2.1 High Magnetic Fields

The development of commercially available superconducting magnets cooled by liquid helium [1], the so-called cryomagnets, has made it possible to record NMR spectra with magnetic field strengths of currently up to 18.8 Tesla, corresponding to a $^1$H resonance frequency of 800 MHz. Compared to conventional electromagnets, with their maximal field strength of about 2.3 Tesla ($^1$H frequency of 100 MHz), superconducting magnets offer several advantages. First, under the influence of the higher external magnetic field, the population difference between possible spin states of NMR-active nuclei is increased, leading to a significant improvement in sensitivity$^1$.

Fig. 2.1.1. $^1$H NMR spectra of 4'-bromo adamantane (1), in CDCl$_3$, at a 400 MHz and b 80 MHz, both on the same $\delta$ scale and in identical solutions.
2.1 High Magnetic Fields

This is associated with a considerable shortening of the time required to achieve a certain signal/noise ratio. Moreover, a better resolution between the signals of nuclei with similar chemical shifts is obtained, whereas coupling constants remain unchanged since they are constants. For example, if $\Delta \delta / J$ – the relation between chemical shifts (in Hertz) and the coupling constant in a two-spin system – is 3 at 80 MHz, it is increased at 400 MHz by the factor $400/80 = 5$, reaching a value of 15. Thus, a strongly coupled AB spectrum at the lower field is converted to a weakly coupled AX spectrum at the higher.

This is demonstrated impressively in Fig. 2.1.1. It is hard to believe that both $^1$H NMR spectra belong to the same compound. Only by comparison with the 400 MHz spectrum can it be seen that, for example, the broad peak that appears between $\delta = 2.8$ and 2.6 in Fig. 2.1.1.b does not correspond to one single proton but to an overlap of two signals that can be identified separately in Fig. 2.1.1.a, namely, that at $\delta = 2.70$ and the left part of the doublet at $\delta = 2.55$ (see dotted lines). The second peak in Fig. 2.1.1.b ($\delta = 2.6 - 2.4$) is a composite of even three signals or signal parts. In the 80 MHz spectrum any signal splitting appears five times larger on the $\delta$-scale compared to the 400 MHz spectrum.

This example demonstrates clearly that not only does a high magnetic field considerably simplify the interpretation of high-order spectra, but often it is the only way of achieving a reliable assignment of signals close to each other in the spectrum. Thus, even $^1$H NMR spectra of such complex aliphatic molecules as steroids or triterpenoids became interpretable in the mid-eighties [2 - 5]. Nowadays, a full $^1$H and $^{13}$C signal assignment of such molecular systems is routine (cf. Sect. 3.2).

References


---

1 Sensitivity is an everlasting problem in NMR spectroscopy and a decisive parameter for the spectrometer time required for an experiment. It depends on the sample concentration, gyromagnetic ratios of the nuclei involved, the number of acquisitions (scans), temperature, relaxation times, and – last but not least – the field strength applied [5]. Recently available micro-probeheads allow NMR measurements of sample quantities in the sub-milligram range.
2.2 One-Dimensional $^1$H and $^{13}$C NMR Spectra (INEPT, DEPT)

$^1$H NMR spectra offer three important parameters which can be extracted, the $^1$H chemical shift ($\delta$), the $^1$H,$^1$H coupling constants ($^{J}_{HH}$; $n =$ numbers of intervening bonds, generally 2 - 4), and the signal intensity (integral). The importance of applying high magnetic fields has been emphasized before (Sect. 2.1) in cases where signals are crowded in narrow resonance areas so that overlap and high-order effects are expected. But the multiplicities of well-isolated signals may be difficult to identify if the splittings are small compared to the natural line width. Then, the splittings are no longer resolved, and only the envelope is visible. In such cases it is advantageous to apply a mathematical procedure to the FID resulting in an artificial narrowing of the line width (resolution enhancement). This is achieved by multiplying the FID with a shifted Gaussian function. Line narrowing may be accompanied by wiggles at the flanks of the signals; moreover, a considerably increased noise level has to be tolerated. An impressive example for the merits of resolution enhancement is shown in Fig. 2.2.1.

![Figure 2.2.1. Section of the 500 MHz $^1$H NMR spectrum of anti-11-methyl-7,11-diazatricyclo[7.3.1.0$^2$]tridecan-13-ol (19, cf. exercise 8), in CDCl$_3$: a without (line broadening LB: +0.1 Hz) and b with resolution enhancement (LB: -2.5 Hz, GB = 0.3); digital resolution: 0.08 Hz/data point.](image)
$^{13}$C NMR spectra are routinely recorded under $^1$H broad-band (BB) decoupling [1]. Thus, a significant improvement of the signal/noise ratio is achieved, because the signals of the insensitive $^{13}$C nuclei appear as narrow singlets without any splitting due to $^{13}$C,$^1$H coupling. In addition, the nuclear Overhauser effect (NOE) may enhance the signal intensities thereby as much as threefold (cf. Sect. 2.7). However, this is accomplished by a complete loss of $^{13}$C,$^1$H coupling information so that, for example, the number of hydrogen atoms adjacent to a carbon can no longer be determined.

In $^1$H coupled spectra obtainable by the so-called gated decoupling technique [2] the carbon signals are split owing to the large one-bond $^{13}$C,$^1$H coupling constants ($J_{CH}$, between 120 and 200 Hz), and doublets are observed for $CH$, triplets for $CH_2$, and quartets for $CH_3$ fragments, possibly over a range of several parts per million (ppm). Often these multiplets contain further fine splitting from couplings over more than one bond and may overlap severely so that an unambiguous assignment is impossible. To escape this dilemma, the so-called off-resonance decoupling technique was invented at the beginning of routine $^{13}$C NMR spectroscopy. The effect of partial $^1$H decoupling is achieved by irradiation of a selective proton frequency near to the $^1$H resonance range (off-resonance) [2]. All signal splittings due to $^{13}$C,$^1$H couplings are reduced to such an extent that only the large one-bond couplings give rise to a relatively small amount of residual splitting, and their multiplicities indicate the number of hydrogen atoms attached to carbons. Unfortunately, off-resonance techniques have a number of severe drawbacks. For instance, signal splittings are not always clear enough to determine multiplicities. Moreover, it may be difficult to distinguish a doublet (CH) from a quartet (CH$_3$) signal if the signal-to-noise ratio is not good. The most serious disadvantage, however, becomes apparent when many $^{13}$C signals exist in a narrow chemical shift range, a situation often occurring in the spectra of steroids, triterpenoids, and other molecules containing many carbon atoms in similar chemical environment. Inspite of the relatively small amount of residual splitting, there is still considerable signal overlap, which may easily obscure any identification of multiplets.

Modern multipulse NMR techniques offer methods that replace off-resonance experiments and are able to overcome these problems. The information – separation of $^{13}$C signals according to the number of attached hydrogens – is the same; however, it does not reside in residual splittings, but in signal intensities exclusively. Peaks may be positive or negative, or they may be absent (zero intensity). This effect is obtained by the so-called $J$-modulation [3]. Experiments based upon this principle are called $J$-modulated or $J$-coupled spin-echo measurements and are sometimes referred to under the purely descriptive acronym APT (Attached Proton Test).

There is another important technique called INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) [3], in which a $J$-modulation is accompanied by a Polarization Transfer (PT) from the protons to coupled carbons, leading to a significant improvement in sensitivity. With this method, however, signals of quaternary carbons do not appear because the experiment is generally optimized to accomplish PT via large one-bond coupling. Nevertheless, such quaternary carbon signals can easily be detected by comparison of the INEPT spectrum with the normal $^1$H broad-band decoupled $^{13}$C NMR spectrum.

A further improvement has been introduced by the DEPT technique (Distortionless Enhancement by Polarization Transfer) [4]. Its advantage, compared with INEPT, is a shorter pulse sequence so that during the evolution time the loss of magnetization due to transversal relaxation is less severe. Moreover, DEPT is clearly less sensitive to missettings of parameters such as pulse widths or delays (as functions of coupling constants) [4].
The so-called spectral editing enables us to prepare DEPT spectra in such a way that only CH, CH\_2 or CH\_3 signals are displayed. This technique, however, requires six separate DEPT measurements. The same APT information can also be obtained more economically by two experiments, DEPT135 and DEPT90, as demonstrated in Fig. 2.2.2; this is the method of choice for DEPT spectra in this book.

![Fig. 2.2.2. Aliphatic region of the 100.6 MHz \(^{13}\text{C}\) DEPT spectra of 3-acetyloleanolic acid methyl ester (2), in CDCl\_3; aliphatic region only: a broad-band \(^1\text{H}\) decoupled spectrum; b DEPT135: CH\_3 and CH signals positive, CH\_2 signals negative; c DEPT90: CH signals only.](image-url)
2.2 One-Dimensional $^1H$ and $^{13}C$ NMR Spectra

Fig. 2.2.3.a. 125.7 MHz $^1H$ broadband decoupled $^{13}C$ NMR spectrum of vanillin (3), in CDCl$_3$; b selective INEPT experiment with $^1H$ pulse on H-5, optimized to $^3J_{CH} = 8$ Hz; c heteronuclear two-dimensional J,$\delta$-spectrum (horizontal: $^{13}C$ chemical shifts in $\delta$-scale as in a and c; vertical: $^{13}C$,$^1H$ coupling constants in Hz); d $^1H$-coupled $^{13}C$ signals of the quaternary carbon atoms C-1, C-3 and C-4; e 500 MHz $^1H$ NMR spectrum for comparison.
In INEPT/DEPT experiments PTs are simultaneously accomplished for all $^1$H and $^{13}$C nuclei. In general, the delays between pulses are adjusted to generate PT via one-bond $^{13}$C,$^1$H couplings. An interesting variant of the INEPT pulse sequence [5] involves a „soft“, i.e., selective pulse on one single proton so that $^{13}$C signals appear only for those carbons that are coupled to the irradiated proton. This method is of particular interest if the delays are optimized to a long-range $^{13}$C,$^1$H coupling so that quaternary carbons can be identified. This method is only feasible, however, if the signal of the irradiated proton is isolated from other signals. In Fig. 2.2.3 the application of this technique is demonstrated using vanillin (3) as an example.

It is apparent from Fig. 2.2.3.b that, with a proton pulse on H-5 (Fig. 2.2.3.e) and a delay adjusted for long-range $^{13}$C,$^1$H couplings of 8 Hz, the signals of C-1 and C-3 appear with significant intensities because the respective three-bond coupling constants are the only ones meeting the 8 Hz value in a benzene ring (Fig. 2.2.3.c). The C-4 signal appears with reduced intensity because the corresponding two-bond coupling constant, although having a substantial value, is clearly smaller than the optimal 8 Hz. This example shows that it is easy to differentiate the two oxygen-bearing quaternary carbon atoms C-3 and C-4. The same information can also be obtained by heteronuclear two-dimensional long-range correlation methods (cf. Sect. 2.5).

Selective decoupling techniques have been developed with great effort and applied successfully during recent years [6].

References

2.3 Two-Dimensional $^1H,^1H$ Correlation
(Homonuclear COSY, Linear Prediction, Pulsed Field Gradients, TOCSY)

One of the most important 2D techniques is homonuclear ($^1H,^1H$) COSY (correlation spectroscopy), the spectra which display $^1H$ chemical shifts in both dimensions. COSY spectra are obtained by a series of individual measurements that differ from each other by an incrementally changed delay ($t_1$) between two $90^\circ$ pulses [1,2]. Thus, interferograms are obtained in the time domain $t_2$ (free induction decays or FIDs) which are differently modulated because of the variable $t_1$ time. By this procedure $^1H$ chemical shift information is present not only in the FIDs themselves, but also in their modulation. In the first step the FIDs are Fourier transformed (as is usual in 1D NMR spectroscopy) to create spectra in the frequency domain $F_2$. A second Fourier transformation in the $t_1$ direction provides the second frequency dimension ($F_1$) of the 2D NMR spectra [1 - 3].

One-dimensional NMR spectra are, of course, "two-dimensional", the second dimension being the signal intensity. Correspondingly, 2D NMR spectra are "three-dimensional". Therefore, reproducing such spectra on paper is a problem because the spectra have to be reduced by one dimension. There are two principal ways of achieving this: either the spectrum is depicted in a perspective view (Fig. 2.3.1.b), or the intensity dimension is eliminated and the lost information restored, at least in part, by the introduction of contour lines like in a topological map (Fig. 2.3.1.a).

In the first case one obtains the so-called stacked plot (Fig. 2.3.1.b) which contains the complete intensity information and catches one's eye because of its appearance. Unfortunately, stacked plots suffer from several drawbacks. First, an interpretation is hampered by the perspective distortion. Second, it cannot be determined whether small signals are hidden behind large ones owing to the "white-washing" of peaks. In case of doubt a second plot is necessary from a different angle of perspective.

The second alternative is the so-called contour plot (Fig. 2.3.1.a). As already mentioned, intensity information is partly lost; in cases of doubt, however, it can be regained by plotting traces (horizontal rows or vertical columns) in any desired direction or - more accurately - by applying volume integration. The contour lines are obtained by intersecting the spectrum with planes parallel to the $F_1,F_2$ plane at consecutive heights. The lowest level of the planes and their number determine to which extent intensity information is restored. If the lowest level is too low, many noise peaks will appear, obscuring the real signals. If it is too high, there is the risk that small but real peaks will be ignored. The main advantages of contour plots are that they are very easy to survey and signal hiding, as in stacked plots, is impossible. Furthermore, there is no perspective distortion.

In theory, COSY spectra are symmetrical with respect to the diagonal, since both frequency domains contain the same $^1H$ chemical shift information. In practice, however, such symmetry is seldom observed because the digital resolution is quite different in both dimensions (cf. the two projections in Fig. 2.3.1.a). Moreover, artifacts without any symmetrical counterpart frequently exist which originate in incorrect pulse widths, too short relaxation delays, longitudinal relaxation during the evolution time $t_1$, and other experimental imperfections. In order to eliminate these imperfections, a mathematical algorithm, the so-called symmetrization, can be applied. This procedure compares the memories of data points that are symmetrical pairwise and uses the lower one for both, thereby eliminating all signals that do not possess a symmetrical counterpart. Symmetrization of the 2D data matrix facilitates interpretation of the spectra and, in addition, leads to an improvement in the signal-to-noise ratio by a factor of $\sqrt{2}$, a welcome bonus especially if only a small quantity.
of substance is available. It should not be ignored, however, that symmetrization may also have disadvantages. Artifacts that by chance have a symmetrical counterpart will not be removed and will give the impression that they are real. Many COSY spectra in this book are symmetrized (cf. Fig. 2.3.2 and 2.3.3), whereas TOCSY, NOESY and ROESY spectra are not.

Fig. 2.3.1. 400 MHz COSY spectra of N-methylisocarbostyril (4), in CDCl₃; aromatic region only, no symmetrization; a contour plot; b stacked plot.
A severe problem in 2D NMR spectroscopy is the low digital resolution – at least in $F_1$ – which, generally, is much smaller than in 1D spectroscopy. Consequently, signals are broad and of low resolution. A convenient method for moderate improvement is the so-called Zero-Filling where zeros (data points with information 0) are added at the ends of the FIDs. However, enhancement of data points beyond factor 2 does not provide further resolution. A better computational technique for improving the resolution is Linear Prediction (LP) [4]. Generally, FIDs are truncated in 2D spectroscopy because the number of data points is too low for an acquisition until transversal magnetization had been completely decayed. LP recognizes frequencies and coefficients of an FID and can thereby predict, i.e., extend it [5].

During the nineties a new technique using pulsed field gradients (PFG) spectroscopy [4] was introduced which allows one to record many 2D spectra in a much shorter spectrometer time. Phase cycling to suppress unwanted signal distortions and select coherence pathways has to be executed for each experiment in the series of a 2D experiment without PFG, leading to 8, 16, 32 or even 64 transients. Artifact reducing can be achieved by PFGs applied within the pulse sequence so that the number of transients can be reduced to an absolute minimum necessary for a sufficient signal-to-noise ratio. This may be only one single scan if enough material is available. Thus, the recording time may often be reduced from 45 - 60 to only a few minutes. Spectra obtained by using PFG are denoted by "gs" (gradient-selected), e.g., gs-COSY.

Two basically different types of signals appear in COSY spectra. Those at the diagonal (diagonal peaks) represent the original 1D spectrum, as obtained in a 1D experiment. The off-diagonal signals are the so-called cross peaks, which prove the existence of scalar (through-bond) couplings between nuclei. The corresponding coupling partners can be found by drawing horizontal and vertical lines starting at the cross peak until the diagonal is intersected, and these positions are the signals of the coupling partners. Owing to the symmetry of the spectrum, this procedure can be performed in either the upper left or the lower right triangle.

The evaluation of a COSY spectrum is explained in the following using quinidine as an example (Figs. 2.3.2 and 2.3.3). Fig. 2.3.2 clearly shows how to separate spin systems. The dotted lines show how to establish the connectivities of the aromatic protons: H-2/H-3 and H-5/H-7/H-8. It should be noted that the cross peak belonging to H-5 and H-7 (marked by a little arrow) is quite small because the corresponding $^1H,^1H$ coupling is weak (four-bond meta-coupling: $^4J_{HH} = 2.7$ Hz). A cross peak representing the even weaker five-bond para-coupling between H-5 and H-8 ($^5J_{HH} < 1$ Hz) is not visible. In addition, the connectivities within the vinyl group (H-20/H-21) and the neighboring H-15 is indicated in Fig. 2.3.2. The horizontal dotted line between the cross peak 15-20 and the H-15 diagonal peak passes two more cross peaks belonging to the coupling between H-15 and H-14 or H-14', respectively. These connectivities can be identified easily by inspecting the section of the COSY spectrum (Fig. 2.3.3). Finally, H-11 which is geminal to the hydroxy group and thereby has a large chemical shift ($\delta = 5.57$) identifies its coupling partner H-12 by the corresponding cross peak. The reader himself is invited to establish the connectivities of the protons within the quinuclidine residue (H-12 to H-19) by evaluating the expansion of the COSY spectrum as depicted in Fig. 2.3.3. As help, the assignment is presented in the horizontal spectrum on top of the 2D plot; the stereochemical position of the H-14 vs. H-14', H-17 vs. H-17', H-18 vs. H-18' and H-19 vs. H-19' can be taken from the structure drawing on top of Fig. 2.3.3. or in section 2.8. It is emphasized that cross peaks respond to significant scalar coupling constants regardless of the number of bonds between. Thus, it cannot distinguished between geminal (two-bond, $^2J_{HH}$) and vicinal (three-bond, $^3J_{HH}$) couplings.
Fig. 2.3.2. 500 MHz gs-COSY spectrum of quinidine (5), in CDCl₃; for details of the $^1$H and $^{13}$C data see Sect. 2.8; signals marked by "x" indicate an impurity.
Fig. 2.3.3. Section of the 500 MHz gs-COSY spectrum of quinidine (5), in CDCl₃; for details of the ¹H and ¹³C data see Sect. 2.8; signals marked by "x" indicate an impurity.
Fig. 2.3.4. Section of the 500 MHz TOCSY spectrum of quinidine (5), in CDCl₃, same expansion as in Fig. 2.3.3; for details of the $^1$H and $^{13}$C data see Sect. 2.8; signals marked by "×" indicate an impurity.
If the signals of coupling partners are close to each other, i.e., if they have very similar chemical shifts, the corresponding cross peak is located very close to the diagonal and may be obscured by overlap of the diagonal peaks (cf. H-19/H-19' in Fig. 2.3.3). For such cases there are variants and improvements of the COSY pulse sequence to alleviate the situation. In the so-called COSY45 variant the second pulse is not a 90° but a 45° pulse, decreasing the extension of the diagonal peaks [2]. Therefore, all COSY spectra in this book have been recorded using the COSY45 pulse sequence. Further improvements can be achieved in digital resolution, and even coupling constant values may be extracted from the spectra of more sophisticated COSY versions, e.g., double-quantum filtered (DQF) phase-sensitive COSY spectra or E.COSY and others [6].

During the eighties the potential of COSY spectroscopy was expanded by the introduction of the so-called RELAY technique [1,7]. To understand its principle, let us imagine a three-spin proton system (A--B--C) in which A and B, as well as B and C, are coupled pairwise, but A and C are not. The RELAY experiment creates a PT from proton A to proton B, which is the relay and passes it on to C. Thus, in a RELAY spectrum a cross peak connecting A and C is observed, although these nuclei do not have a significant common coupling. A comparison with the respective COSY spectrum that does not display such a peak can provide further 1H,1H connectivity information.

Later, in the nineties RELAY spectroscopy was outrated by TOCSY (total correlation spectroscopy) [8], also known as HOHAHA (Homonuclear Hartmann Hahn). This technique involves a spin-lock during which all spins are locked in the y'-direction of the rotating frame by a train of 180° pulses or, alternatively, by nonselective composite pulse sequences such as MLEV, WALTZ or others [8]. During the spin-lock time coherence transfer is possible between nuclei within a network of coupling 1H nuclei rendering multiple-relay cross peaks. The result is a 2D spectrum similar to COSY or RELAY. The duration of the spin-lock time and the magnitude of the 1H,1H coupling constants determine how far coherence transfer is achieved along a chain of hydrogen-bearing carbon atoms. In addition, TOCSY offers a higher sensitivity. It should be noted that coupling constants can be read from the rows and columns of TOCSY spectrum only, if it has been recorded in its z-filtered version.

TOCSY application is demonstrated in Fig. 2.3.4 which displays the same section as in Fig. 2.3.3. Compare, for example, the cross peaks for H-16, the bridge-head hydrogen of the bicyclic quinuclidine ring system (horizontal dotted line): whereas there are only two in the COSY spectrum (H-16/ H-19 and H-16/H-17'), five more can be found in the TOCSY spectrum. They are from the left, H-16/H-12, H-16/H-14', H-16/H-18', H-16/H-15, and H-16/H-17.

TOCSY spectroscopy is particularly useful if the molecule consists of several individual spin systems isolated from each other. This is, for example, the case in oligosaccharides. As a demonstration, Fig. 2.3.5 shows the saccharide portion of the 400 MHz TOCSY spectrum of a quilliac acid glycoside with a tetrasaccharide side-chain [9]. It is obvious that the position of the sugar protons of each monosaccharide subunit (A - D) can be identified starting from the individual anomeric proton signals (A1 - D1).

References
Fig. 2.3.5. Section of the 400 MHz TOCSY spectrum of a quillaia acid glycoside (6), containing a tetrasaccharide side-chain, in pyridine-d$_5$; taken from ref. [9].


2.4 Two-Dimensional $^{13}\text{C},^1\text{H}$ One-Bond Correlation

(HETCOR, Inverse Detection, HSQC, HMQC, HMQC-TOCSY)

Heteronuclear COSY (often called HETCOR for Heteronuclear Correlation) is extremely important, since it connects $^1\text{H}$ signals in the $F_1$ dimension with $^{13}\text{C}$ signals in $F_2$ via the large one-bond $^{13}\text{C},^1\text{H}$ coupling ($J_{\text{CH}}$) [1 - 4]. Since in this case the chemical shift information is different for the two dimensions, there is, of course, no symmetry in the spectrum.

In HETCOR spectra only signals for CH$_n$ fragments with $n \geq 1$ are visible, i.e., there is no information about quaternary carbons. The reason is a delay time in the pulse sequence that is proportional to the inverse of $^{13}\text{C},^1\text{H}$ coupling constants and that is calibrated for the large one-bond coupling constants ($J_{\text{CH}} = 120 - 200$ Hz). This delay time can be optimized for smaller couplings. It then, however, has to be increased to such an extent (several hundred milliseconds) that measurements are only feasible if the transversal relaxation time of the protons ($T_2^*$) is relatively large. Otherwise, the magnetization will have decayed more or less at the end of the pulse sequence (i.e., when the FID is to be sampled), and the experiment will be very insensitive.

Fig. 2.4.1 shows a HETCOR spectrum of 4-methoxycarbonyladamantan-2,6-dione (7) [5]. Note that the resolution of the $F_2$ domain is high enough to separate the close-by $^{13}\text{C}$ signals of C-4 and the methoxyl carbon.

All HETCOR spectra in this book have been recorded using a technique affording a quasi-$^1\text{H}$ decoupled spectrum in the $^1\text{H}$ dimension. This is advantageous because the $^1\text{H}$ signals have a higher $F_1$ dispersion that improves the signal-to-noise ratio. The signals of methylene groups with two diastereotopic, and hence anisochronous protons (e.g., those at C-10 in Fig. 2.4.1), however, tend to give small signals because the total intensity is distributed in two parts. Moreover, these signals often display a splitting, or at least a broadening, since the relatively large geminal coupling between the diastereotopic protons remains visible. In addition, an artificial signal may appear exactly midway between the two partial signals.

An even better resolution in the $F_1$ dimension can be achieved by incorporating a BIRD pulse (Bi-linear Rotation Decoupling). Thereby splittings of the $^{13}\text{C},^1\text{H}$ cross peak due to $^1\text{H},^1\text{H}$ couplings in H-$^{13}\text{C}-^{12}\text{C}-^1\text{H}$ fragments can be avoided (X-filter) [6].

Provided that sufficient material is available, i.e., the concentration in the sample is 0.5 M or more, the time demand for a HETCOR experiment is similar to that for a COSY spectrum. If it is possible to obtain a 1D broad-band decoupled $^{13}\text{C}$ NMR spectrum of a given sample within a few minutes with a reasonable signal-to-noise ratio, a HETCOR spectrum can be obtained in less than one hour. Spectrometer time problems arise when the concentration is lower. In such cases the classical HETCOR experiment is inferior to variants using inverse detection mode [7], such as HSQC (Heteronuclear Single Quantum Correlation) and HMQC (Heteronuclear Multiple-Quantum Correlation). These experiments require some hardware modifications and specially designed probeheads.

Whereas HETCOR is a method observing $^{13}\text{C}$ nuclei correlated to $^1\text{H}$ spins, the inverse techniques record $^1\text{H}$ nuclei coupled to $^{13}\text{C}$ spins. Preferably, HMQC spectra are recorded under $^{13}\text{C}$ decoupling using the GARP (Global Optimized Alternating-Phase Rectangular Pulses) technique. This provides two advantages. First, the sensitivity is greatly increased and hence the spectrometer time reduced; it can be shown that the sensitivity gain as compared to the HETCOR experiment is $(\gamma_H/\gamma_C)^{3/2} \approx 8$ [7,8], i.e., the time requirement for achieving the same signal-to-noise ratio is reduced to $(1/8)^2 = 1/64$. Second, longitudinal $^{13}\text{C}$ relaxation times, which may be quite long, are of no significance anymore giving additional spectrometer
time reduction if $^{13}$C relaxation times are expected to be large. It should be noted that HMQC is less affected by pulse flip angle missettings than HSQC so that it is favored in practice. On the other hand, it has been found that HSQC is somewhat more sensitive. In particular, coupled HSQC spectra allow one to view $^1$H multiplet structures [9]. There are versions of so-called edited HMQC and HSQC which allow the differentiation of CH/CH$_3$ and CH$_2$ cross peak signals having opposite signs (positive and negative, or negative and positive, respectively). Thereby, the separation of these two types of signals is possible rendering clearer and safer assignments. Examples of edited HSQC can be found in Figs. 3.2.3, 4.12.3, and 4.12.4.

Fig. 2.4.1. Section of the 100.6 MHz HETCOR spectrum of 4-methoxycarbonyladamantan-2,6-dione (7) [5], in CDCl$_3$. The spectrum in the $^1$H dimension (vertical) is the projection, not the original 1D spectrum.
Fig. 2.4.2. 125.7/500 MHz $^{13}$C-decoupled HMQC spectrum of quinidine (5), in CDCl₃; for details of the $^1$H and $^{13}$C data see Sect. 2.8; signals marked by "x" indicate an impurity.

Fig. 2.4.2 shows the similarity of HETCOR and $^{13}$C-decoupled HMQC spectra. It should be noted that according to common agreement the orientations of the $^1$H vs. $^{13}$C domain in the plots are in
2 Methodology

verted. Thereby, $F_1$ (modulating nucleus) is vertical and $F_2$ (observed nucleus) is horizontal in both cases.

An interesting and useful variant is the $^{13}\text{C}$-coupled HMQC spectrum providing doublets in the $^1\text{H}$ dimension so that $^{13}\text{C},^1\text{H}$ coupling constants can be read from the spectrum, although generally with moderate resolution. Fig. 2.4.3 shows a comparison of both variants.

![Fig. 2.4.3. Aromatic/olefinic section of the 500 MHz HMQC spectra of quinidine (5), in CDCl$_3$: a $^{13}\text{C}$-decoupled; b $^{13}\text{C}$-coupled; for details of the $^1\text{H}$ and $^{13}\text{C}$ data see Sect. 2.8.]

The RELAY technique already mentioned in Sect. 2.3 can be applied in a heteronuclear experiment as well [10]. A proton $^1\text{H}^a$ can transfer polarization via a coupled proton $^1\text{H}^b$ (relay nucleus) to a carbon $^{13}\text{C}^b$ directly attached to $^1\text{H}^b$. Thus, it is possible to monitor $^{13}\text{C},^1\text{H}$ connectivities in a molecule, which otherwise could only be detected – if at all – by a long-range $^{13}\text{C},^1\text{H}$ experiment, such as COLOC or HMBC (Sect. 2.5).

Again, the RELAY experiment has been outrated by the advent of inverse and spin-lock techniques. Nowadays, the experiment of choice is the HMQC-TOCSY combining $^1\text{H},^1\text{H}$ and $^{13}\text{C},^1\text{H}$ connectivity information [11]. For each HMQC cross peak a horizontal line can be drawn leading to the TOCSY partner of the proton involved. Consequently, rectangles (dashed lines in Fig. 2.4.4) can be identified connecting neighboring carbon atoms.
by the vicinal \(^1\text{H},^1\text{H}\) couplings of their attached protons (see e.g. the C-2/H-2 – C-3/H-3 pair in Fig. 2.4.4). That means, proof for a carbon-carbon connectivity is obtained indirectly if the corresponding \(^1\text{H}\) assignment is correct. Another network is C-20/H-20 – C-21/H-21/H-21’ which give further extensions (dotted lines) to H-15 and the two H-14 protons. The reader is encouraged to identify the network of the benzenoic hydrogens and carbons in Fig. 2.4.4 himself.

Fig. 2.4.4. Section of the 500 MHz HMQC-TOCSY spectrum of quinidine (5), in CDCl\(_3\); for details of the \(^1\text{H}\) and \(^{13}\text{C}\) data see Sect. 2.8, signals marked by "*" indicate an impurity.
References

2.5 Two-Dimensional $^{13}$C, $^1$H Long-Range Correlation (COLOC, HMBC)

HETCOR and HSQC/HMOC are largely limited to one-bond couplings. As mentioned in Sect. 2.4, HETCOR becomes insensitive if it is optimized to small long-range $^{13}$C, $^1$H coupling constants. Among the direct methods, a pulse sequence offering a way out of this dilemma is COLOC (Correlation via Long-Range Couplings) [1]. This technique is particularly suitable if the molecule contains quaternary carbons. Since $^1$H, $^1$H couplings influence signal intensities of COLOC peaks in a way not easy to predict, COLOC should preferably be applied to molecules or molecular fragments bearing only few hydrogen atoms.

Fig. 2.5.1. 400 MHz COLOC spectrum of vanillin (3), in CDCl$_3$; encircled peaks are from one-bond $^{13}$C, $^1$H couplings (HETCOR).
In Fig. 2.5.1 the COLOC spectrum of vanillin (3) is shown; the signal assignment is based on $^{13}$C NMR data taken from the literature [2], from a selective INEPT experiment (Fig. 2.2.3.b), and from a HETCOR spectrum. In general, peaks originating from one-bond $^{13}$C,$^1$H couplings are found in COLOC spectra as well; in Fig. 2.5.1 these have been marked by circles. In order to separate these peaks accurately from those representing long-range couplings, it is always advisable to compare the COLOC with the HETCOR spectrum of the same compound. In the COLOC spectrum of vanillin the pulse sequence parameters have been adjusted so that $^{13}$C,$^1$H coupling constants in the range of 4 - 8 Hz will give rise to significant signals. This is the typical range for three-bond couplings in coplanar arrangements, and, indeed, there are corresponding peaks, for instance, C-1/H-5, C-2/H-7, C-3/H-5, C-4/H-2, C-4/H-6, C-7/H-2, and C-7/H-6. The signal connecting C-3 with the methoxy protons is of special interest since it proves that the methoxy group is attached to C-3 and the hydroxy group to C-4, and not vice versa.

The sensitivity of COLOC is lower than that of HETCOR since both are direct, i.e., $^{13}$C-detecting methods. Therefore, COLOC has been substituted by an inverse, i.e., $^1$H-detection technique called HMBC (Heteronuclear Multiple-Bond Correlation) [3]. Fig. 2.5.2 shows a section of an HMBC spectrum of quinidine.

In contrast to HMQC, the HMBC experiment is generally conducted without $^{13}$C decoupling so that correlations via one or more bond can be discerned: one-bond correlations affords double cross peaks in the $^1$H dimension; these are marked by brackets in Fig. 2.5.2. The spectrum clearly shows the connectivities of all hydrogen and carbon atoms involved, including quaternary carbons.

HMBC is greatly superior to COLOC, not only by its reliability in representing long-range $^{13}$C,$^1$H couplings by cross peaks and its clear distinction between one-bond and long-range correlations, but mainly due to its enormous spectrometer time advantage. Even if only a few milligrams of a compound with a molecular weight up to 1000 Dalton is available and a reasonable 1D $^1$H-decoupled $^{13}$C NMR spectrum cannot be obtained even overnight, an interpretable HMBC spectrum can still be recorded in a few hours. Since in the most cases all carbon atoms – quaternary ones included – give at least one signal, the $F_1$ projection can be taken as a 1D $^{13}$C spectrum. Although the digital resolution is often quite low (a few Hz), it is still good enough for routine data acquisition. This performance of the HMBC experiments prompts more and more NMR laboratories in the world to refrain from the insensitive direct $^{13}$C observation when only a small amount of material is available.

References
Fig. 2.5.2. Section of the 500 MHz HMBC spectrum of quinidine (5), in CDCl₃; the first number in the pairs i-j corresponds to $^{13}$C (F₁), the second to $^1$H (F₂); for details of the $^1$H and $^{13}$C data see Sect. 2.8; signal marked by "×" indicate an impurity.
2.6 $^{13}$C,$^{13}$C-Correlation (INADEQUATE)

Structural elucidation of an unknown organic compound or a natural product implies establishing the connectivity of the atoms in the carbon skeleton. The methods described in the previous sections achieve this goal only indirectly, although with remarkable success. For example, first by evaluation of the homonuclear COSY spectrum the connectivity of the protons is established, then, in a second step, HETCOR/HSQC/HMQC and COLOC/HMBC experiments show to which carbons these protons are bonded so that the carbon-carbon connectivity is finally obtained. It was shown in Sect. 2.4 that the combination of HMQC and TOCSY is a further improvement.

![Diagram](image)

Fig. 2.6.1.a 100.6 MHz 1D $^{13}$C INADEQUATE spectrum of cyclooctanol (8); b signal of C-2 expanded; c signal of C-3 expanded. In these expansions the signal line widths were decreased by resolution enhancement. With the INADEQUATE pulse sequence, the partial signals of the doublets have opposite signs (no refocussing). In c the appearance of two doublets does not indicate that the two couplings (C-3/C-2 and C-3/C-4) are of different magnitudes. Rather, the C-3 signals in the two isotopomers ($C_8H_{16}$-$^{2,3}$-$^{13}$C$_2$ and $C_8H_{16}$-$^{3,4}$-$^{13}$C$_2$) do not coincide, i.e., here a $^{13}$C neighbor isotope effect is observed.
Of course, it would be much more elegant to arrive at the carbon-carbon connectivity directly from $^{13}$C,$^{13}$C couplings. Since, however, the natural abundance of the $^{13}$C isotope is ca. 1.1%, only one in about 8000 molecules contains two $^{13}$C nuclei in two ascertained positions. Thus, the sensitivity of such a measurement is extremely low, even at high concentrations. Moreover, the signals from $^{13}$C satellites in the $^{13}$C NMR spectrum can easily be overlapped by the main signal arising from molecules containing only a single $^{13}$C nucleus. In addition, rotational sidebands (if the probe rotates) and peaks from traces of impurities may obscure the identification of the $^{13}$C satellites.

These problems can be overcome by the $^{13}$C INADEQUATE technique (Incredible Natural Abundance Double Quantum Transfer Experiment) [1 - 3], which suppresses the main signals so that only $^{13}$C satellites appear in the spectrum; this technique also removes rotational sidebands and signals from impurities. Thus, in a 1D $^{13}$C INADEQUATE spectrum there are doublets for each carbon, one or more according to its topological position, from which the $^{13}$C,$^{13}$C coupling constant(s) can be taken.

Unfortunately, however, one-bond $^{13}$C,$^{13}$C coupling constants are very uniform in CH$_2$ fragments without further electronegative substituents ($J_{CC} = 30 - 40$ Hz), so it is very often difficult or even impossible to establish a $^{13}$C,$^{13}$C connectivity from these data alone. In cyclooctanol (Fig. 2.6.1) all $J_{CC}$ values are between 34.2 and 34.5 Hz; the only exception is the coupling between C-1 and C-2, which is larger (37.5 Hz) because C-1 has the hydroxy group. It can be seen, even in such a simple example, that the determination of $^{13}$C,$^{13}$C connectivities is not possible from a 1D experiment alone.

Here the transition from 1D to 2D spectroscopy is very helpful. It is possible to obtain 2D $^{13}$C,$^{13}$C INADEQUATE spectra (see Fig. 2.6.2) that resemble the homonuclear COSY spectra introduced in Sect. 2.3. The only difference is that the diagonal peaks appearing in the homonuclear COSY spectra are absent from those of the 2D $^{13}$C,$^{13}$C INADEQUATE spectrum because diagonal peaks represent peaks from $^{13}$C atoms having $^{12}$C neighbors, and these have been filtered out by the INADEQUATE technique. In an evaluation similar to that of the COSY spectra, the connectivity of all carbons in cyclooctanol (8) can be obtained starting with the signal obvious to assign, namely, C-1. Below the corresponding doublet in the trace above the 2D plot, there is a cross peak leading to C-2 if we follow the horizontal dashed line until it intersects the diagonal (dotted line). From here a vertical dashed line identifies the C-2 signal. For C-2 there is another cross peak from which a new horizontal dashed line is drawn marking another intersection with the diagonal. From there we end up at C-3. The signals of C-4 and C-5 can be identified analogously.

Like any other 2D method, this technique requires a series of individual FID measurements. Thus, even with a very large amount of material available for the sample solution, a 2D $^{13}$C,$^{13}$C INADEQUATE experiment is very time-consuming; often it requires one or two days of spectrometer time. Consequently, such measurements are performed only when other methods for establishing carbon-carbon connectivities fail.

The cross peaks in a 2D INADEQUATE spectrum (Fig. 2.6.2) appear as doublets. If however, the $^{13}$C spectral width is high, for example, by measuring the full $^{13}$C absorption range from $\delta = 0 - 220$, the digital resolution $\Delta v$ may easily become too small to resolve these doublets. This is generally the case if $\Delta v > 0.5 J_{CC}$. Then, the cross peaks are singlets, but this does not affect the information content of the spectrum.

The sensitivity of the experiment can be enhanced by a combination of PT (for instance INEPT or DEPT) and INADEQUATE pulse sequences [3] or by GROPE-16 compensation [4].
Fig. 2.6.2. 100.6 MHz 2D INADEQUATE spectrum of cyclooctanol (8), in CDCl₃; the 1D spectrum at the top of the plot is the projection, not the normal \(^1\)H decoupled \(^1^3\)C NMR spectrum.

References
2.7 Dipolar Coupling Experiments (NOE difference, NOESY, ROESY)

The ability to measure nuclear Overhauser effects (NOEs), which enhance signal intensities, has existed for many years, and measurements have been performed using older generation continuous-wave (CW) spectrometers [1]. In the early 1960s it was shown in a double-resonance experiment that the irradiation of a proton $S$ may lead to an up to 50% enhancement of the signal intensity of another proton $I$ [2,3]. The most important condition for such an observation is that nucleus $I$ be greatly relaxed by the dipolar mechanism [2,3]. It is also important that the ability of the irradiated nucleus $S$ to influence the population difference of the transitions of nucleus $I$ fades with the inverse of the sixth power of the distance between both nuclei. Thus, in contrast to scalar spin-spin couplings, the appearance of NOE signal enhancements provides information about the spatial proximity of nuclei in a molecule regardless of the number of bonds between them.

Under the assumption that dipolar relaxation is dominating, the maximal signal enhancement as a consequence of an NOE can be described by

$$\eta_{i}(\text{max}) = \gamma_S / 2 \gamma_I$$

If both nuclei are protons, the maximal intensity gain is 50%, i.e., the signal may become 1.5 times as large. If the observed nucleus is $^{13}$C, the signal can be enhanced as much as threefold in an optimal case since $\gamma (^{1}H) \approx 4 \cdot \gamma (^{13}C)$. This fact has been welcomed in $^1$H broad-band decoupled $^{13}$C NMR spectroscopy from its beginning [4] (cf. Sect. 2.2).

Measurements of NOEs using CW spectrometers have been based on intensity comparisons; in experiments both with and without selective decoupling, the different heights of integration curves have been observed and evaluated. This method is rather limited if the NOE is small. Since the early 1980s, the so-called NOE difference technique has been used to subtract free induction decays (FIDs) obtained with pulse Fourier transform (PFT) spectrometers, both with or without double resonance irradiation. These difference spectra contain only signals of such nuclei which suffer from NOE-induced intensity changes; all others are cancelled. Thus, even very small intensity differences can be reliably monitored, and there is no overlapping of uninvolved signals.

The foregoing is demonstrated in Fig. 2.7.1: the acetyl derivative of a benzodiazepinone derivative [5] has been nitrated. The question is whether the newly introduced nitro group is situated at position 7 or 8. This problem cannot be solved easily by establishing the $^1$H,$^1$H connectivity, since there are no detectable couplings between the aromatic and aliphatic protons. Irradiation of the acetyl methyl protons affords significant intensity enhancements for $H$-$4$ and for one of the aromatic protons, which apparently does not possess an ortho-positioned $^1$H neighbor, since the signal is a narrow singlet and lacks a 7 - 9 Hz splitting. Owing to spatial proximity, this can only be $H$-$6$. So, it has to be concluded that the nitro group is attached to C-$7$. This simple experiment, requiring only a few minutes of spectrometer time, gives an answer to a question that could have been solved alternatively only by establishing $^{13}$C,$^{13}$C connectivities. This, however, would involve the use of time-consuming direct (2D $^{13}$C,$^{13}$C INADEQUATE, Sect. 2.6) or indirect methods (COSY and HMBC, Sect. 2.3 - 2.5).

Often, in such experiments, it is possible to observe artifacts whose origin the user should know for the sake of a correct interpretation. Minor temperature or field strength deviations during the measurement can lead to residual signals with a dispersion-type appearance, even in the absence of an NOE (see, e.g., the signal at $\delta = 7.33$ in Fig. 2.7.1.b).
Occasionally intensities of partial peaks in multiplet signals are severely changed as compared with the unperturbed case; these peaks may even be negative. Such situations occur if the observed and the irradiated nuclei share a significant scalar coupling – for example, diastereotopic protons within a methylene group or vicinal antiperiplanar protons. This is caused by a polarization transfer between transitions with common energy levels, an effect that is successfully used in experiments involving SPT (selective population transfer) [6], INEPT, or DEPT (cf. Sect. 2.2). If the total intensity of such a multiplet, as indicated by the integration curve, is significantly different from zero, the signal can be regarded as NOE positive [3].

If in conformationally mobile molecules some atoms are chemically interchanging (dynamic NMR), an NOE enhancement may occur at atomic positions far from the site of the irradiated nucleus. In such cases the nucleus may have received its signal intensity enhancement in the vicinity of the irradiated nucleus and then changed its position by a fast conformational rearrangement before the original population difference in its energy levels is retained by relaxation.

It is tempting to evaluate an NOE difference experiment quantitatively in order to obtain the magnitudes of internuclear distances within a molecule, and, indeed, it is easy to extract relative intensity values (in percentages) from the computer-stored spectrum. However, the extent of a signal
intensity enhancement depends on many experimental parameters, such as decoupler power, duration of decoupler irradiation, presence of relaxation mechanisms other than dipolar, and correlation times of the molecule. The existence of other ("third") protons $A$ close in space to the target proton $I$ can also influence NOE intensity enhancements, because $A$ can also contribute to the dipolar relaxation of proton $I$, thereby diminishing the effect of the irradiated proton $S$ (direct effect) [3]. This mechanism is one of the major reasons why it is often observed that a comparison of an NOE with its reverse (nucleus 1 $\rightarrow$ nucleus 2 vs. nucleus 1 $\leftarrow$ nucleus 2) is not equal, even allowing for substantial experimental error limits. Simply, the arrangement of "third protons" around the two nuclei involved is different.

It may even occur that a negative NOE difference signal for a proton $I$ is encountered, for example, if a third proton $A$ is positioned in line between $S$ and $I$. Then, $A$ suffers from an NOE itself and transmits the effect further to $I$, but in the reversed sense (indirect effect) [3].

For all these reasons, a quantitative evaluation should be restricted, if made at all, to molecules very similar in structure, to spectra obtained under identical external conditions, and to experiments for which the signal enhancements obtained can be calibrated using known interatomic distances. A semiquantitative interpretation, however (signals indicated as strong, medium, weak, or absent), is significant, often very useful, and in most cases sufficient.

Rarely found in the literature are heteronuclear variants of NOE difference experiments in which protons are irradiated selectively and signal enhancements for $^{13}$C nuclei are observed. The main problem is that carbon nuclei are very efficiently relaxed by their own directly attached protons (direct effect) so that NOEs from other protons farther away cannot produce additional significant signal enhancements. Thus, heteronuclear NOE experiments are largely restricted to the observation of signals belonging to quaternary carbons.

![Diagram](image)

**Fig. 2.7.2.a** $^1$H broadband decoupled $^{13}$C NMR spectrum of fenchone (10), in CDCl$_3$; **b** heteronuclear ($^{13}$C, $^1$H) NOE difference spectrum with H-4 irradiated.
The preceding is demonstrated in Fig. 2.7.2, showing the differentiation between the aliphatic quaternary C-I and C-3 in fenchone. If H-4 is irradiated, a significant NOE is observed for C-3 but not for C-I. Among the hydrogen-bearing carbons only C-4 directly attached to H-4 and C-5 are also affected.

Spatial proximities can also be derived from 2D, the so-called NOESY experiments preferably applied in their phase-sensitive version allowing a separation of NOESY from COSY cross peaks. In their appearance these spectra are very similar to COSY spectra (Sect. 2.3); NOESY cross peaks, however, do not indicate scalar (through-bond) but rather dipolar (through-space) couplings.

The application of NOESY may be exemplified by the determination of the relative configuration of C-12 in quinidine which is opposite to that of quinine, a stereoisomer of quinidine. Fig. 2.7.3 proves that in quinidine (5) it is the H-17 atom (cross peak marked by an arrow), not H-17' (encircled area), which is close in space to the olefinic hydrogen H-20 within the quinuelidine moiety. Another section of the same NOESY spectrum (Fig. 2.7.4) shows the pairwise proximity of H-12 and H-17' (cross peak marked by an arrow), not that of H-12 and H-17 (encircled area). Therefore, it is clear that both substituents, the vinyl and the Ar–CH(OH)– groups, are on the same side of the quinuelidine residue. This is reversed in quinine (structure on the right in Fig. 2.7.3 and 2.7.4); for that molecule a consecutive spatial connectivity H-12/H-17/H-20 (double arrows) would be expected.

Both methods, NOE-difference spectroscopy and NOESY, suffer from the fact that signal intensity changes depend strongly on the molecular mobility. Whereas rapid motion (extreme narrowing limit) affords positive NOE effects as discussed above, their magnitudes decrease, pass through zero and even turn to negative values (the maximum is -100%) when the molecules become larger and/or the solution more viscous. In addition, spin diffusion has a significant influence in the negative NOE regime [3], i.e., propagation of the population disturbances leads to signal intensity changes even for far remote spins. In other words, NOE experiments lose their information about close spatial relationships between spin pairs in the slow motion regime.

An example for negative NOE effects due to restricted mobility is presented in Fig. 2.7.5 for the saponin 6 with a molecular weight of 1134 Dalton [7]. Irradiation of the anomeric proton of glucose C leads to negative NOE difference signals of the indicated protons in close proximity. In this particular case, spin diffusion does not yet play a major role.

1 The fact that the C-4 signal appears at all is surprising, since only the H-4 protons that are attached to 13C-4 nuclei have been irradiated. Those at 13C-4 atoms are represented by the 1H satellites, which are approximately 60 to 70 Hz away from each side of the main signal. Probably, the decoupler power was strong enough to affect not only the main signal but also these satellites.
Fig. 2.7.3. Expanded section of the 500 MHz NOESY spectrum of quinidine (5), in CDCl₃; for details of the $^1$H and $^{13}$C data see Sect. 2.8; signals marked by "x" indicate an impurity. Since the section is unsymmetric, the diagonal is marked by a dotted line for an easier orientation.
Fig. 2.7.4. Expanded section of the 500 MHz NOESY spectrum of quinidine (5), in CDCl₃; for details of the $^1$H and $^{13}$C data see Sect. 2.8; signals marked by "x" indicate an impurity.
The ROESY experiment (Rotating-Frame Overhauser Enhancement Spectroscopy) [8], a variant of NOESY, offers a solution. It is a spin-lock experiment similar to TOCSY (see section 2.3). Whereas in classical NOE experiments, population disturbances are effected by longitudinal relaxation (z-direction), this takes place in ROESY by transversal relaxation during the spin-lock time in the y'-direction of the rotating frame. Since this is another kind of relaxation, its dependence on mobility is different. In fact, the signal intensity changes remain positive whatever the molecular mobility is. Moreover, the time demand for a ROESY spectrum is generally less than for a NOESY because of the shorter period needed during the spin-lock.

A possible appearance of TOCSY correlation peaks is a disadvantage of ROESY which, however, has been greatly removed by some recent modifications of the pulse sequence [8].

In case of the existence of interconvertable molecular species signals may arise which pretend to indicate spatial proximity although the nuclei involved are far away from each other. This is a consequence of an exchange between the species (EXSY) involved and is observed only if the lifetime of the species is comparable to the presaturation time in the NOE difference or the mixing time in the NOESY or the spin-lock time in the ROESY experiments, respectively [8].

References

2.8 Appendix: $^1$H and $^{13}$C NMR Data of Quinidine (5)

$^1$H chemical shifts (in CDCl$_3$): $\delta = 8.33$ (H-2), 7.90 (H-8), 7.50 (H-3), 7.25 (H-7), 7.14 (H-5), 6.05 (H-20), 5.57 (H-11), 5.05, and 5.02 (two H-21) (AB-part of an ABX spectrum; X-part is H-20; vicinal coupling constant cannot be read directly from the signals; therefore, a stereochemical assignment was not made), 3.81 (OCH$_3$), 3.36 (H-14), 2.99 (H-12), 2.88 (H-14'), 2.85 (H-18'), 2.73 (H-18), 2.21 (H-15), 2.05 (H-17), 1.73 (H-16), 1.49 (H-19), 1.46 (H-19'), 1.09 (H-17).

Selected $^1$H,$^1$H coupling constants (in Hz): 4.6 (H-2,H-3), 2.7 (H-5,H-7), 9.2 (H-7,H-8), 3.9 (H-11,H-12), 1.5 (H-15,H-21).

$^{13}$C chemical shifts (in CDCl$_3$): $\delta = 157.5$ (C-6), 148.0 (C-4), 147.3 (C-2), 143.9 (C-9), 140.7 (C-20), 131.2 (C-8), 126.5 (C-10), 121.3 (C-7), 118.4 (C-3), 114.4 (C-21), 101.2 (C-5), 71.8 (C-11), 59.6 (C-12), 55.5 (OCH$_3$), 50.1 (C-18), 49.6 (C-14), 40.1 (C-15), 28.2 (C-16), 26.4 (C-19), 20.9 (C-17).

The commercial sample used contained traces of ethanol and grease which were not removed prior to the NMR measurements. The corresponding peaks are marked by "x" (impurity).
Fig. 2.8.1. 500 MHz $^1$H NMR spectrum of quinidine (5) in CDCl$_3$; signal marked by "x" indicate an impurity.