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Publisher *Taylor & Francis*

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Spectroscopy Letters

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597299>

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To cite this Article Niccolai, Neri and Rossi(1989) 'Proton Detection of Heteronuclear Dipolar Couplings', Spectroscopy Letters, 22: 5, 561 — 568

To link to this Article: DOI: 10.1080/00387018908053905

URL: <http://dx.doi.org/10.1080/00387018908053905>

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PROTON DETECTION OF HETERONUCLEAR DIPOLAR COUPLINGS

Key words: nuclear relaxation, satellite peaks, molecular motion.

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SUMMARY

A method is proposed for the calculation of heteronuclear dipolar coupling between two $\frac{1}{2}$ nuclei, X and Y, by measuring the spin-lattice relaxation rates of the abundant Y nucleus and of the satellite peaks (^1H , ^{31}P , ^{19}F) due to the scalar coupling of Y with the less abundant X nucleus. The ^1H - ^{13}C dipolar interaction has been evaluated from the proton spin-lattice relaxation rates of tyrosine in water solution and the effective correlation times of the aromatic moiety have been calculated.

INTRODUCTION

Magnetic relaxation parameters are now commonly used for investigating molecular structures and dynamics of complex spin systems.

Proton homonuclear dipolar couplings, observed in one or two dimensional Overhauser effect experiments, have been extensively correlated to molecular dynamics and conformations¹⁻⁵. Recently, also heteronuclear dipolar connectivities have been quantitatively interpreted in terms of molecular structure and motions⁶⁻⁸.

Spin-lattice relaxation rates of low natural abundance $I=1/2$ nuclei have been extensively analyzed since their relaxation pathway is relatively simple. In fact, for these X nuclei, any inter- or intra-molecular X-X dipolar coupling does not significantly contribute to the relaxation process. If the X nucleus is bound to hydrogens, its relaxation pathway is very often dominated by this one-bond dipole-dipole interaction. Therefore, spin-lattice relaxation rates of protonated X nuclei and correlation times, i.e. molecular motions, may be directly correlated.

A strong limitation for investigating molecular dynamics by measuring R_{XH} 's from partially relaxed X spectra, is given by the usual low sensitivity of these X nuclei.

In the present report, a method for a proton detection of proton-carbon dipolar coupling from 1H spin-lattice relaxation measurements is proposed.

Tyrosin in water solution was used as model system to explore the applicability of this experimental approach for large biomolecules where, in many cases, the characteristic pattern of the tyrosyl aromatic proton resonances can be easily recognized.

EXPERIMENTAL

A 0.05 M D₂O solution of tyrosine was used for ¹H T₁ measurements. The latters were performed by using a standard inversion recovery pulse sequence. Partially relaxed spectra were recorded on a Varian XL-200 spectrometer. Computer fitting of the relaxation curves was used to calculate relaxation rates. All the measurements were performed at 25 °C and temperature was controlled at +/- 1 °C. The rms error on relaxation rates, calculated by the computer fitting program, was typically 1% and 5% respectively for ¹²C-H and ¹³C-H signals.

RESULTS AND DISCUSSION

For X=1/2 nuclei, the X-H dipolar interaction yields a relaxation contribution which is described the following relationship :

$$R_{XH} = \frac{n}{10} \frac{\hbar^2 \gamma_H^2 \gamma_X^2}{r_{XH}^6} \left[\frac{3 \tau_c}{1 + \omega_0^2 \tau_c^2} + \frac{6 \tau_c}{1 + (\omega_H + \omega_X)^2 \tau_c^2} + \frac{\tau_c}{1 + (\omega_H - \omega_X)^2 \tau_c^2} \right] \quad [1]$$

where n is the number of protons bound to the X nucleus, ω_0 is the Larmor frequency of the observed nucleus, ω_X , ω_H , γ_X and γ_H are the Larmor frequencies and the magnetogyric ratios of X and ¹H nuclei, $r_{(XH)}$ is the internuclear distance and τ_c is the rotational correlation time which modulates the X-H dipolar interaction⁹⁻¹¹.

The R_{XH} rate can be calculated from partially relaxed spectra of the X nucleus if the fractional effectiveness of the X-H

dipolar interaction to the total relaxation process can be independently estimated.

An alternative approach for the measurement of the R_{CH} contributions is given by the different relaxation pathway of a proton signal and of its X satellite peaks. The spin-lattice relaxation rate, R_a , of a proton resonance and of its satellites, R_s , can be described by the following equations :

$$R_a = R + \sigma + R^* \quad [2]$$

$$R_s = R + \sigma + R^* + R_{\text{CH}} \quad [3]$$

where R and σ terms refer to direct and cross relaxation contributions arising from proton-proton dipolar interactions, R^* accounts for other dipolar and non-dipolar contributions, and R_{CH} is the relaxation term described in Eq.[1]. It is apparent that, from the difference $(R_s - R_a)$, R_{CH} can be calculated and information on molecular dynamics can be obtained. It should be noted that, outside the extreme narrowing condition $(\omega_0\tau_c)^2 \ll 1$, R_{CH} depends on the Larmor frequency of the observed nucleus and different values of this relaxation contribution have to be expected from proton and X relaxation spectroscopies, as shown in Fig. 1. The sensitivity of the observed nucleus and the natural abundance of the X isotope are of critical importance for detecting heteronuclear dipolar couplings. As far as the $^{13}\text{C}-^1\text{H}$ interactions are concerned, these can be directly observed from ^{13}C spectra with a sensitivity $S_0 = 1.76 \times 10^{-4}$ lower than the proton one or, in a reverse way, from proton ^{13}C satellite peaks with a

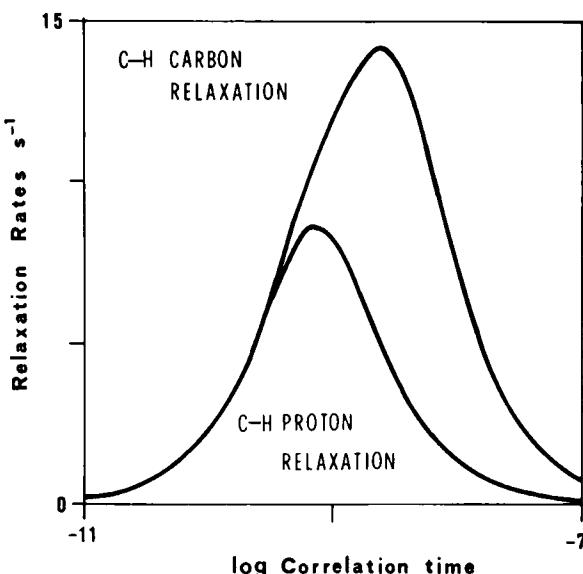


Figure 1: - Proton-Carbon dipolar relaxation curves determined on the basis of equation [1].

sensitivity $S_R = 5.54 \times 10^{-3}$. The S_R factor takes into account the J_{CH} splitting and the ^{13}C natural abundance. For proton singlets, therefore, a sensitivity enhancement of 31.5 is obtained by detecting carbon-proton dipolar interactions from the relaxation rates of $^{1}H-^{13}C$ satellites instead of measuring $^{13}C T_1$'s.

As reported in Table I, ^{13}C satellite peaks of aromatic protons of tyrosine relax much faster than the respective central proton resonances. As commonly observed, the four lines of the AA'BB' spin system of tyrosine relax differently and the same behaviour is found for the satellite peaks. Only one satellite

TABLE I

PROTON SPIN-LATTICE RELAXATION RATES OF TYROSINE AROMATIC SIGNALS OBSERVED FOR A 50 mM SOLUTION IN D₂O

	R _a	R _s	R _{CH}	τ _c (ns)
C _δ H (7.29)	0.77	3.64	2.87	0.124
C _δ H (7.25)	0.73	3.57	2.84	0.122
C _ε H (6.88)	0.46	3.61	3.15	0.136
C _ε H (6.84)	0.43	3.44	3.01	0.130

Values in parenthesis are proton chemical shift (ppm) from external DSS; experimental errors on relaxation rates are 2 and 10 % respectively for R_a's and R_s's. Effective correlation times were calculated from Eq.[1] assuming that the ¹H-¹³C internuclear distance is 1.07 Å.

doublet was detected for each aromatic proton since the chemical shift difference between H_δ and H_ε (0.41 ppm) is very close to ¹J_{CH}/2. The R_{CH}'s evaluated from each component of the two aromatic protons are identical as expected. The difference between the two set of R_{CH}'s and, hence, correlation times reported in Table I suggests that the reorientation of C_δ-H and C_ε-H internuclear vectors is not fully isotropic. Furthermore, the calculated values of τ_c's indicate the tyrosine molecules in water solution have molecular motions which satisfy the extreme narrowing condition : (ω₀ + ω_c)²τ_c² << 1.

It can be concluded that information on molecular dynamics in systems with strong limitations on sample concentration can be obtained by using the method described here. It is also apparent that the latter is confined to the observation of satellite peaks of proton signals with low multiplicity. It should be noted that simplification of the proton J pattern can be achieved by using standard single or multiple decoupling techniques. Thus, this relaxation method can be used for studing the motional behavior of biopolymers in very dilute solutions and as an alternative to more complex pulsed techniques as the reverse detection experiments recently proposed^{1,2}.

ACKNOWLEDGMENTS

This work was supported in part by grants from the Italian Council of Reaserch (C.N.R. Italy/U.K. cooperative agreement).

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Date Received: 09/12/88
Date Accepted: 03/01/89