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Correlation of Proton Chemical Shifts by Two-Dimensional Fourier Transform NMR

The first two-dimensional Fourier transform NMR experiment of Jeener (*1*) has proved to be of considerable historical importance in the development of two-dimensional NMR spectroscopy (*2*, *3*) but in its original form (a 90°–t_1–90°–t_2 sequence) it has been surprisingly little used. On the other hand, the corresponding heteronuclear chemical shift correlation experiments (*4*) have been quite popular (*5–8*). Recently Nagayama *et al.* (*9*) have proposed a modification of the Jeener experiment in which acquisition is delayed by a further period t_1 so that it starts at the peak of the spin echo. They have demonstrated that this "spin-echo correlated spectroscopy" technique (SECSY) can be extremely useful in the assignment of proton spectra of fairly large molecules, since it identifies the resonances connected by a scalar spin–spin coupling.

The purpose of the present communication is to show that the original Jeener experiment can have some advantages in this application. For the proton spectroscopy of large molecules, the refocusing effect which occurs at time $2t_1$ is not usually an important consideration since the linewidths are largely determined by T_2 relaxation; sensitivity can thus be improved by starting acquisition immediately after the second 90° pulse. The size of the data matrix can be reduced by the equivalent of quadrature phase detection in the F_1 dimension. It is also possible that the form of the two-dimensional spectrum obtained in the Jeener experiment is better suited to the task of sorting out the complicated correlation networks appropriate to large molecules.

A detailed theoretical analysis of the Jeener experiment has been given by Aue *et al.* (*3*), showing that in general the spectrum $S(F_1,F_2)$ contains three kinds of resonance response: axial, diagonal, and cross peaks. Axial peaks appear along the F_2 axis and arise from transverse nuclear magnetization created by the second 90° pulse from longitudinal magnetization; they are weak when $t_1 \ll T_1$. Consider the simple example of a first-order AX spin system with chemical shift difference δ and coupling constant J. The second 90° pulse (called a mixing pulse) interchanges transverse nuclear magnetization between the four resonance frequencies. If the resulting frequency change is only zero or $\pm J$ Hz, the corresponding responses lie on or near the diagonal $F_1 = F_2$. With the convention that all responses of a two-dimensional spin multiplet constitute a "peak," these are diagonal peaks. The frequency change may, however, be of the order of δ, and then there are responses far away from the diagonal; these "cross peaks" are of particular interest because they correlate the shifts of groups of spin-coupled nuclei.

One of the factors governing the efficiency of magnetization transfer from A to X is a term $\sin(2\pi Jt_1)\sin(2\pi Jt_2)$, which implies that both time dimensions should extend to at least $1/(4J)$. Another way of visualizing this requirement notes that the cross peaks in the frequency-domain spectrum consist of two pairs of

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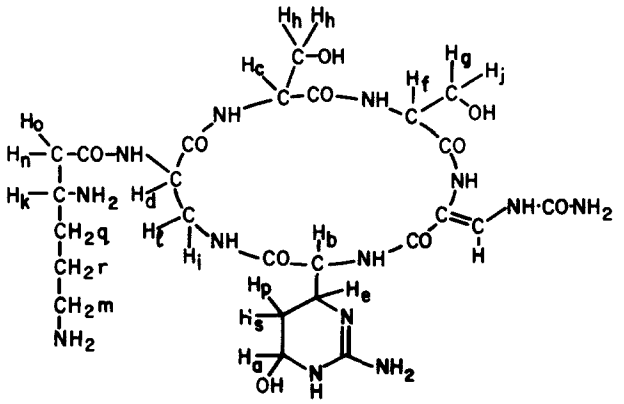

FIG. 1. The cyclic peptide viomycin with the protons labeled according to the assignment of the NMR spectrum shown in Fig. 2.

antiphase signals, so that mutual cancellation occurs if there is poor digitization or excessive line broadening. In this manner it is possible to determine which values of J are large enough to generate appreciable cross peaks.

The principal drawback of the Jeener experiment in its simple form is the large size of the data matrix needed to cover the full range of chemical shifts and yet still resolve the multiplet structure of the cross peaks. Because there is pure amplitude modulation of the signals as a function of t_1, it is not possible to determine the sign of the F_1 coordinates of the peaks, and to avoid ambiguity it would normally be necessary to set the transmitter frequency outside the spectrum and employ single-phase detection. A simple modification circumvents this problem by converting the amplitude modulation into phase modulation. This is achieved by shifting the phase of the second 90° pulse by 90° on alternate transients, while the pulse sequence is being repeated for time-averaging purposes. A two-step sequence suffices for this, but it is convenient to extend the sequence to four steps to suppress the axial peaks arising from longitudinal magnetization at the end of the evolution period. The complete cycle may be written

$$90°(+X)\text{–}t_1\text{–}90°(\Phi)\text{–acquisition}(t_2),$$

where $\Phi = +X, +Y, -X, -Y$. This discriminates the signs of the frequency components in the F_1 dimension, allowing the transmitter frequency to be set near the middle of the spectrum, thus reducing the sampling frequency and the size of the data matrix. A closely related phase-cycling procedure has been used by Nagayama *et al.* (*9*) in spin-echo correlated spectroscopy. In that experiment an additional alternation of the receiver phase is used in order to allow for the alternation in the sense of the Hahn echo; in the present experiment no such alternation is needed.

An illustration of the effectiveness of this correlation technique is provided by the 200-MHz proton spectrum of a D_2O solution of the cyclic peptide antibiotic viomycin (Fig. 1), which has been extensively studied by ^1H, ^{13}C, and ^{15}N NMR by Hawkes *et al.* (*10*). Within a range of 4 ppm there are 19 separate proton resonances, labeled a to s in the conventional spectrum shown in Fig. 2.

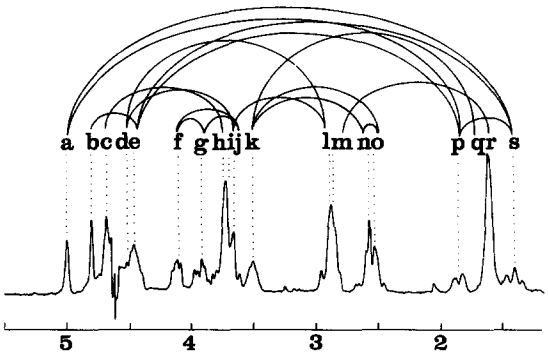

FIG. 2. The 200-MHz proton NMR spectrum of viomycin with the strong HDO peak suppressed (4.65 ppm). The pattern of spin couplings, shown above the spectrum, was determined from the two-dimensional correlation spectrum.

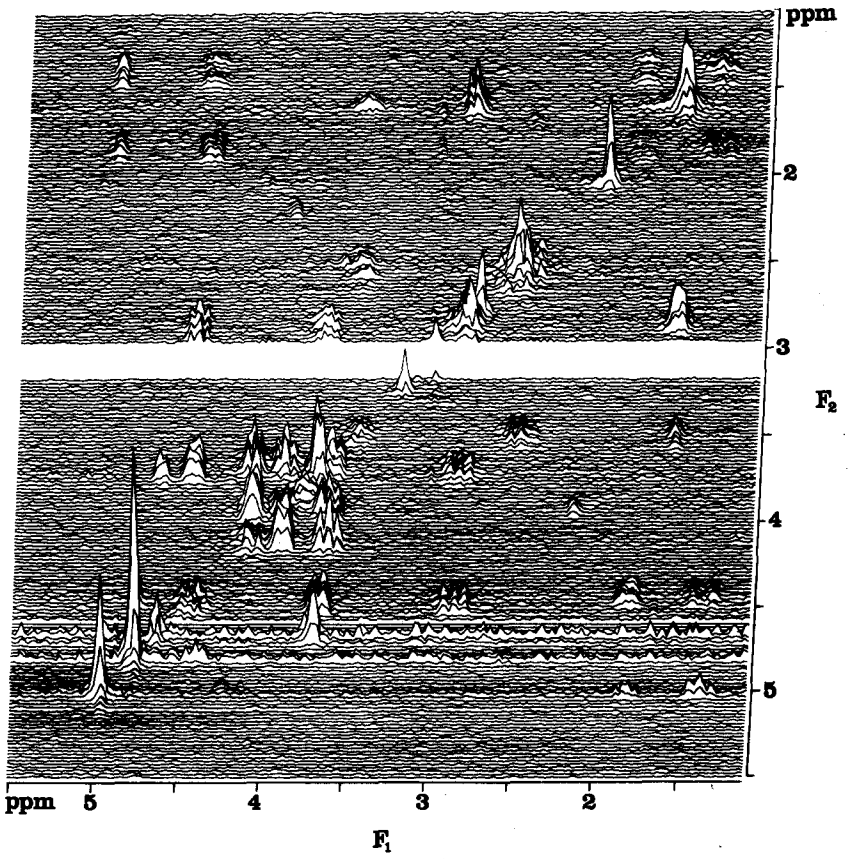

FIG. 3. The two-dimensional proton shift correlation spectrum of viomycin at 200 MHz. For the sake of clarity artifacts near F_2 = 3.1 ppm and near F_2 = 4.6 ppm have been omitted. The conventional proton spectrum runs along the diagonal $F_1 = F_2$, with cross peaks indicating which of these resonances are spin coupled.

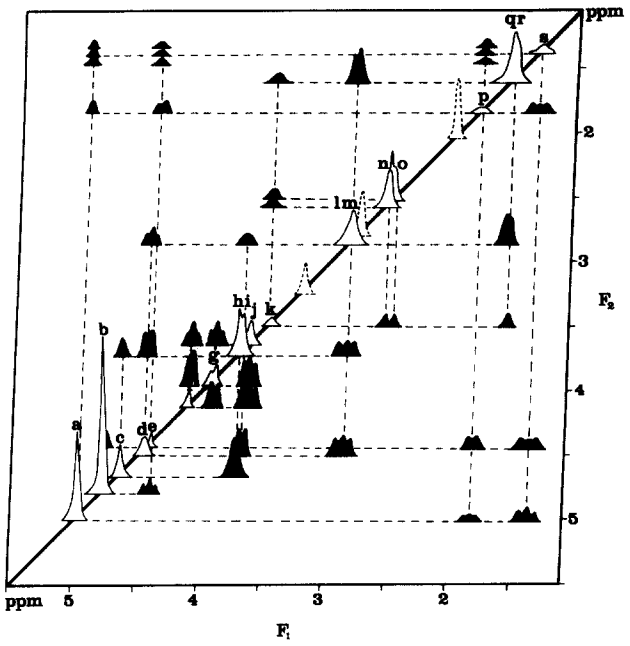

FIG. 4. A tracing of the two-dimensional spectrum of Fig. 3, showing the diagonal peaks (white), the cross peaks (black), and the pattern of shift correlation (broken lines).

The pattern of scalar coupling established by the two-dimensional autocorrelation experiment is shown schematically above the spectrum; there are 18 couplings involved.

Measurements were made on a Varian XL-200 spectrometer modified for two-dimensional Fourier transformation, and equipped with a Zeta fast digital plotter. The data matrix consisted of 256 × 256 complex data points stored in a floating-point format. In correlation experiments of this type it is an advantage to reduce the intensities of the diagonal peaks compared to those of the cross peaks. This can be achieved by exploiting the antiphase nature of the multiplet components which make up a given cross peak. Strong exponential weighting in both time dimensions causes a degree of mutual cancellation of multiplet components within the cross peaks, and therefore convolution–difference (*11*) emphasizes these cross peaks at the expense of the diagonal peaks. Axial peaks are eliminated through the phase-cycling procedure.

The two-dimensional autocorrelation map of viomycin is shown in Fig. 3. The strong HDO line near 4.65 ppm was suppressed by selective irradiation during the period immediately preceding the pulse sequence. It was nevertheless necessary to delete a few traces in this region of the two-dimensional spectrum together with some traces near 3.1 ppm where there was a zero-frequency artifact attributable to the different dc levels in the two quadrature receiver channels.

In order to bring out the many different correlations involved, a tracing of the diagonal peaks (white) and cross peaks (black) was made, and the couplings are indicated by broken lines (Fig. 4). Seventeen pairs of cross peaks may be

discerned; there may also be unresolved cross peaks between lines n and o and between lines q and r. This was how the complex coupling pattern above Fig. 2 was obtained. It is consistent with the assignment indicated in Fig. 1 and that previously reported by Hawkes *et al.* (*10*).

Such autocorrelation maps are clearer and less prone to ambiguity than the alternative selective decoupling experiments. Each scalar coupling gives rise to a pair of cross peaks, in mirror-image positions with respect to the principal diagonal, thus helping to reinforce the assignment if there is any doubt. If necessary, correlation through long-range couplings can be suppressed by employing relatively short t_1 and t_2 ranges, or alternatively may be emphasized by introducing fixed delays $\Delta \sim 1/(4J)$ at the beginning of the t_1 and t_2 periods. Above all the experiment is simple to perform. A purely pragmatic approach to the interpretation can be adopted, without any detailed analysis of the form of the peaks, in a manner similar to that used in assignments by double resonance.

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