Two-Dimensional (2D-J) NMR Spectroscopy for Analysis of Isomers and Heterocouplings

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Z. Naturforsch. 36b, 488–491 (1981); received November 27, 1980

2-Dimensional NMR, Stereoisomers, Heterocouplings

The use of two-dimensional NMR spectroscopy (2D-J) for the identification of isomers and heterocouplings is demonstrated. The routine application of this new method to practical NMR problems is discussed.

Introduction

NMR spectra can be recorded by displaying the intensity of absorption not only as a function of one frequency but also as a function of a second or third variable. This original idea has been suggested by Jeener in 1971 [1] and since then the basic principles and some pioneer experiments of multidimensional NMR spectroscopy have been developed by several groups [2, 3]. In particular, it has become possible to record two-dimensional NMR spectra (a two-dimensional NMR spectrum is a plot of intensity versus two frequency variables). The basic principles of this technique (2D-NMR) have been worked out by Ernst [4] and Freeman [5]. Recently it has been shown that 2D-NMR is suitable for the study of a variety of phenomena, such as multiquantum transitions [6], 13C–13C coupling constants in natural abundance spectra [7] or exchange phenomena [8]. A special case of 2D-spectroscopy is 2D-J resolved spectroscopy [4, 5]. For weakly coupled spins in 2D-J NMR the chemical shift of a nucleus is displayed in one dimension and the coupling of the nuclei with each other (or with an other magnetically active nucleus) is displayed in the second frequency dimension.

The basis of a 2D–J resolved spectrum is a spin echo experiment [9] consisting of a 90° — τ/2 — 180° — τ/2 — FID(t2) pulse sequence. The spin echoes are modulated by homonuclear couplings since the coupled nuclei experience the effects of the refocusing pulse. If the period τ is varied the phase error becomes a phase modulation and this can be transformed into an amplitude modulation. The second half of the spin echo FID(t2) resembles a free induction decay (the FID is a function of t2). The detected signal therefore is a function of t1 and t2. It is transformed first with respect to t2 and then with respect to t1 to produce the two dimensional spectrum F(ω1, ω2). The ω2 frequency dimension is that of conventional NMR (i.e. the chemical shift axis). The homocouplings appear in ω1 frequency dimension. Thus orthogonal projection in ω1 direction on the ω2 dimension results in a type of “broad band 1H decoupled” proton spectrum consisting of singlets (in the absence of heterocouplings) whereas projection in ω2 direction on the ω1 axis yields the homonuclear multiplet patterns of the corresponding resonances.

Although this new technique has been known for some years its application to practical NMR is still rather limited. The aim of this paper is to show the use of 2D-J NMR spectroscopy in routine NMR analysis: in particular the utility of 2D-J NMR in the identification of (stereo)isomers and heterocouplings will be emphasised.

Experimental

All spectra were recorded on a Bruker WH 400 spectrometer, equipped with an Aspect 2000 computer with a 80 K memory and a double drive high density disk. The 2D-J resolved spectra were recorded with a development version of the FT-NMR–2D program. The number of data points in the t1 (ω1) dimension was 256 and in the t2 (ω2) dimension 4096; the corresponding spectral width was ± 17.816 Hz and 570.125 Hz leading to a digital
resolution of 0.139 Hz in the \( t_1 \) (\( \omega_1 \)) and 0.278 Hz in the \( t_2 \) (\( \omega_2 \)) dimension. The 90° pulse was 5.1 \( \mu \)s. The number of transitions for each time \( t_1 \) was 16 (the initial \( t_1 \) value was 250 \( \mu \)s). The repetition rate was ten seconds. (A \( T_1 \)-measurement {inversion recovery} yielded \( T_1(I_1) = 2.3, T_1(I_2) = 2.3 \) and \( T_1(I_3) = 2.1 \) s.) The total acquisition time was 9.7 h and the computing time was about 1.5 h. Before carrying out the Fourier transformation in the \( t_2 \) and \( t_1 \) dimension each FID was multiplied by a certain window function. In the \( t_2 \) dimension the window function \( \exp (2t/AQ_T) \cdot \cos (2\pi t/AQ_T) \), and in the \( t_1 \) dimension the window function \( \sin (\pi t/AQ_T) \) \( (AQ_T = \text{acquisition time}) \) were used. Both window functions improve the resolution and, in addition, the second filter function suppresses strong tails and broad peaks (\textit{e.g.}, the signal of proton 3). All 2D–J spectra are absolute value spectra.

Results

When NMR is used for analysis of diastereoisomers the corresponding multiplets may lie very close together even at high fields. The application of 2D–J spectroscopy for the identification of diastereoisomers will be demonstrated by taking the molecule (1) as a representative example. In (1) there are three asymmetric carbon atoms, thus four diastereomers should be discernable by NMR; however, by \( ^{13} \text{C} \)-NMR (25.2 MHz) only one and by \( ^2 \text{H} \)-NMR (61.4 MHz) only two isomers could be detected. The 400 MHz \( ^1 \text{H} \)-NMR spectrum of (1) dissolved in CDCl\(_3\) is also complex (\textit{cf.} Fig. 1a): there is one major isomer I and its signals for the protons 1, 2, and 3 at \( \delta(I_1) = 3.78, \delta(I_2) = 3.29 \), and \( \delta(I_3) = 3.65 \) can be easily identified as two doublets of quartets, and a “triplet of triplets”, respectively. The latter splitting results from the coupling of 3 with the diastereotopic benzyl protons and with deuterium. The deuterium coupling should give a triplet splitting with the intensity ratio 1:1:1; however, the H,D coupling is small and partly hidden in the line width, therefore a deceiving 1:2:1 intensity ratio is observed for each component of \( I_3 \). The other signals cannot be assigned directly, in particular the resonances around \( \delta = 3.55 \) are unresolved. The assignment becomes clear from the 400 MHz 2D–J spectrum of (1) (\textit{cf.} Fig. 1b and Fig. 2). For clarity and experimental reasons (\textit{cf.} experimental part) only the signals of the protons 1, 2, and 3 are presented. The major isomer I can be easily identified by its signals for the protons 1, 2 and 3, and by the splittings of these resonances. Two further stereoisomers II and III can be detected in the two-dimensional spectrum of (1) (\textit{cf.} the assignment in Fig. 2). They are most easily identified in the projection of the 2D–J spectrum (\textit{cf.} Fig. 1b). In contrast to the one-dimensional 400 MHz \( ^1 \text{H} \)-NMR
The resonances of the protons II\textsubscript{1} and II\textsubscript{3} are now clearly separated, and one signal is recognized as an impurity (marked with *). Furthermore, a signal III\textsubscript{3} of the third stereoisomer can be detected at $\delta(\text{III}_{3}) = 3.76$. These assignments are confirmed by the homonuclear coupling patterns of the seven signals (I\textsubscript{1}, I\textsubscript{2}, I\textsubscript{3}, II\textsubscript{1}, II\textsubscript{2}, II\textsubscript{3}, and III\textsubscript{3}). The multiplets can be obtained from the two dimensional plot (cf. Fig. 2) by projection in the $\omega_{2}$ ($\delta$) direction on the $\omega_{1}$ (J) axis. These projections are shown in Fig. 3.

The line width (homonuclear couplings) is controlled by spin-spin relaxation rather than by magnetic field inhomogeneity. Therefore homonuclear coupling constants which are obtained from 2D-J spectra are of high accuracy, as long as the magnetic field remains constant with time.

The relative intensity of each of the resonances 1, 2, and 3 should be one. The deviation from this ratio (cf. Fig. 1 and Fig. 2) results from apodisation of the FID in $t_{1}$ and $t_{2}$ dimension with certain window functions (cf. experimental part). In particular the apodisation which was chosen in the $t_{2}$ dimension suppresses broad peaks and therefore the intensity of the resonances of proton 3 (with partly unresolved deuterium coupling in the $\omega_{2}$ dimension) is reduced. However, when the intensity of corresponding multiplets is compared (i.e. I\textsubscript{3}; II\textsubscript{3}; III\textsubscript{3}), the real ratio of the stereoisomers (I (82%), II (15%), and III (3%)) should be obtained. The assignment of the stereoisomers I–III is based on their NMR data and on chemical arguments. In I (the major stereoisomer) the relative configuration of the methyl groups is believed to be threo (R*R*) and in II erythro (R*S*), since in I the coupling of the protons 1 and 2 is $^{3}J_{1,2}(\text{I}) = 5.15$ Hz and in II $^{3}J_{1,2}(\text{II}) = 7.24$ Hz. The minor stereoisomer (III) must therefore have the relative configuration of the methyl groups identical to either that of I or II (having only H and D of the OHD group interchanged). No signals for the protons III\textsubscript{1} and III\textsubscript{2} are observed, since they must be virtually degenerate with the corresponding signals of either I or II. Precise integration showed that III\textsubscript{1} and III\textsubscript{2} coincide with the corresponding signals of I, thus establishing the relative configuration of the methyl groups of III as threo. This interpretation agrees well to the chemistry of (1) [10]: (1) was prepared from the corresponding 2-benzyl-4,5-dimethylidioxolane which consisted of 85% of racemic isomer (threo) and of 15% of meso isomer (erythro).

As pointed out earlier the homonuclear couplings exclusively are displayed in the $\omega_{1}$ (J) frequency dimension whereas heterocouplings in general are displayed in the $\omega_{2}$ ($\delta$) dimension. This results from the fact that only the transitions of the proton spin system are affected by the refocusing 180° pulse. In the case of a homonuclear AX spin system the A$_{1}$
and A2, as well as X1 and X2 transitions are changed, while in a heteronuclear AX spin system (A = ^1H and X = ^2H) only the A1 and A2 transitions are interchanged by the 180° pulse. In (1) the splitting of proton 3 should be a triplet of a triplet, due to coupling with the diastereotopic benzyl protons and with deuterium. As illustrated in Fig. 2 and more clearly in an expansion (cf. Fig. 4) the homonuclear couplings of II1 and II3 are displayed in the ω1 (J) dimension, whilst the coupling of II3 with deuterium is displayed in the ω2 (δ) dimension. The singlet at δ = 3.49 originates from an impurity * (cf. Fig. 1b).

![Fig. 4. Expansion (δ = 3.43–3.50) of the 2D-J-resolved spectrum of Fig. 2. The homonuclear couplings of II1 and II3 are displayed in the ω1 (J) dimension, whilst the coupling of II3 with deuterium is displayed in the ω2 (δ) dimension. The singlet at δ = 3.49 originates from an impurity * (cf. Fig. 1b).](image)

Thus 2D-J NMR can be used as a method for identification of heterocouplings especially in those cases where the heteroresonance frequency is unknown or where decoupling experiments cannot be performed. (For instance it is not possible to observe protons and to decouple deuterium while locked on deuterium. Experiments in which ^13C is observed and ^31P is decoupled while locked on deuterium can also not be carried out since the frequency difference of ^31P and ^2H comes close to the ^14C resonance frequency.)

**Discussion**

In many cases it is possible to record NMR spectra over of a small spectral range of interest (with the help of suitable filters folded frequencies can be largely suppressed. Consequently 2D-J spectra can be obtained with high digital resolution in ω1 and ω2 directions (a typical data matrix consists of 4096 × 128 data points) and within reasonable recording and computing time (typically in overnight runs). Of special value is the projection of a 2D-J spectrum in the ω1 (J) direction: it results in a type of broadband proton decoupled one dimensional proton spectrum with true intensity ratios of the singlets (if the appropriate window functions for the apodisation of the FID are used). From the resulting spectrum of singlets symmetry arguments for structure elucidation as well as frequencies for homonuclear decoupling experiments can be obtained. Furthermore mixtures of different compounds or isomers can be easily investigated by means of 2D-J NMR (even at moderate field strength) as long as each isomer has a spectrum which is nearly first order. In particular stereoisomers can be easily detected by 2D-J NMR spectroscopy. Other techniques for identifying stereoisomers such as labeling with deuterium and studying the deuterium resonances may be unfavourable since the high frequency dispersion of the protons is reduced in the deuterium spectrum. The use of shift reagents can also become difficult since the lines broaden (especially in the case of high field spectrometers) and stereoisomers of low intensity are difficult to detect. Hetero-couplings can be readily detected with the help of 2D-J NMR, when only the multiplets of interest are recorded.

[7] R. Freeman,
  b) A. Bax, R. Freeman, and S. P. Kampsell, J. Am. Chem. Soc. 102, 4849 (1980).