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Evaluation of errors of interproton distances and correlation time determined from NMR cross-relaxation rates

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SUMMARY

We have analyzed a combined use of the two-dimensional nuclear Overhauser effect in the laboratory frame (NOESY) and in the rotating frame (ROESY) to determine interproton distances and correlation time in medium-sized rigid molecules (Davis, 1987). This method can be applied in the intermediate motional regime, $0.2 < \omega_o\tau_c < 5$, (τ_c correlation time, ω_o resonance frequency). Error limits depend on the motional regime and are smallest near $\omega_o\tau_c = 1.14$.

The method was tested on six geminal proton pairs in the bicyclic octapeptide (S-deoxo-γ-[R]-OH-Ile[3] amaninamide, Mw = 870) for which at 297 K in DMSO, a correlation time of 1.0 ns, with a standard deviation of 0.12 ns, and an interproton distance of 1.87 Å, with standard deviation of 0.04 Å, are obtained.

INTRODUCTION

The primary aim of this work is to test the limits of the elucidation of interproton distances and correlation times by the quantitative measurement of cross-relaxation rates from NOESY (Wagner and Wüthrich, 1979; Kumar et al., 1980, 1981; Macura and Ernst, 1980; Ernst et al., 1987) and ROESY (Bothner-By et al., 1984; Davis and Bax, 1985) spectra. This method has been proposed and applied to peptides using 1D difference spectroscopy (Davis, 1987). Due to fluctua-

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Abbreviations: NOESY, nuclear Overhauser enhancement spectroscopy; ROESY, rotating frame Overhauser spectroscopy.

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tions of experimentally obtained cross-relaxation rates, this method applies only in favorable cases. Here, we propose to use the 2D method and statistically average correlation times over many spin pairs. Such improvement provides correlation time and interproton distances for all spin pairs.

We have applied the method to S-deoxo-γ-[R]-OH-Ile[3] amaninamide, whose structure in the solid state has been recently solved (Zanotti et al., 1989) by diffractometric studies. All NMR experimental data obtained in solution point to a compact structure of the bicyclic framework with very little internal flexibility (Zanotti et al., 1986, 1988, 1990).

Cross-relaxation rates between two spins, experimentally measured in the laboratory, σ^n, and in the rotating frame, σ^r, depend on the interspin distance, r, and the correlation time, τ_c, that modulates dipole–dipole interaction (Solomon, 1955):

$$\sigma^{n,r} = f^{n,r}(\tau_c)\, r_{n,r}^{-6} \tag{1}$$

Two functions, $f^n(\tau_c)$ and $f^r(\tau_c)$, depend on the type of molecular motion, and are well known for a rigid body with isotropic motion (Davis, 1987; Farmer et al., 1988).

For $\omega_o\tau_c > \sqrt{5}/2$, σ^n is negative; at $\omega_o\tau_c = \sqrt{5}/2 \approx 1.14$, the cross-relaxation disappears, i.e., σ^n is zero. For $\omega_o\tau_c < 1.14$, σ^n is positive and shows a distinct maximum at $\omega_o\tau_c = \sqrt{\sqrt{21}/4 - 1} \approx 0.382$. Cross-relaxation in the rotating frame, σ^r, monotonously increases with correlation time. For $\omega_o\tau_c < 0.382$, both cross-relaxation rates, σ^n and σ^r, have similar dependence on correlation time, and in the limit, when $\omega_o\tau_c \to 0$, $\sigma^n/\sigma^r \to 1$. A distinct behavior of the two cross-relaxation rates at $\omega_o\tau_c > 0.382$ in the two frames is vital to determine correlation times.

From cross-relaxation rate measurements, interproton distances, r_n and r_r, can be calculated directly from Eq. 1, provided a value of $f(\tau_c)$ is known from other measurements. Davis has proposed a method that uses cross-relaxation rates in both frames, σ^n and σ^r, and enables estimates of both r and $f(\tau_c)$ for the observed spin pair (Davis, 1987; Farmer et al., 1988). This method uses a ratio of cross-relaxation rates from the two frames, $S(\tau_c)$. Since interproton distances are identical ($r_r = r_n$), $S(\tau_c)$ does not depend on the distance:

$$S(\tau_c) = \frac{\sigma^n}{\sigma^r} = \frac{f^n(\tau_c)}{f^r(\tau_c)} \tag{2}$$

In two limiting cases, i.e., spin diffusion $\omega_o\tau_c \gg 1$ and extreme narrowing $\omega_o\tau_c \ll 1$, the ratio takes the values $-\frac{1}{2}$ and $+1$, respectively. In the intermediate range, it smoothly depends on correlation time and therefore can be used for its estimate (Davis, 1987; Farmer et al., 1988). Thus, from the measured cross-relaxation rates, σ^n and σ^r, one gets $S(\tau_c)$ and from it the corresponding correlation time, τ_c. Then, one calculates $f^n(\tau_c)$ or $f^r(\tau_c)$ and finally, r_r or r_n.

Theoretically, r_n is equivalent to r_r. It means that σ^n or σ^r can be interchangeably used to calculate interproton distances if the correlation time is calculated from their ratio. However, if the correlation time is modified by any means (for example, by statistical averaging) or obtained by other methods, then the calculated r_n and r_r will not be the same. Quantification of these differences is essential to establish a practical rule for optimal data evaluation.

LIMITATIONS TO SIMULTANEOUS DETERMINATION OF CORRELATION TIME AND INTERPROTON DISTANCES

Taking the derivative of the logarithm of Eq. 1, we obtain an expression that relates the relative differential change in cross-relaxation rates, $d\sigma/\sigma$, with the relative differential changes of the correlation time, $d\tau_c/\tau_c$, and interproton distances, dr/r:

$$\left(\frac{d\sigma}{\sigma}\right)=\tau_c[\ln f(\tau_c)]'\left(\frac{d\tau_c}{\tau_c}\right)-6\left(\frac{dr}{r}\right) \tag{3}$$

[ ]′ denotes the first partial derivative with respect to τ_c. For $dr/r=0$ we get:

$$\varepsilon^{n,r}=\frac{\left(\frac{d\sigma^{n,r}}{\sigma^{n,r}}\right)}{\left(\frac{d\tau_c}{\tau_c}\right)}=\tau_c[\ln f^{n,r}(\tau_c)]' \tag{4}$$

ε is the relative differential change of the cross-relaxation rate for the unit relative change of correlation time. It shows how sensitive cross-relaxation rates are to small changes in correlation times. The more sensitive cross-relaxation rates are to correlation times, the higher the value of ε. In two limiting motional regimes (spin diffusion and extreme narrowing), we have $\varepsilon \doteq 1$, which means that, in this case, uncertainty in the cross-relaxation rate propagates directly into uncertainty of correlation time. Dependence of $\varepsilon^{n,r}$ on correlation time in the intermediate regime can be readily calculated, replacing respective function $f^{n,r}(\tau_c)$ into Eq. 4. Figure 1 shows ε^n and ε^r as a function of correlation time.

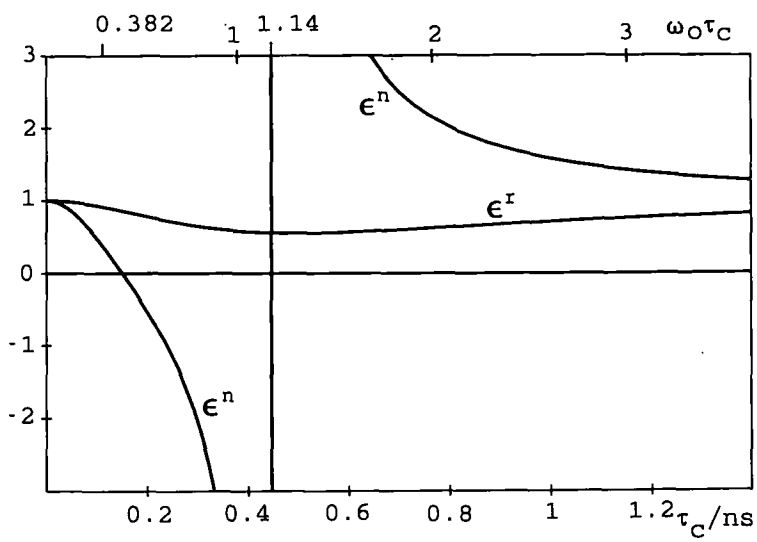

Fig. 1. Sensitivity of cross-relaxation rates to correlation times: $\varepsilon^n=(d\sigma^n/\sigma^n)/(d\tau_c/\tau_c)$, $\varepsilon^r=(d\sigma^r/\sigma^r)/(d\tau_c/\tau_c)$. Linear time scale corresponds to $\omega_o/2\pi=400$ MHz.

It is important to note the significant difference in sensitivity to correlation time of the two cross-relaxation rates in the intermediate regime. Variability in correlation time propagates into the rotating frame cross-relaxation rate directly. However, in the laboratory frame cross-relaxation rate, it propagates with amplification. In the intermediate motional regime, σ^r is more suitable to interproton distance determination than σ^n, since it is less prone to correlation time variability.

It is also important to evaluate the variability of estimated τ_c with respect to the variability of the ratio S. In the complete analogy to derivation of Eq. 4, from Eq. 2 we obtain:

$$\varepsilon^S = \frac{\left(\frac{dS}{S}\right)}{\left(\frac{d\tau_c}{\tau_c}\right)} = \varepsilon^n - \varepsilon^r \tag{5}$$

Since S is 'known' and τ_c is calculated, $1/\varepsilon^S$ is more appropriate to analyze. When $\varepsilon^n \rightarrow \varepsilon^r$ (i.e. $\omega_o\tau_c \ll$ or $\gg 1.14$, Fig. 1), the variability of S propagates into correlation time with immense amplification. Under this condition, S is not suitable for τ_c determination.

This analysis asserts only error limits set up by the variable sensitivity of the method without taking into account experimental sources of errors. To rationalize a real situation, experimental errors must be included.

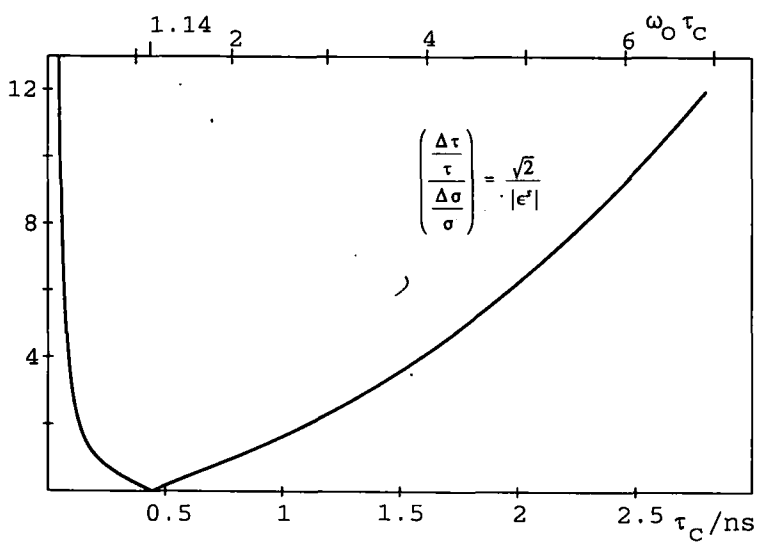

Fig. 2. Relative error of correlation time as a function of molecular mobility according to Eq. 7. Equal relative errors in NOESY and ROESY cross-relaxation rates are assumed: $(d\tau_c/\tau_c)/(d\sigma/\sigma) = \sqrt{2}/|\varepsilon^S|$. Linear time scale corresponds to $\omega_o/2\pi = 400$ MHz.

PROPAGATION OF EXPERIMENTAL ERRORS

As an input we have cross-relaxation rates, σ^n and σ^r, with estimated standard deviations $\Delta\sigma^n$ and $\Delta\sigma^r$. The relative error of the cross-relaxation rate ratio, $\Delta S/S$, is a function of the relative errors of input variables (Bevington, 1969):

$$\left(\frac{\Delta S}{S}\right)^2 = \left(\frac{\Delta\sigma^n}{\sigma^n}\right)^2 + \left(\frac{\Delta\sigma^r}{\sigma^r}\right)^2 \tag{6}$$

We assume that the errors are small enough, i.e., $\Delta S \approx dS$, $\Delta\sigma^{n,r} \approx d\sigma^{n,r}$ and $\Delta\tau_c \approx d\tau_c$. Combining Eqs. 5 and 6, we get an expression for the relative error of the correlation time:

$$\left(\frac{\Delta\tau_c}{\tau_c}\right)^2 = \left[\left(\frac{\Delta\sigma^n}{\sigma^n}\right)^2 + \left(\frac{\Delta\sigma^r}{\sigma^r}\right)^2\right]\frac{1}{(\varepsilon^S)^2} \tag{7}$$

The most important consequence of Eq. 7 is that the relative error of the calculated correlation time depends on the actual motional regime, as depicted in Fig. 2. When $\omega_o\tau_c > 5$ or $\omega_o\tau_c < 0.2$, parameter ε^S tends to zero, and an adequate correlation time estimate can hardly be obtained.

Once the correlation time is determined, the interproton distance can be calculated with relative errors:

$$\left(\frac{\Delta r_{n,r}}{r_{n,r}}\right) = \frac{1}{6}\left[(\varepsilon^{n,r})^2\left(\frac{\Delta\tau_c}{\tau_c}\right)^2 + \left(\frac{\Delta\sigma^{n,r}}{\sigma^{n,r}}\right)^2\right]^{1/2} + \text{cov.} \tag{8}$$

where the cov. term takes into account a possible correlation between $\Delta\tau_c$ and $\Delta\sigma^{n,r}$. For uncorrelated errors, the cov. term is zero. However, if the same values of $\sigma^{n,r}$ are used to calculate τ_c and $r_{n,r}$, then $\Delta\tau_c$ and $\Delta\sigma^{n,r}$ are correlated (more precisely, they are functionally related according to Eq. 7) and the cov. term cancels out the first term in Eq. 8. For each pair of cross-relaxation rates, a unique pair of correlation time and interproton distance is defined, and strictly speaking, errors cannot be estimated. For practical purposes, the correlation between errors can be neglected and the cov. term dropped from Eq. 8. Then the estimated error should be used as the upper limit.

Replacing the correlation time error from Eq. 7, we finally obtain an expression for the propagation of cross-relaxation rate errors into errors of distances:

$$\left(\frac{\Delta r_{n,r}}{r_{n,r}}\right) = \frac{1}{6}\left\{\left(\frac{\varepsilon^{n,r}}{\varepsilon^S}\right)^2\left[\left(\frac{\Delta\sigma^n}{\sigma^n}\right)^2 + \left(\frac{\Delta\sigma^r}{\sigma^r}\right)^2\right] + \left(\frac{\Delta\sigma^{n,r}}{\sigma^{n,r}}\right)^2\right\}^{1/2} \tag{9}$$

Again, a possible correlation between errors is completely ignored. The dependence of relative errors of interproton distances on correlation time is shown in Fig. 3.

Interproton distance errors are smaller than cross-relaxation rate errors only when $0.2 < \omega_o\tau_c < 5$. Outside this range, relative errors of calculated distances sharply increase. The theoretical limit $\Delta r/r = (d\sigma/\sigma)/6$ can be achieved only when $\varepsilon^{n,r}\Delta\tau_c \approx 0$, Eq. 8. For r_r, this happens when $\omega_o\tau_c \approx 1{,}14$ and for r_n, when $\omega_o\tau_c \approx 0.382$.

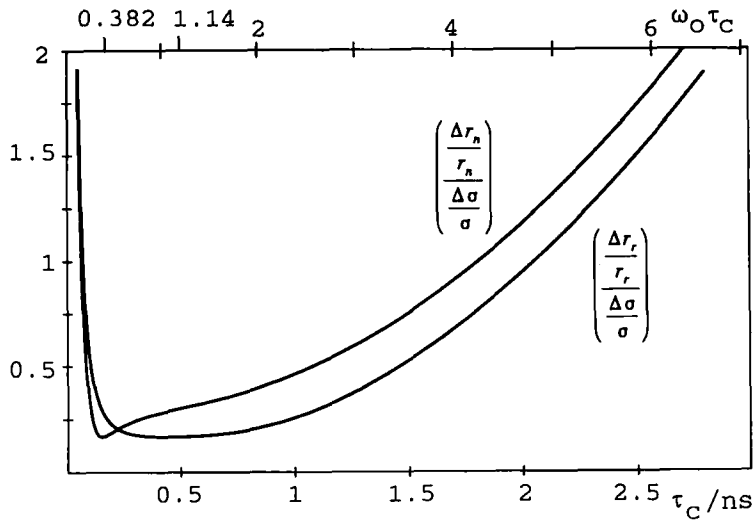

Fig. 3. Relative errors of interproton distances according to Eq. 9. Equal relative errors in NOESY and ROESY cross-relaxation rates are assumed: $(dr_n/r_n)/(d\sigma/\sigma)=\frac{1}{6}\{2(\varepsilon^n/\varepsilon^S)^2+1\}^{1/2}$, $(dr_r/r_r)/(d\sigma/\sigma)=\frac{1}{6}\{2(\varepsilon^r/\varepsilon^S)^2+1\}^{1/2}$. Linear time scale corresponds to $\omega_o/2\pi=400$ MHz.

Equation 7 and Fig. 2 show that a reasonable estimate of the correlation time can be obtained only in a very limited motional range around $\omega_o\tau_c \approx 1.14$. To apply the method in wider limits, it is necessary to have more precise cross-relaxation data. This may not always be feasible. One way to handle this problem is to statistically average correlation times obtained for many spin pairs. Respective errors can be estimated from Eq. 8, where $\Delta\tau_c$ is now acquired from statistical analysis of the obtained data set. Of course, this is possible only for structurally rigid molecules.

It would be the most effective to perform averaging before error propagates, i.e., to average original input data. In the present situation, it would mean that one should average original cross-relaxation rates and use their mean for further calculation. Then, from Eq. 2 we obtain:

$$S_{av.}=\frac{\sigma^n_{av.}}{\sigma^r_{av.}} \tag{10a}$$

We can extend 'averaging' over *all* spin pairs by summing up all cross-relaxation rates between protons that have the same correlation time but not necessarily the same distances:

$$S_{AV}=\frac{\Sigma\sigma^n}{\Sigma\sigma^r} \tag{10b}$$

This averaging enhances the role of short distances but is properly weighted with respect to total magnetization transferred.

EXPERIMENTAL

Experiments were performed on a Bruker AM 400 NMR spectrometer ($\omega_0/2\pi = 400$ MHz) with degassed and sealed 16.1 mM solution of S-deoxo-γ-[R]-OH-Ile[3] amaninamide in DMSO-d_6 at 297 K. The NOESY experiments were recorded with mixing times of 50, 100, 200, 300, 400, 500, and 800 ms, with a mixing time variation of $\pm 10\%$. The ROESY experiments were performed with CW spin lock, $B_1 = 3.57$ kHz, with mixing times of 30, 50, 75, 100, 150, and 200 ms. In both sets of experiments, 48 scans for each of 256 t_1 increments were acquired. Upon zero-fill, data were doubly Fourier transformed, yielding 1024 × 512 ReRe 2D spectra.

RESULTS AND DISCUSSION

For the experimental test of the method, we have chosen to analyze cross-relaxation rates of six geminal proton pairs in S-deoxo-γ-[R]-OH-Ile[3] amaninamide. The main advantages in using geminal protons are the well-known interproton distances, 1.75Å, fast cross-relaxation due to their proximity, and associated with that, absence of second-order transfer effect in the original build-up curve analysis. Disadvantages include the possible influence of coherent magnetization transfer and difficult quantification of cross-peaks due to overlap with the diagonal.

Table 1 shows results obtained for individual geminal spin pairs and structural parameters calculated from them. Parameters are calculated for each pair separately. Error limits are calculated from the statistical analysis of six pairs of independent build-up curves.

The first four geminal proton pairs listed in Table 1 have the same values for all parameters within the experimental errors. We obtain interproton distances of approximately 1.85 Å with the

TABLE 1
CROSS-RELAXATION DATA AT 400 MHz FOR GEMINAL PROTONS IN S-DEOXO-γ-[R]-OH-ILE[3] AMANINAMIDE

	Asn[1] β–β′	Hyp[2] β–β′	Gly[7] α–α′	Cys[8] β–β′	Gly[5] α–α′	Trp[4] β–β′
$\sigma^n(s^{-1})$ [a]	−1.0	−0.9	−0.9	−1.0	−0.6	−1.3
$\Delta\sigma^n/\sigma^n$ [b]	0.22	0.24	0.24	0.22	0.39	0.17
$\sigma^r(s^{-1})$ [a]	+3.2	+2.8	+3.2	+3.0	+3.7	+2.7
$\Delta\sigma^r/\sigma^r$ [b]	0.10	0.12	0.10	0.11	0.09	0.12
τ_c(ns) [c]	0.97	1.0	0.89	1.0	0.61	3.5
$\Delta\tau_c/\tau_c$ [d]	0.29	0.35	0.26	0.32	0.15	3.0
$r_{n,r}$(Å) [e]	1.87	1.91	1.84	1.89	1.73	2.31
$\Delta r_n/r_n$ [f]	0.08	0.10	0.09	0.09	0.11	0.50
$\Delta r_r/r_r$ [f]	0.04	0.05	0.04	0.04	0.02	0.48

Calculated parameters were obtained for each pair separately.
[a] From individual build-up curves.
[b] Standard deviation; obtained from data in previous row.
[c] From explicit form of $S(\tau_c) = \sigma^n/\sigma^r$.
[d] From Eq. 7.
[e] From Eq. 1, using σ and τ_c from above.
[f] From Eq. 9.

relative error of 10% when σ^n is used and 5% from σ^r. These distances match well with the known geminal protons distance of 1.75 Å. Residues Trp[4] and Gly[5], however, have distinctly different properties, although their individual σ^n and σ^r relaxation rates are within estimated limits. Incidentally, their σ values are on opposite extremes of the error limits and, consequently, produce extreme values of the ratio S and correlation time.

One possible solution to this problem is to take average values according to Eq. 10 and then to calculate the correlation time common for all involved spin pairs. Table 2 shows interproton distances generated in this way from the data shown in Table 1. Using Eq. 10 for six pairs shown in Table 1, we obtain $\tau_c = 0.94$ ns with $\Delta\tau_c/\tau_c = 0.12$. Interproton distances obtained from each σ^n and σ^r value are within the estimated error range from σ^n (9%) and σ^r (4%) around the true value of 1.75 Å. Even the values obtained for Trp[4] now fall in the much narrower error limits. This clearly indicates that the main source of errors is a poor estimate of correlation time. Once the correlation time is fixed (with statistical averaging of the data over all spin pairs with the same motional properties), even cross-relaxation rates of modest precision will yield good estimates of interproton distances. The requirement for statistical averaging of the data is exemplified in Fig. 4. It shows a (σ^n, σ^r) plot of 25 spin pairs in S-deoxo-γ-[R]-OH-Ile[3] amaninamide. The molecule is a small, bicyclic octapeptide and is not likely to possess a detectable degree of internal mobility. The large spreading of the data along the correlation time axis suggests modest precision of the data set rather than actual spread of correlation times. This indicates that any attempt to extract motional and structural parameters from the *individual* (σ^n, σ^r) pairs would require the original data to have much higher precision.

Besides random errors, experimental data may contain systematic errors as well. This is most likely due to homonuclear Hartmann–Hahn transfer in the ROESY experiment (Bax, 1989; Brown and Farmer, 1989). Hartmann–Hahn transfer tends to reduce σ^r values calculated for coupled spin pairs since the effect is of the opposite sign with respect to cross-relaxation. Underestimation of σ^r increases both correlation time and interproton distance. In the data presented here, the overestimation of geminal proton distances, 1.87 Å vs. 1.75 Å, could be attributed to this

TABLE 2
INTERPROTON DISTANCES FOR GEMINAL PROTONS BASED ON A SINGLE AVERAGED VALUE OF CROSS-RELAXATION RATES*

	Asn[1] β–β′	Hyp[2] β–β′	Gly[7] α–α′	Cys[8] β–β′	Gly[5] α–α′	Trp[4] β–β′
r_n(Å)	1.85	1.88	1.88	1.85	2.03	1.77
$\Delta r_n/r_n$	0.09	0.09	0.09	0.09	0.09	0.09
r_r(Å)	1.86	1.90	1.86	1.87	1.81	1.91
$\Delta r_r/r_r$	0.04	0.04	0.04	0.04	0.04	0.04

*$\sigma^n = -0.943\ s^{-1}$, $\Delta\sigma^n/\sigma^n = 0.10$; $\sigma^r = +3.11\ s^{-1}$, $\Delta\sigma^r/\sigma^r = 0.05$.
From these values other parameters were calculated as described in Table 1:
$S = -0.303$, $\Delta S/S = 0.11$; $\varepsilon^n = 1.66$;
$\tau_c = 0.94$ ns, $\Delta\tau_c/\tau_c = 0.12$; $f^r(0.94\ ns) = 130.8\ Å^6\ s^{-1}$;
$\varepsilon^s = 0.98$; $\varepsilon^r = 0.68$.
$f^n(0.94\ ns) = -39.6\ Å^6\ s^{-1}$;

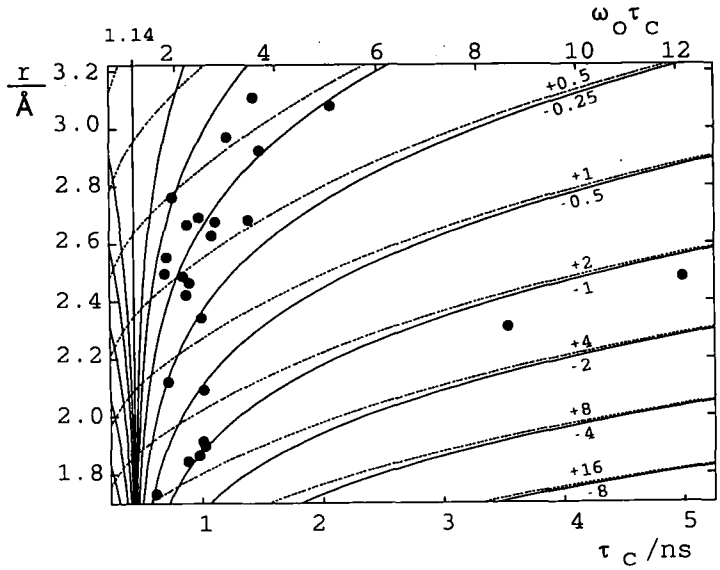

Fig. 4. Cumulative (σ^n,σ^r) plot as a function of interproton distances and correlation times. Full line: contour plot of $\sigma^n = f^n(\tau_c)\cdot r^{-6}$; dotted line the same for σ^r. Numbers indicate cross-relaxation rates (positive: σ^r, negative: σ^n) in s^{-1}. Experimental points for 25 spin pairs in S-deoxo-γ-[R]-OH-Ile[3] amaninamide at $\omega_0/2\pi = 400$ MHz are placed in diagram according to their σ_i^n and σ_i^r values. Their orthogonal projection on the axis gives individual r_i and τ_i values. Use of the average correlation time is equivalent to projecting a point along the $\sigma_i^{n,r}$ contour to $\tau_c = \langle\tau_c\rangle$ and then orthogonal to r_i. Points at $\tau_c > 3$ are associated with coarse errors. These points would return erroneous distance and correlation times if used individually. Used with common, average correlation times, these points can produce meaningful distances with reasonable errors. For example, point at $\tau_c = 3.5$ ns (Trp[4] β-β') by itself yields $r_{r,n} = 2.3 \pm 0.5$ Å (Table 1) but with an average correlation time, $\langle\tau_c\rangle = 0.94 \pm 0.1$ ns, the same cross-relaxation rates give $r_n = 1.8 \pm 0.2$ Å and $r_r = 1.9 \pm 0.1$ Å.

effect. Systematic error is still within the margin of statistical errors as can be seen from Fig. 4 where points from spin pairs not influenced by Hartmann–Hahn transfer cannot be distinguished from those which are. In more precise data sets, this source of systematic error can be eliminated by use of pulse sequences designed for elimination of the Hartmann–Hahn effect (Cavanagh and Keeler, 1988; Hwang and Shaka, 1992).

In conclusion, we have shown that in rigid, medium-sized molecules, interproton distances and correlation times can be obtained by comparative analysis of cross-relaxation rates in the two frames only in a very restricted range of intermediate motional regime and that for reliable estimates of the distances, statistical averaging of the input cross-relaxation rates is required. By statistical averaging, even cross-relaxation rates of modest precision can produce a consistent set of interproton distances.

The disadvantages of this method are that it assumes a model of rigid body isotropic motion and that it is applicable only for a narrow range of correlation times. The main advantage is that it produces a set of interproton distances without calibration, i.e., without prior knowledge of any of the distances.

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