(11) D. W. Hart, T. F. Blackburn, and J. Schwartz, *J. Am. Chem. Soc.*, **97**, 679 (1975).
(12) In each case, the yield[9] of bromide (or of both bromides combined) was ca. 85%.
(13) These bromides were readily separated on an 8 ft × ¼ in. column packed with 5% Carbowax 20M on Chromosorb P (NAW).
(14) S. F. Brady, M. A. Ilton, and W. S. Johnson, *J. Am. Chem. Soc.*, **90**, 2882 (1968).
(15) Preliminary results indicate that the reactivity of 2,4-dienes is not analogous. Deuterium labeling studies show that these species react with *3* equiv of **1** to yield *saturated*, terminally functionalized alkyl complexes.
(16) For example, see H. O. House, "Modern Synthetic Reactions", 2nd ed, W. A. Benjamin, New York, N.Y., 1972, Chapter 10.

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Nonexponential Methyl Proton Spin–Lattice Relaxation in the C-Terminal Tetrapeptide of Gastrin

Sir:

In this communication we report the observation of nonexponential spin–lattice relaxation for the methyl protons of the C-terminal tetrapeptide of gastrin. The most likely source of this nonexponentiality is cross-correlations between the methyl proton spin pairs. This observation is important in view of the recent increasing interest in the effects of cross-correlations in NMR relaxation. Cross-correlations have been discussed by a number of authors and have been shown to result in a slower nonexponential relaxation mechanism.[1-4] With few exceptions,[5,6] the observations of these effects have been in solid systems. In physiologically active molecules the effects have not been observed.

The C-terminal tetrapeptide of gastrin has the primary structure TRP-MET-ASP-PHE-NH_2 and has the same physiological range of activity as gastrin itself. The material[7] used in this study was synthesized by the method of Davey et al.[8] and isolated as the gastrin tetrapeptide amide trifluoroacetate, purity ≥95%. The material was dissolved in 100% DMSO-d_6, deoxygenated via at least three freeze–pump–thaw cycles, and sealed with pressure caps under nitrogen in 5-mm NMR tubes.

Spin–lattice relaxation measurements at 30 ± 1°C were performed in the Fourier transform mode at 100 MHz on a JEOL-PFT-100 spectrometer with a disk storage system. The conventional inversion recovery method (180°-τ-90°-t) was used with the phase of the 90° pulse changed by 180° on every scan.[9] The value of t was 17.0 sec for all measurements and was intentionally kept long relative to the time scale of the relaxation. A bandwidth of 2.0 kHz was employed with 8192 data points. Peak heights were taken to be proportional to magnetization. Resonance assignments are available in the literature.[10,11] It should be noted that we use "aromatic" to designate the partially overlapping resonances of the aromatic protons of phenylalanine and tryptophan.

The relaxation of the aromatic and methionine methyl protons is shown in Figure 1. It is evident from the curvature exhibited in Figure 1A that the methyl relaxation cannot be characterized by a single exponential. The linearity exhibited in Figure 1B establishes that the curvature in Figure 1A does not result from nonlinearities in our detection system. Furthermore, since Figure 1 is a composite (without scaling of the vertical axis) of three separate data runs, the scatter depicted therein is indicative of the reproducibility of the results. One of these runs was taken with 40 accumulated transients and the other two with ten accumulated transients per spectrum. Two of the runs were made using a

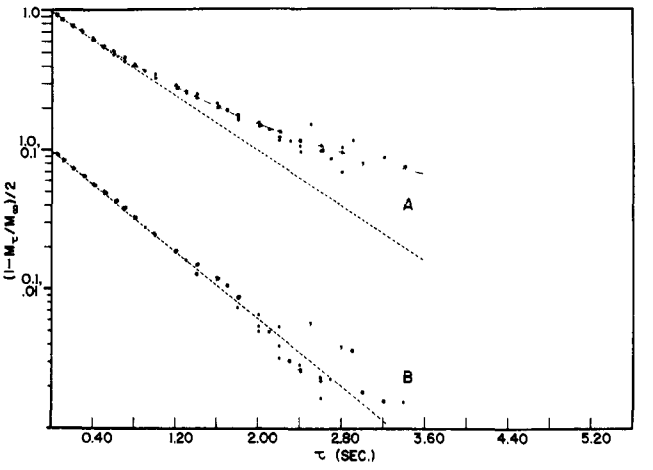

Figure 1. Spin–lattice relaxation in the C-terminal tetrapeptide of gastrin (0.03 *M* in DMSO-d_6, 30 ± 1°C): A, methionine methyl proton relaxation (dotted line denotes results of nonlinear regression analysis using the weighted sum of two exponentials, dashed line denotes initial slope calculated from the results of the double exponential fit); B, aromatic proton relaxation (dashed line denotes results of nonlinear regression analysis using a single exponential).

computer program which sequentially increments the time interval τ in the inversion recovery method. The third run was performed manually with τ values chosen at random and the equilibrium spectrum at effectively infinite pulse separation measured before and after each measurement at finite τ. This procedure ensures that any instrument drift appears as scatter and not as curvature in Figure 1.

There are alternative reasons, in addition to cross-correlations, why the methyl protons might relax nonexponentially. One alternative is that of slow exchange between various molecular conformations which are characterized by different spin–lattice relaxation rates. We reject this alternative on the grounds that although the protons all have T_1's in the same time domain, nonexponentiality is not reflected in both plots in Figure 1. Another possibility is that there is cross-relaxation[12] between the methyl protons and other protons on the same or neighboring molecules. We reject the alternative of intermolecular cross-relaxation between solute molecules on the grounds that results for a 0.015 *M* solution are identical with those shown in Figure 1 for a 0.03 *M* solution. The possibility remains that intramolecular cross-relaxation contributes to the curvature depicted in Figure 1A. We consider this possibility unlikely (but not rigorously excludable in view of the absence of accurate and complete structural information) on the basis of internuclear separations between nearest proton neighbors as seen in a space filling model of the tetrapeptide arranged to minimize steric interactions.

We are thus led to the conclusion that the observed nonexponential methyl relaxation is most likely due to cross-correlations. Theory predicts[3] in general that the magnetization M relaxes nonexponentially to its equilibrium value M_0, and the quantity $(M_0 - M)/2M_0$ is given by the sum of three decaying exponentials, each with its own rate constant λ_i and preexponential weighting factor A_i. We note that the scatter in our data prevents the determination of reliable values of six parameters. The values of the four parameters determined via nonlinear regression analysis using the weighted sum of two exponentials are $A_1 = 0.817 \pm 0.162$, $\lambda_1^{-1} = 0.762 \pm 0.126$ sec, $A_2 = 0.155 \pm 0.168$, and $\lambda_2^{-1} = 3.77 \pm 4.65$ sec. For comparison a fit of the aromatic data to a single exponential yields $A_1 = 0.969 \pm 0.008$ and $\lambda_1^{-1} = 0.724 \pm 0.010$ sec. The ± figures denote approximate *95% confidence limits* (approximately two standard deviations) provided by the computer analysis. The

large confidence limits for the four parameter fit indicate that a greater density of more accurate data over a larger range of pulse separations is required to determine even four parameters precisely from relaxation experiments such as these.

Using a computer program written to follow the treatment of proton cross-correlations for this geometry,[3] we calculate to first approximation (isotropic overall tumbling) that the intramolecular reorientation rate of the methyl group is large compared to that for overall tumbling. In this case theory indeed predicts a biexponential curve with preexponential terms $A_1 = 0.833$ and $A_2 = 0.167$ with $\lambda_2^{-1} \gg \lambda_1^{-1}$.[13] The initial slope ($\lambda_{init}^{-1} = 0.873$ sec) gives the relaxation time in the absence of cross-correlation. Exact comparison with theory, however, awaits a full treatment of the effect of a spin-rotation relaxation mechanism in the presence of dipole cross-correlations. It is clear that experiments on ^{13}C relaxation of the methyl carbon should confirm the interpretation of the data presented above. We have shown this to be true and it will be discussed as part of a more complete study of ^{1}H and ^{13}C relaxation in tetragastrin presented in a forthcoming publication.

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Gas Phase Proton Affinities of Molecules in Excited Electronic States by Ion Cyclotron Resonance Spectroscopy

Sir:

We wish to report a straightforward method for determining the gas phase acid-base properties of molecules in excited electronic states using the techniques of ion cyclotron resonance spectroscopy (ICR).[1] The factors important in determining acid-base properties of molecules in the gas phase have been elucidated in the past several years. In the absence of complications due to solvation, the energetics of protonation can be directly related to electron distributions

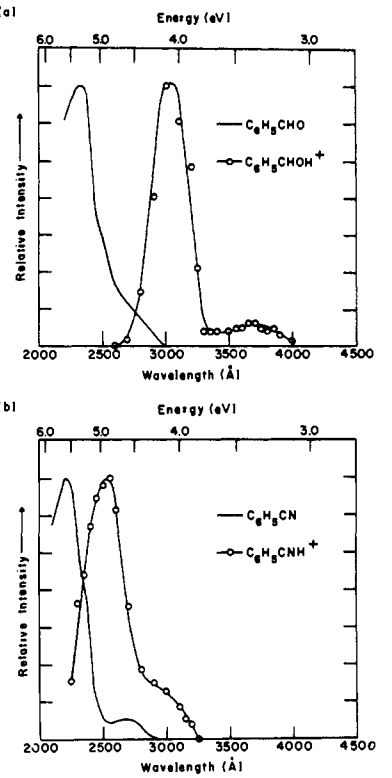
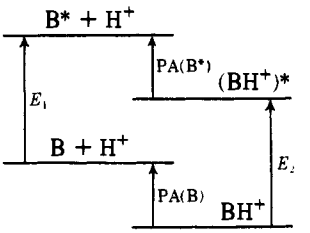


Figure 1. (a) Comparison of the gas phase absorption spectrum of benzaldehyde with the photodissociation spectrum of its conjugate acid. (b) Comparison of the gas phase absorption spectrum of cyanobenzene with the photodissociation spectrum of its conjugate acid. The absorption spectra of the neutrals were recorded at low resolution to facilitate comparison.

Scheme I



in neutral molecules and their conjugate acids and bases. Changes in electron distribution which accompany electronic excitation can be probed by determining the energetics of protonation in the excited state. The determination of proton affinities of molecules in excited electronic states follows directly from the thermochemical cycle (Scheme I).[2,3] In accordance with eq 1,

$$PA(B^*) = PA(B) + (E_1 - E_2) \quad (1)$$

PA(B*) can be calculated if the proton affinity of B in its ground state, PA(B), and the excitation energies of the base, E_1, and its conjugate acid, E_2, are known.

ICR techniques have been developed for examining photodissociation of ions in the gas phase,[4-6] the phenomenon being generalized in eq 2.

$$A^+ + h\nu \rightarrow B^+ + C \quad (2)$$

These experiments yield the product of the extinction coefficient and the quantum yield for dissociation as a function of wavelength (relative photodissociation probability.) A comparison of the absorption spectrum of a molecule with the relative photodissociation probability of its conjugate acid reveals in many instances quite similar spectra, which may be analyzed to determine E_1, E_2, and hence PA(B*).