

Determination of C-C-H bond angles and models for internal rotation of side chains of biopolymers in solution*

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We describe a method for the determination of the C-C-H bond angle of a side chain with one degree of internal motion, which is attached to a rigid polymer backbone with isotropic rotational reorientation in solution. The method would be applicable, for example, to the methyl group of an alanine residue in a globular protein. The suggested experiment also distinguishes between the random-jump model and the stochastic diffusion model of internal rotation.¹

The proposed experiment involves the measurement of the ^{13}C spin-lattice relaxation time (T_1), ^{13}C spin-spin relaxation time (T_2), and nuclear Overhauser enhancement² (NOE) of a protonated side-chain carbon, by means of ^{13}C Fourier transform (FT) nuclear magnetic resonance (NMR). For a protonated carbon of a large molecule, relaxation is overwhelmingly dominated by the ^{13}C - ^1H dipole-dipole interaction.³ T_1 , T_2 , and the NOE are functions of the correlation time for over-all molecular reorientation (τ_R), the correlation time for internal rotation of the side chain (τ_G), the angle between the C-H vector of the side chain and the axis of internal rotation (θ), the C-H distance, and the magnetic field. The pertinent relations are Eqs. (37)-(43) and (25)-(29) of Ref. 4. It should be noted that Eq. (26) of Ref. 4 applies to the stochastic diffusion model for internal rotation,¹ with $6\tau_G$ arbitrarily defined as the reciprocal of the diffusion constant for internal rotation. For the random jump model, Eq. (26) must be replaced by¹ $\tau_G^{-1} = \tau_B^{-1} = \tau_R^{-1} + 3\tau_G^{-1}$, where τ_G^{-1} is the frequency of jumps between discrete angular positions of 0° , 120° , and 240° .

In Fig. 1 we show the dependence of the linewidth ($W = 1/\pi T_2$) and the NOE upon θ , for τ_R of 20 nsec (a typical value for a small protein⁵), and a T_1 of 50 msec, at 14.1 kG. The curves for W and the NOE vs θ have been plotted for fixed τ_R and T_1 and not fixed τ_G . The linewidth is nearly independent of the model for internal rotation, while the NOE is strongly dependent upon it. Clearly, an accurate measurement of the linewidth, when τ_R and T_1 are known, will yield an accurate value of θ , even when the model is not known. A measurement of the NOE will give two possible values of θ . In this case, even a relatively inaccurate determination of W is enough to distinguish between the two models and the two solutions for θ .

As an example, we propose a study of a protein specifically ^{13}C -enriched (e.g., 20%) in one alanine residue, so that very accurate ^{13}C T_1 , T_2 , and NOE measurements could be made without interference

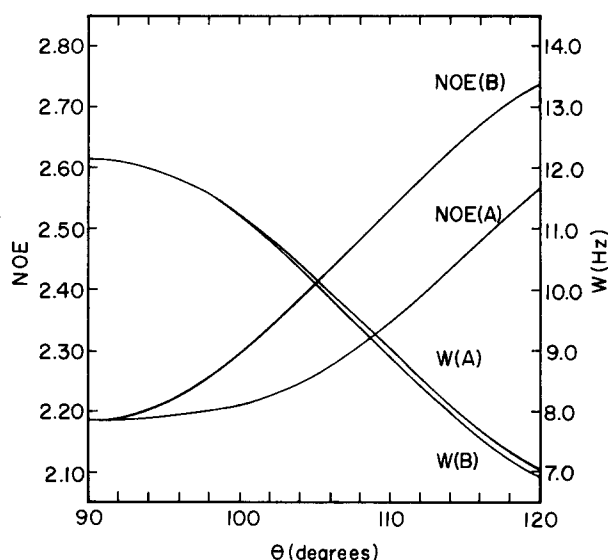


FIG. 1. Plot of the ^{13}C linewidth and nuclear Overhauser enhancement vs θ , for a CH group with a measured ^{13}C T_1 value of 50 msec which is undergoing internal rotation while attached to a molecule with isotropic rotational reorientation (with $\tau_R = 20$ nsec), under conditions of complete proton decoupling. The CH distance is taken as 1.09 Å, the magnetic field is 14.1 kG. Curves labelled (A) and (B) are for a stochastic diffusion model and random jump model, respectively.

from other ^{13}C signals. A determination of the α -carbon T_1 by means of partially relaxed Fourier transform (PRFT) spectra³ would yield τ_R .⁶ PRFT spectra of the methyl carbon would provide the necessary T_1 value for constructing the appropriate set of theoretical curves for W and the NOE, as in Fig. 1, from which θ and the model for internal rotation would be extracted after measuring the experimental W and NOE. It should be noted that the equations of Ref. 3 are valid for a carbon with a single attached hydrogen. T_1 and T_2 must be replaced by NT_1 and NT_2 , where N is the number of directly attached hydrogens, for the more general case. The equation for the NOE is not affected.

Finally, we wish to point out that the above method is not applicable to small molecules, because then $1/\tau_R$ is large with respect to the resonance frequency. In this case, $T_1 = T_2$, and the NOE has an asymptotic maximum of 2.988.^{2,3} Neither can this method be used for random-coil polymers which undergo rapid segmental motion. It appears that its use will be limited to biopolymers in their native state.

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¹D. E. Woessner, *J. Chem. Phys.* **36**, 1 (1962); A. G. Marshall, P. G. Schmidt, and B. D. Sykes, *Biochemistry* **11**, 3875 (1972).

²K. F. Kuhlmann, D. M. Grant, and R. K. Harris, *J. Chem. Phys.* **52**, 3439 (1970).

³A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.* **55**, 189 (1971).

⁴D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.* **56**, 3683 (1972).

⁵A. B. Rawitch, *Arch. Biochem. Biophys.* **151**, 22 (1972).

⁶A. Allerhand, D. Doddrell, V. Glushko, D. W. Cochran, E. Wenkert, P. J. Lawson, and F. R. N. Gurd, *J. Am. Chem. Soc.* **93**, 544 (1971).

Comments

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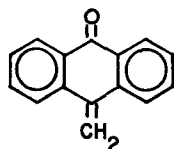
Impurity phosphorescence in methylene anthrone*

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In a recent study the polarization and absorption spectra of methylene anthrone (I) were reported.¹ Based on these spectral measurements the assignments



of the electronic states appearing in the near ultraviolet spectrum were proposed. Their results may be summarized as follows. (1) The lowest singlet and triplet electronic states have an n, π^* configuration. (2) The more intense electronic transitions in the near ultraviolet region are π, π^* states; moreover, these states are due to perturbed 1L_a and 1L_b benzene states split by inter-ring exciton interaction. The basis of these assignments was the polarization of the excitation spectrum of methylene anthrone with respect to its phosphorescence.

We have attempted to duplicate this work and have been unable to reproduce these results using a similar spectrophotofluorimeter. Our first results obtained from a crude preparation of methylene anthrone were very similar to those given previously.^{1,2} However, after careful recrystallization from ethanol we obtained solutions which were free of detectable luminescence.

We now propose that the emission observed by Smith and Dearman¹ and by us in our early studies was that of a photo-oxidation product, anthraquinone. The basis of this proposal is threefold. First, only samples which were irradiated with the xenon or ordinary room lights gave this emission. Second, it has been reported that methylene anthrone can be photochemically converted

to anthraquinone on a preparative scale.³ Finally, comparison of the phosphorescence and polarized phosphorescence excitation spectra of anthraquinone⁴ with those of methylene anthrone^{1,2} shows them to be, with experimental error, identical. No phosphorescence lifetimes or excitation spectra were given so these comparisons cannot be made.

The point that the previous investigators did not purify their material prior to use virtually assures that some anthraquinone was present in their samples. The intense phosphorescence which is observed from anthraquinone requires that it be rigorously excluded.⁵

On the basis of our results we suggest the following spectral scheme. The first singlet-singlet transition having its 0-0 band at 400 nm is weak, $\epsilon = 30 M^{-1} \cdot \text{cm}^{-1}$. Its low extinction coefficient and spectral position suggest that this band is due to an $^1A_2(n, \pi^*) \leftarrow ^1A_1$ transition assuming C_{2v} symmetry. The higher absorption bands at 345 nm ($\epsilon = 4000 M^{-1} \cdot \text{cm}^{-1}$), 272 nm ($\epsilon = 16\,000 M^{-1} \cdot \text{cm}^{-1}$) and 235 nm ($\epsilon = 36\,000 M^{-1} \cdot \text{cm}^{-1}$) are in accord with the spectrum of Smith and Dearman. The polarization and group theoretical assignments of the excited states, however, cannot be made.

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¹J. C. Smith and H. H. Dearman, *J. Chem. Phys.* **57**, 1308 (1972).

²I. S. Krull and D. I. Schuster, *Tetrahedron Lett.* **2**, 135 (1968).

³A. Mustafa and A. B. Islam, *J. Chem. Soc., Suppl.* **1**, 1 (1949).

⁴H. R. Drott and H. H. Dearman, *J. Chem. Phys.* **47**, 1896 (1967).

⁵C. A. Praker and C. S. Hatchard, *Analyst (Lond.)* **87**, 664 (1962).