

As mentioned in the previous section and also in Sec. 9.3.2, "The NMR method and the spectrometer", ^{13}C NMR spectra normally consist only of singlets because couplings to protons are eliminated by broad-band (BB) decoupling. Consequently the only data available for the assignment of signals are the chemical shifts. In many cases it would be very helpful to know how many hydrogen atoms are bonded directly to each carbon atom. A spectrum recorded without decoupling would, of course, contain this information in the form of the multiplicities of the signals. But, as explained in Sec. 9.3.2, to measure such non-decoupled spectra is very time-consuming and the spectra are often difficult to analyze, especially when the molecule contains many carbon atoms. Up to the early 1980s, ^{13}C, ^{1}H coupling information was nearly always obtained by the ^{1}H



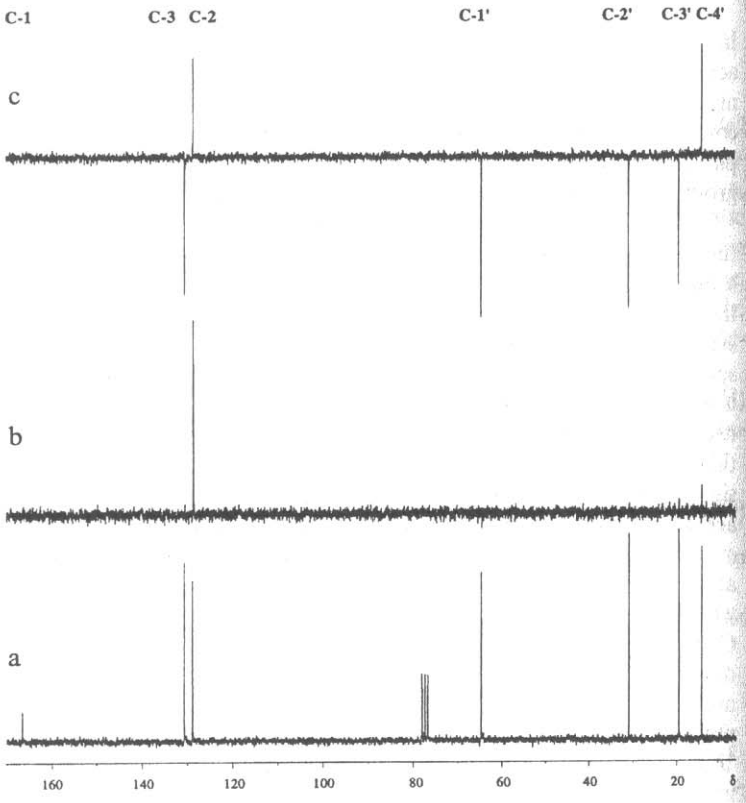


Fig. 9.3-32. Examples of DEPT experiments. (a) 50.3 MHz ^{13}C NMR spectrum of acrylic acid n-butyl ester with ^{1}H broad-band decoupling. (b) DEPT(90) spectrum; CH sub-spectrum of (a). (The small signal at $\delta = 13.9$ corresponds to the residual signal of the CH_3 group. A complete suppression of all CH_2 and CH_3 signals is sometimes difficult because it is necessary to make compromises in selecting the experimental conditions). (c) DEPT(135) spectrum; positive signals arise from CH and CH_3 groups, negative signals arise from CH_2 groups. (*Experimental conditions*: ≈50 mg in 0.5 ml $CDCl_3$ as solvent, total time approximately 15 min)

off-resonance decoupling technique. In this procedure the C,H coupling constants are not averaged to zero by decoupling, but are effectively reduced to typically one-tenth of their normal value. This means that the multiplet structures can still be recognized: quartets for CH_3, triplets for CH_2, doublets for CH and singlets for quaternary carbons (C_q). Couplings to protons which are two bonds or more away disappear completely. However, with the new generation of NMR experiments the DEPT technique (acronym for **D**istortionless **E**nhancement by **P**olarization **T**ransfer) has been developed as a better alternative. This technique uses a complex pulse sequence which need not concern us here; for our purpose, only the result is of importance. By carrying out two further experiments (DEPT(90) and DEPT(135)) in addition to the recording of the normal BB-decoupled ^{13}C NMR spectrum, we obtain the same information as from the off-resonance spectrum, but much more easily. Figure 9.3-32 shows the three ^{13}C NMR spectra for acrylic acid n-butyl ester. Spectrum (a) is the BB-decoupled ^{13}C NMR spectrum. Spectrum (b) is the DEPT(90) spectrum, which theoretically should contain only signals for CH groups, all with positive amplitude. In our example we find only the resonance signal for one CH group. Spectrum (c) is the DEPT(135) spectrum. As can be seen it is a sub-spectrum of (a). Besides the CH signal already seen in spectrum (b) we find one additional signal with positive amplitude and four with negative amplitudes. These additional signals can be assigned to the ^{13}C resonances of a CH_3 group (positive amplitude) and four CH_2 groups (negative amplitude). Neither the DEPT(90) nor the DEPT(135) spectrum shows a signal for the quaternary carbon, that of the carboxylic group –COO–. We therefore know that our molecule contains one CH group, four CH_2 groups, one CH_3 group, and one quaternary carbon, C_q.

In most practical cases it is sufficient to record only the BB-decoupled ^{13}C NMR spectrum and the DEPT(135) spectrum. It is true that we cannot then differentiate between signals of CH and CH_3 groups, because both have positive amplitudes, but one can usually distinguish between them using other criteria (for example, indications from chemical shifts). The signals of the four CH_2 groups above can be easily assigned on the basis of substituent increments such as those mentioned earlier. However, the following section describes two procedures which enable one to complete the assignments without recourse to these other sources of information.

Two-dimensional experiments

Two-dimensional (2D) NMR spectroscopy represents a new generation of NMR experiments. Whereas a one-dimensional spectrum has just one frequency axis, the abscissa, with the intensities as ordinate, in a two-dimensional spectrum both axes, the abscissa and the ordinate, are frequency axes, with the intensities constituting a third dimension. In the following we will first discuss a 2D spectrum in which ^1H chemical shifts along both frequency axes are correlated with each other. This technique has become known as H,H-COSY (from correlated spectroscopy). Next we will learn about the kinds of information that can be deduced from a 2D spectrum in which ^1H and ^{13}C chemical shifts are correlated, by the method of heteronuclear $^1H,^{13}C$-correlated spectroscopy, or H,C-COSY. Without going into the theory we can understand the results obtained by these techniques and learn how such spectra can be analyzed.

All two-dimensional methods are based on the couplings between nuclear dipoles. These interactions need not necessarily be scalar couplings (the "indirect spin-spin coupling"); dipolar interactions through space can also be involved, through their effects on nuclear relaxation (see Sec. 9.3.2, "Free induction decay and relaxation"). However, the examples considered here will be restricted to scalar coupling. In our first example we treat the homonuclear case, H,H-COSY.

Two-dimensional homonuclear (H,H)-correlated NMR spectroscopy (H,H-COSY)

The analysis of a 2D spectrum begins with the identification of diagonal and cross peaks.

In Fig. 9.3-33 the H,H-COSY spectrum of acrylic acid n-butyl ester is shown. The normal one-dimensional ^1H NMR spectrum appears at the top edge and at the left-hand edge of the 2D spectrum. The δ-scales are given below and to the right. The abscissa is called the F_2-axis and the ordinate the F_1-axis. In the 2D spectrum we find peaks on the diagonal, the *diagonal peaks*, and off the diagonal the so-called *cross peaks*. The projections of the diagonal peaks onto the two axes are equal (δ_i/δ_i; the first δ-value corresponds to the value on the F_2-axis, the second that on the F_1-axis). When we draw a vertical or a horizontal line, we come to the corresponding signal of proton i in the one-dimensional spectrum (top or left). In our example we find seven diagonal peaks, which correspond to the seven multiplets in the one-dimensional spectrum. These diagonal peaks do not give us any new information. But the cross peaks do! Their positions are (δ_i/δ_j) or (δ_j/δ_i) where δ_i corresponds to the chemical shift of proton i, and δ_j to the chemical shift of proton j. The cross peaks indicate a *correlation* between the chemical shifts of the two coupled protons (or groups) i and j. Each pair of coupled nuclei gives two cross peaks, with the diagonal and the cross peaks forming the corners of a square.

With that knowledge we can analyze the spectrum shown in Fig. 9.3-33. The best method is to begin the analysis at a diagonal peak whose assignment is known. This diagonal peak forms one corner from which we start to draw the first square. In our example we use the peak of the $OCH_2(1')$ group at $\delta = 4.16$ (on the F_2- and F_1-axes). By drawing vertical and horizontal lines we find the cross peaks, and, consequently by completing the square, also the diagonal peak of the coupled protons—here those of the $CH_2(2')$ group. Now we start again to draw the next square, beginning at the diagonal peak of $CH_2(2')$ just assigned. The cross peak in the horizontal (or vertical) direction leads us to the diagonal peak of $CH_2(3')$. By repeating this procedure we can also find the chemical shifts of CH_3. We can see

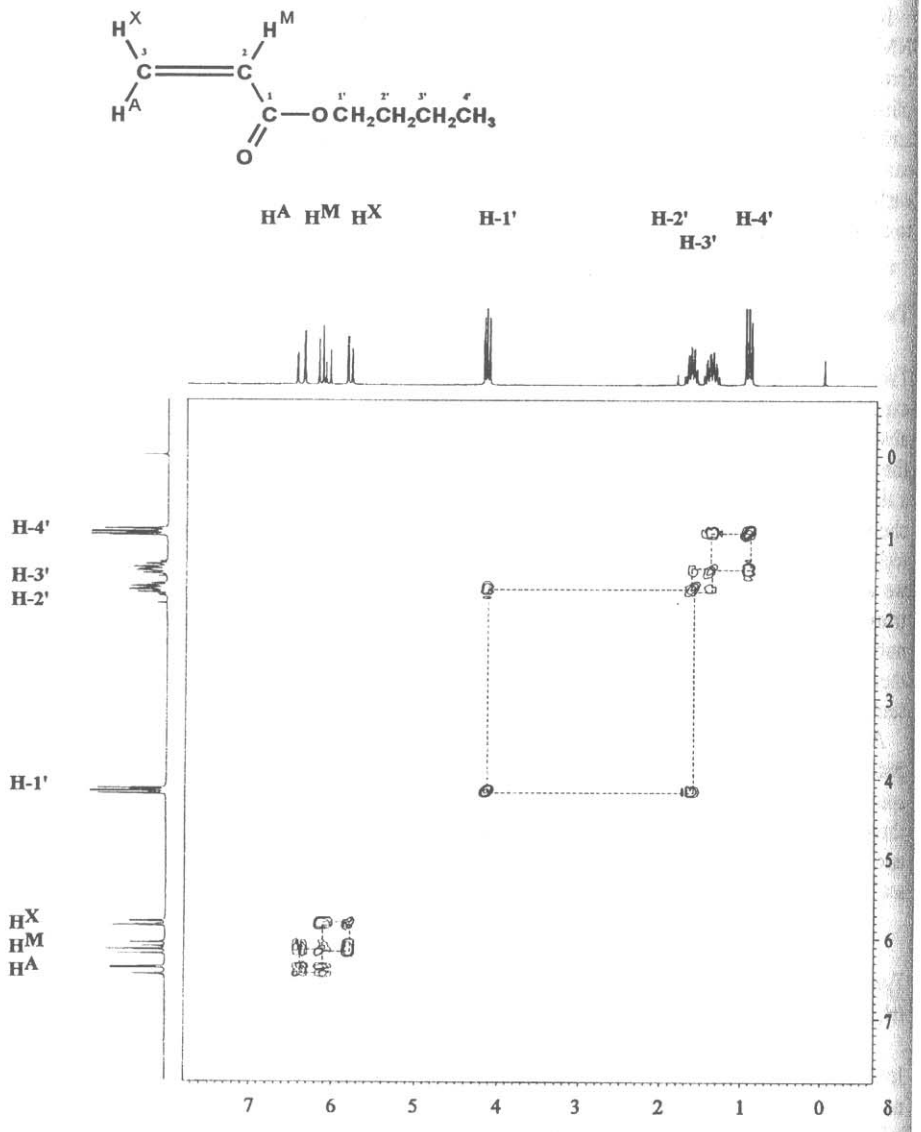


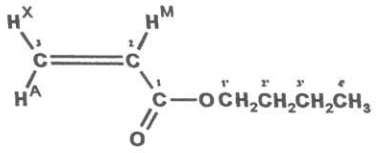
Fig. 9.3-33 200 MHz two-dimensional (H,H)-correlated NMR spectrum of acrylic acid n-butyl ester. At the left-hand edge and at the top is the one-dimensional ^1H NMR spectrum with assignments. The diagonal and cross peaks joined by dashed lines indicate which protons are scalar coupled; they form squares. (*Experimental conditions*: ≈50 mg in 0.5 ml $CDCl_3$ as solvent, total time approximately 1 h)

that diagonal peaks of protons which are coupled to more than one group of protons (here $CH_2(2')$ and $CH_2(3')$) are corners of more than one square. In Fig. 9.3-33, the individual steps of the total analysis are drawn as dashed lines. The result of the assignment is indicated above the one-dimensional spectrum at the top edge.

The same procedure can be used to analyze the peaks of the olefinic protons in the region of $\delta = 5.8$ to $\delta = 6.5$.

The reader will recall that a similar result can be obtained by spin decoupling experiments (see Sec. 9.3.5, "Spin decoupling"). However, as was mentioned there, one may need to perform several decoupling experiments. Moreover, spin decoupling experiments are not possible when multiplets are close together, a limitation which does not apply to the two-dimensional COSY experiment (see, for example, the correlations of the olefinic protons in Fig. 9.3-33). For a fair comparison one must also take into account the measurement time for a two-dimensional COSY experiment. Although this depends on the measurement method, the spectrometer used, and the sample concentration, recording times of one hour or half an hour are fairly typical.

Finally it must be mentioned that diagonal and cross peaks can show fine structures caused by spin-spin couplings, although these cannot be recognized in Fig. 9.3-33. We ignore the fine structure here, since our aim was "only" the correct assignment of signals.



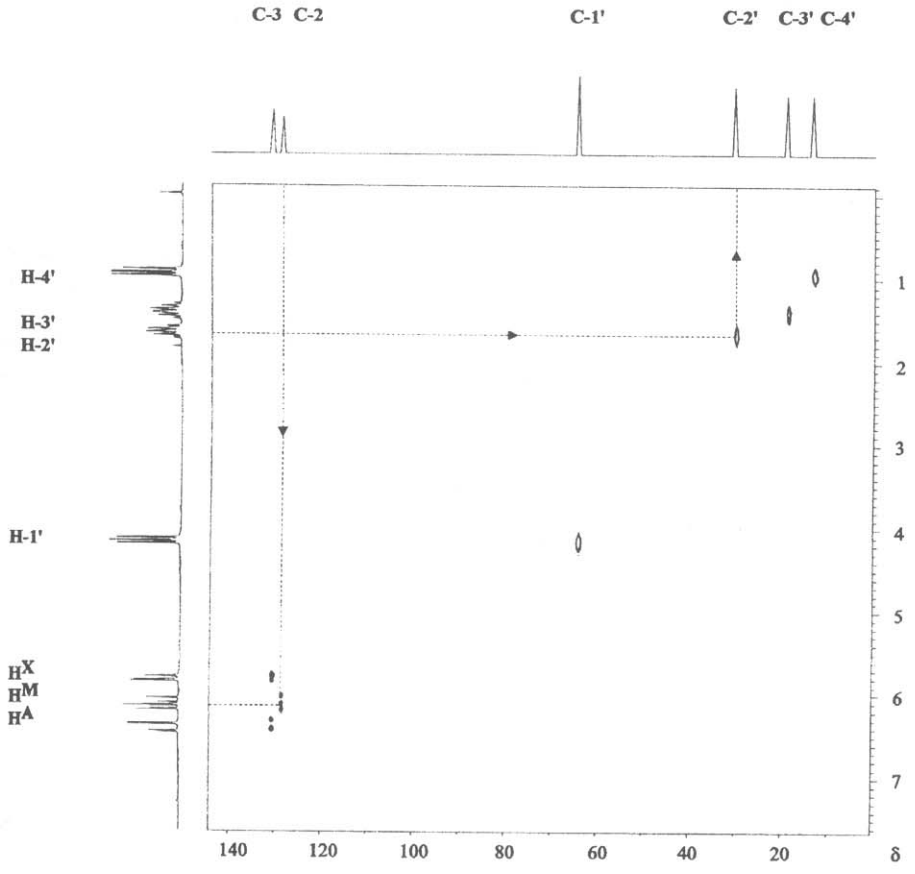


Fig. 9.3-34. Two-dimensional H,C-correlated 50 MHz NMR spectrum of acrylic acid n-butyl ester. The one-dimensional ^1H NMR spectrum is shown at the left-hand edge, while at the top edge is the projection of the two-dimensional spectrum on the F_2-axis. The dashed lines show the analysis procedure for two examples. In the first example the analysis starts at the ^1H NMR signal of CH_2; following the arrows, the ^{13}C resonance of the corresponding nucleus C-2′ can be found. In the second example we start at the signal of =CH to assign the corresponding ^1H NMR signal. (*Experimental conditions*: ≈ 50 mg in 0.5 ml $CDCl_3$ as solvent, total time approximately 1 h)

Two-dimensional heteronuclear (H,C)-correlated NMR spectroscopy (H,C-COSY)

Figure 9.3-34 shows the two-dimensional (H,C)-correlated NMR spectrum of acrylic acid n-butyl ester, the same test molecule which we used in the previous sections. The abscissa (F_2-axis) corresponds to ^{13}C chemical shifts, the ordinate (F_1-axis) to ^1H chemical shifts. As in the H,H-COSY spectrum, the 2D spectrum shows cross peaks which correlate ^1H chemical shifts with ^{13}C chemical shifts. The one-dimensional ^1H NMR spectrum is shown at the left-hand edge, while at the top edge the ^{13}C NMR spectrum is obtained by projecting the peaks of the two-dimensional spectrum onto the F_2-axis. This spectrum shows only six peaks, though the molecule contains seven carbon atoms. Theory shows that cross peaks only appear when carbon nuclei are coupled to directly bonded protons. Therefore a quaternary carbon, such as the carboxy carbon nucleus in this example (at $\delta = 166.3$; see Fig. 9.3-32), does not give a correlation peak. Consequently, in the projection of the two-dimensional spectrum onto the F_2-axis the corresponding peak is missing so that in Fig. 9.3-34 only the range from $\delta = 0$ to $\delta = 145$ is shown.

Usually some of the resonances in the ^1H and ^{13}C NMR spectra can immediately be assigned with confidence on the basis of chemical shifts and multiplicities. In our example we know the assignments of all the ^1H resonances from the H,H-COSY experiment. Also the DEPT(135) experiment discussed earlier allowed us to assign nearly all the ^{13}C signals. Thus, an H,C-COSY experiment would not be

necessary in this case. However, this situation is the exception rather than the rule. Two examples will therefore be described to illustrate the analysis procedure.

In our first example, we start the analysis from the ^{1}H resonance of a proton that has been definitely assigned. For example, we use the ^{1}H signal of $CH_2(2')$ at $\delta = 1.66$ (see Fig. 9.3-34, formula, and spectrum at the left-hand edge). Drawing the horizontal line, there is no difficulty in finding the cross peak and the corresponding ^{13}C resonance of C-2′ at $\delta = 30.58$ in the spectrum at the top edge.

In our second example, we now begin with the ^{13}C resonance at $\delta = 128.5$, which has been assigned on the basis of its chemical shift and the DEPT(135) spectrum to the olefinic carbon nucleus C-2 (=**C**H). By drawing the vertical line, we find the cross peak, and in the horizontal direction (following the line in Fig. 9.3-34) the corresponding ^{1}H resonance of the olefinic proton at $\delta = 6.12$.

Concluding remarks

The development of the pulsed Fourier transform method of recording NMR spectra in the 1960s later provided the basis for a remarkable variety of experimental procedures which could scarcely have been dreamed of at the time. In the space of this article it has only been possible to sketch three of the most important and useful of these, the DEPT experiment and the 2D methods H,H-COSY and H,C-COSY, without giving any experimental details. The armory of multiple pulse methods (with a plethora of acronyms that is bewildering even to experts) continues to grow. On the semiclassical picture used in Sec. 9.3.2, "Free induction decay and relaxation", all these procedures are based on manipulating the macroscopic magnetization vector by means of radiofrequency pulses, alternating with periods of data acquisition. The interested reader can find details in the extensive literature. The hectic pace of development goes on unabated, ensuring the continued fascination of NMR spectroscopy for the specialist and extending its already impressive capabilities for the chemist.

General reading

Introduction to NMR spectroscopy

H. Friebolin, *Basic One- and Two-Dimensional NMR Spectroscopy*, 3rd ed. Weinheim, VCH 1998.

H. Günther, *NMR Spectroscopy. An Introduction*, New York, John Wiley & Sons 1980.

H.-O. Kalinowski, S. Berger, S. Braun, *Carbon-13 NMR Spectroscopy*, Chichester, John Wiley & Sons 1988.

J.K.M. Sanders and B.K. Hunter, *Modern NMR-Spectroscopy. A Guide for Chemists*. Oxford, Oxford University Press 1987.

Physical basis of NMR spectroscopy

R. Freeman, *A Handbook of Nuclear Magnetic Resonance*. New York, Longman Scientific & Technical 1987.

R.K. Harris, *Nuclear Magnetic Resonance Spectroscopy. A Physicochemical View*, New York, J. Wiley & Sons 1986.

C.P. Slichter, *Principles of Magnetic Resonance*, Berlin, Heidelberg, New York, Springer Verlag 1978.

F.J.M. van de Ven, *Multidimensional NMR in Liquids. Basic Principles and Experimental Methods*, New York, VCH Publishers, Inc. 1995.

Theory

R.R. Ernst, G. Bodenhausen and A. Wokaun, *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Oxford, Clarendon Press 1986.

NMR techniques

S. Braun, H.-O. Kalinowski and S. Berger, *100 and More Basic NMR Experiments. A Practical Course*, Weinheim, VCH 1996.

A.E. Derome, *Modern NMR Techniques for Chemistry Research*, Oxford, Pergamon Press 1987.