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COMMUNICATIONS

Improvement of 2D NOE and 2D Correlated Spectra by Symmetrization

In a recent paper (*1*) we described the use of triangular multiplication for improvement of 2D NMR spectra after the Fourier transformation has been performed in both directions. It soon became apparent that the practical advantages of this routine for work with biological macromolecules (e.g., (*2*, *3*)) exceeded our expectations. New experiments have since been developed which depend critically on the use of triangular multiplication (S. Macura, K. Wüthrich and R. R. Ernst, to be published). This prompts us to describe in the present note a variation of triangular multiplication, i.e., "symmetrization" of 2D NMR spectra, which has been found to be more efficient and to yield superior improvements of the spectra.

In 2D NOE spectroscopy (NOESY) (*4–6*) and 2D correlated spectroscopy (COSY) (*3*, *7*, *8*) the desired connectivity information is contained redundantly at symmetry-related locations in the spectra. On the other hand spurious peaks, which may feign real correlation peaks or mask informative spectral features, do not usually occur simultaneously in the different redundant areas of a spectrum. This is illustrated by the spectrum in Fig. 1A, which contains, besides the desired pairs of cross peaks in symmetric locations with respect to the diagonal peaks (*3*, *7*, *8*), a strong vertical noise band at 4.75 ppm, i.e., at the chemical shift of the HDO resonance, and additional vertical noise bands at 10.6, 7.4, 7.0, 3.9, 3.0 ppm, between 2.0 and 0.7, at 0.3 and at −1.1 ppm. Such noise patterns, which correspond to "tails" of strong diagonal peaks, are quite typical for 2D NOE and 2D correlated spectra of biological macromolecules (*1*, *5*, *6*). Triangular multiplication reduces the intensity of such spurious peaks relative to that of the informative cross peaks by first multiplying the resonance intensities of each pair of points in the $\omega_1 - \omega_2$ plane which are in symmetrical locations with respect to the diagonal peaks, and then inserting into these locations either the square root of this product, or the original intensity, whichever is smaller (*1*).

We propose the use of an improved technique of "symmetrization," where the resonance intensities in each pair of symmetry-related locations are compared and the smaller of the two values is inserted into both locations. It may be noted that the symmetrized spectrum corresponds to that obtained after repeated application of the triangular multiplication routine. The selection of the smaller of the two symmetry-related signal amplitudes corresponds to a minimum estimate of the peak intensities.

Symmetrization of 2D NMR spectra is readily accomplished in the submatrix structure of the present version (Winter 1980/81) of the Bruker–Spectrospin 2D program package. The time required for symmetrization of a 512 × 512 data matrix is approximately 40 sec, and is determined mainly by the necessary disk transfers.

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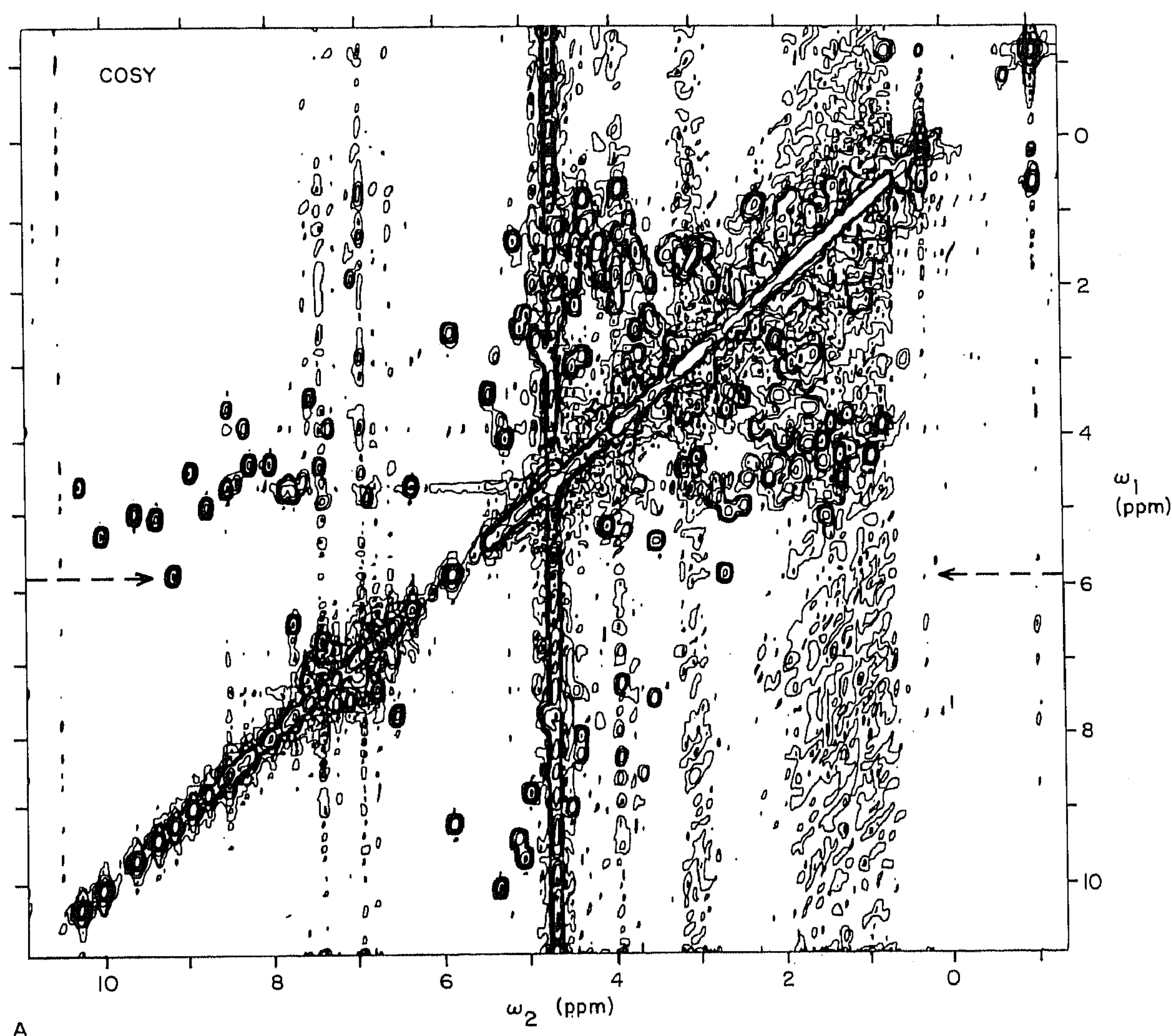

FIG. 1. 2D correlated (COSY) ^1H spectrum of a 0.01 *M* D_2O solution of *Dendroaspis polylepis polylepis* (black mamba) inhibitor K, pD = 3.4, recorded at 25°C on a Bruker HX 360 spectrometer equipped with an Aspect 2000 data system. The spectrum was recorded with quadrature detection in approximately 22 hr. The spectral width is 4464.3 Hz, the data set consists of 1024 × 1024 points. The spectral resolution was improved by multiplication of the free-induction decays with a phase-shifted sine squared bell in the t_2 direction and by a phase-shifted sine bell (*9*) in the t_1 direction. (A) Spectrum without manipulation after Fourier transformation. (B) Spectrum after triangular multiplication. (C) Spectrum after symmetrization. The arrows indicate the location of the cross sections of Fig. 2.

In Figs. 1 and 2 symmetrization is illustrated and compared to diagonal multiplication with a 360-MHz COSY spectrum of *Dendroaspis polylepis polylepis* (black mamba) inhibitor K, a protein with molecular weight 6000 and known amino acid sequence (*10*), which was given to us by Drs. L. Visser and F. J. Joubert of the National Chemical Research Laboratory in Pretoria, South Africa. The experimental conditions used in recording the NMR data are described in the caption to Fig. 1. Comparison of the three spectra in Figs. 1 and 2 clearly shows that the cross peaks are emphasized relative to the spurious peaks by application of

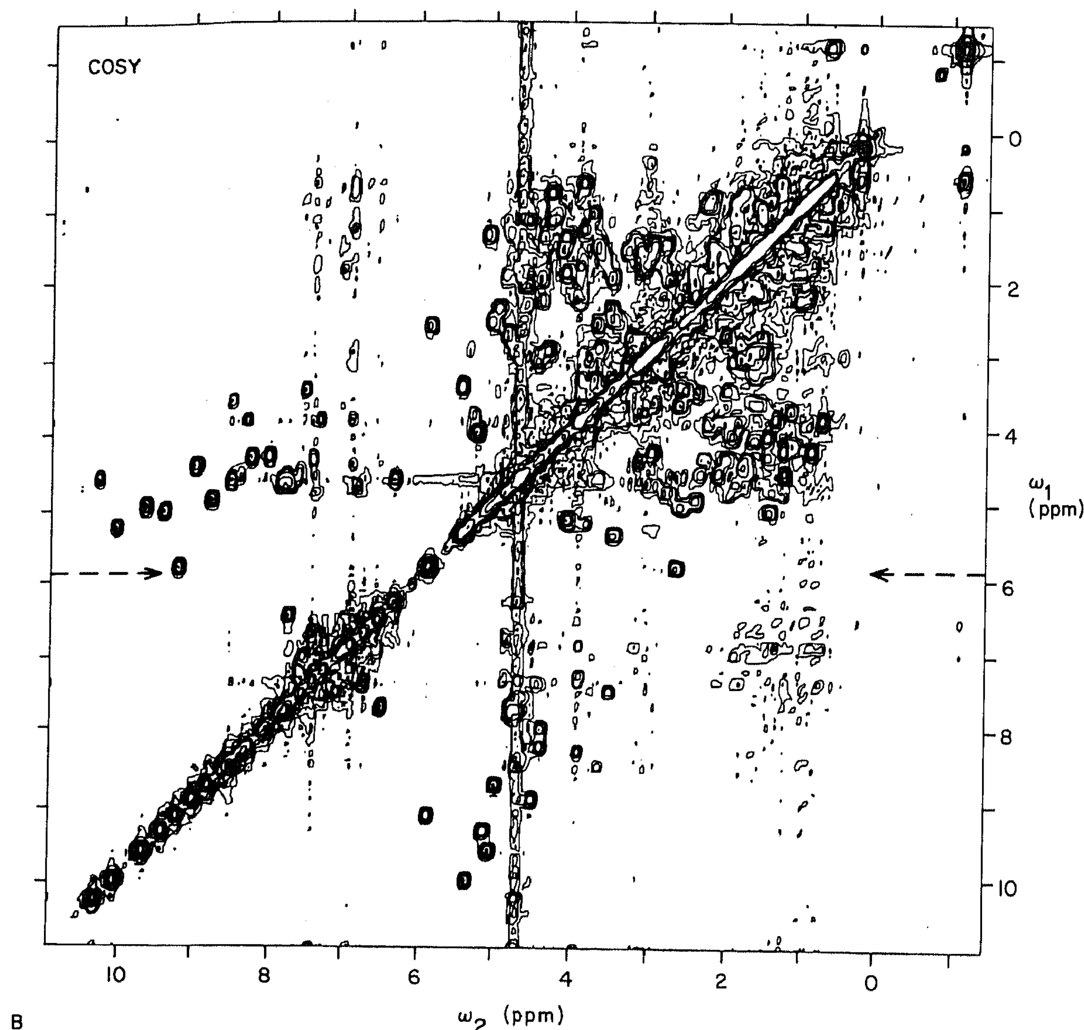

FIG. 1—*Continued*.

both triangular multiplication and symmetrization. However, after symmetrization the vertical noise bands, in particular the spike at the position of the HDO resonance, are more thoroughly eliminated from the spectrum. It is apparent that the symmetrized spectrum is more readily amenable to spectral analysis than the untreated spectrum. Symmetrization might be of particular value in view of future, automated analysis of 2D NMR spectra.

Two additional observations may be pointed out. First, similar to triangular multiplication, symmetrization results in an improvement of the signal-to-noise ratio (Fig. 2). Second, in the original, untreated spectrum (Fig. 1A) the cross peaks are elongated in the direction of ω_1, which is a consequence of the selection of a smaller spectral resolution in t_1 than in t_2 (S. Macura, A. Kumar, G. Wider, K. Wüthrich and R. R. Ernst, unpublished). After symmetrization the peaks have a square shape, for obvious geometrical reasons. Hence, symmetrization apparently reduces detrimental effects caused by the use of low resolution along t_1, which is often indicated in order to avoid excessive performance times when working with biological macromolecules.

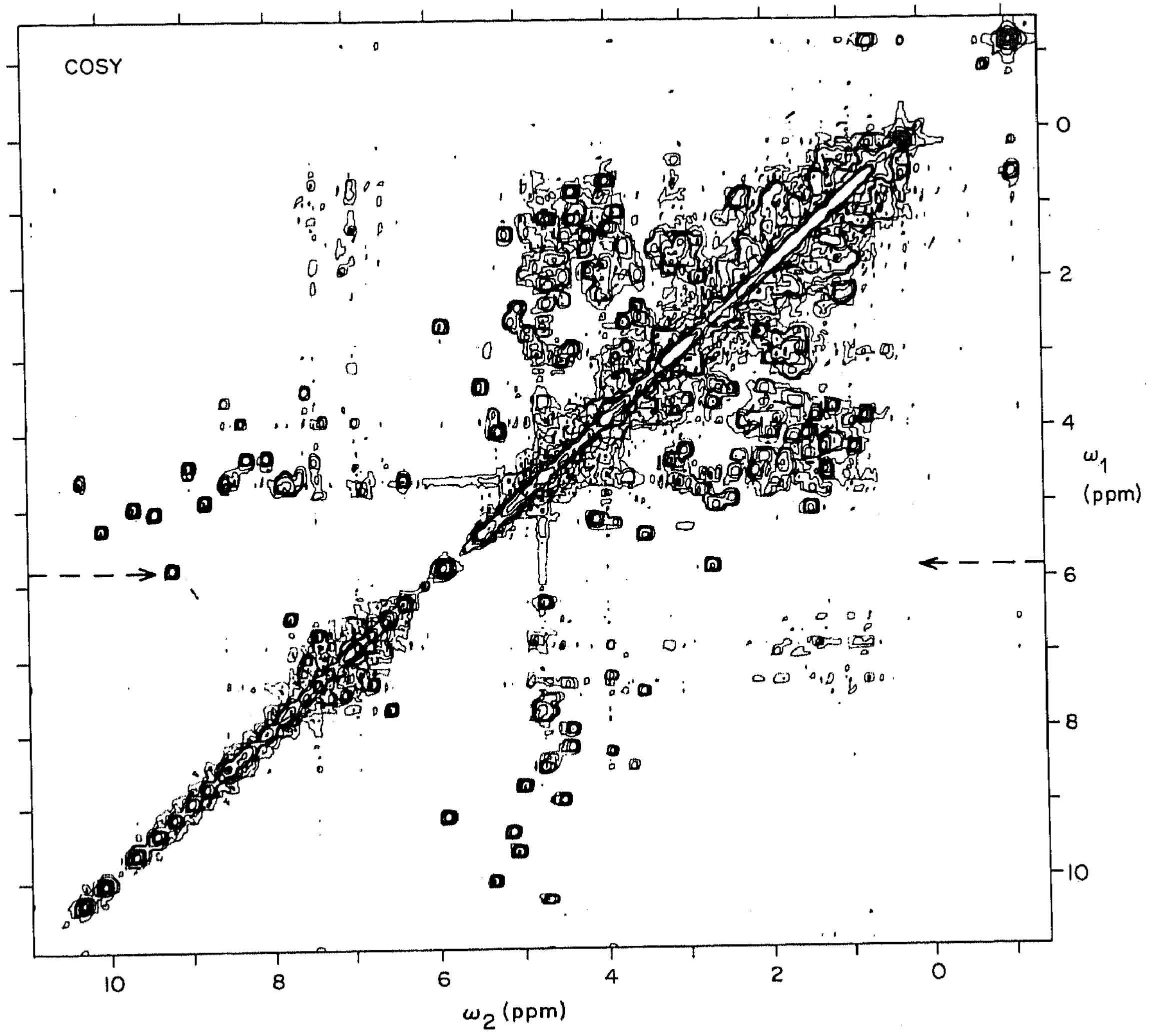

FIG. 1—*Continued.*

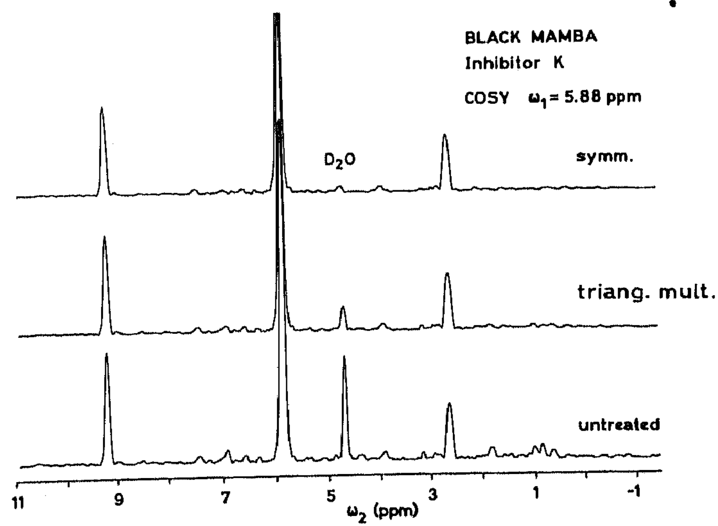

FIG. 2. Cross sections taken at $\omega_1 = 5.88$ ppm through spectra A (untreated), B (triangular multiplication), and C (symmetrized) of Fig. 1.

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