

We emphasize that the observed frequency shifts reflect both electronic changes, which influence bond strength and therefore force constants, and geometric changes, which alter both force constants and kinematic coupling. It is to be anticipated that cases will be found which do not fit the present correlations for oxidation and spin state, because of structural influences of protein conformation or axial ligands.

**Oxidation State of Oxyhemoglobin.** While oxyhemoglobin is known to be diamagnetic, a considerable controversy has developed over the years as to whether the iron-oxygen unit should properly be considered  $\text{Fe}^{2+}\text{-O}_2^{38,39}$  (low spin) or  $\text{Fe}^{3+}\text{-O}_2^-$ ,<sup>40</sup> with the superoxide radical antiferromagnetically coupled to low-spin  $\text{Fe}^{3+}$ , or even as  $\text{Fe}^{4+}\text{-O}_2^{2-}$ .<sup>41</sup> It is therefore of interest to observe that with respect to the oxidation and spin state Raman marker bands, oxyhemoglobin clearly classifies as low-spin Fe(III) (see Table III). Indeed, the resonance Raman spectrum of oxyhemoglobin is essentially identical with that of cyanomethemoglobin, as can be seen in Figure 2, and it is much closer to the spectrum of ferri- than ferrocytochrome c. From the standpoint of the porphyrin vibrational frequencies, which, as we have seen, are quite sensitive to oxidation state, the best formulation is clearly  $\text{Fe}^{3+}\text{-O}_2^-$ . This conclusion has been reached

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(39) J. S. Griffith, *Proc. Roy. Soc., Ser. A*, **235**, 23 (1956).

(40) J. Weiss, *Nature (London)*, **203**, 83 (1964).

(41) H. B. Gray, *Advan. Chem. Ser.*, No. 100 (1971).

earlier by Rimai and coworkers<sup>10</sup> on the basis of the position of the  $\sim 1370\text{ cm}^{-1}$  band for a series of heme proteins, but the present study allows a much more detailed comparison.

On the other hand, it is instructive to note that carbonmonoxyhemoglobin, in which  $\text{O}_2$  is replaced by CO, gives resonance Raman spectra identical with those of oxyhemoglobin. We have studied the carboxyhemoglobin spectrum in some detail, taking care to correct for photodissociation in the laser beam (see Figure 7), and can find no differences, at least in the high-frequency region, with respect to oxyhemoglobin. The formulation  $\text{Fe}^{3+}\text{-CO}^-$  is much less congenial than  $\text{Fe}^{3+}\text{-O}_2^-$ , in view of the instability of the  $\text{CO}^-$  radical. Inorganic chemists are, however, quite accustomed to the notion of relief of charge on a metal atom by back bonding to  $\pi$  acceptor ligands like CO or  $\text{O}_2$ . Indeed, the C-O stretching frequency in carbonmonoxyhemoglobin,<sup>42,43</sup>  $\sim 1950\text{ cm}^{-1}$ , is  $\sim 200\text{ cm}^{-1}$  lower than that of free CO and is typical of transition metal carbonyls. It is reasonable to conclude that  $\pi$  back donation occurs to about the same extent in oxy- and carboxyhemoglobin, and that the resultant charge on the iron atom is about the same as for low-spin ferric heme derivatives.

**Acknowledgment.** We thank Dr. L. Rimai for communicating results of his work prior to publication.

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## Temperature Dependence of the Line-Narrowed $^{19}\text{F}$ Nmr Spectrum of Solid Perfluorocyclohexane<sup>1</sup>

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**Abstract:** The rate of ring inversion in solid perfluorocyclohexane was measured as a function of temperature by analyzing line-narrowed  $^{19}\text{F}$  nmr spectra. The activation parameters describing the inversion depend strongly on a parameter used in the analysis, the residual line width, which could not be measured precisely. It is nonetheless possible to conclude that the enthalpies and entropies of activation for chair-to-chair inversion in the liquid and solid states do not differ radically. The free energy of activation in the solid at the coalescence temperature of  $262^\circ\text{K}$  is 11.5 kcal/mol, roughly 1 kcal/mol higher than the liquid state value. In addition, we present a line-narrowed nmr spectrum of perfluorocyclohexane taken at a sufficiently low temperature to permit an estimation of the anisotropy of the chemical shift tensors of the  $^{19}\text{F}$  nuclei. This spectrum resembles the line-narrowed  $^{19}\text{F}$  nmr spectrum of Teflon.

The rate of interconversion of cyclohexane and related molecules between alternate chair conformations has been studied by many workers.<sup>2</sup> Perfluorocyclohexane is of particular interest because, unlike cyclohexane, it exhibits a negative entropy of activa-

tion.<sup>3</sup> A possible interpretation of this is that perfluorocyclohexane passes through a planar rather than a "skew-boat" intermediate conformation.<sup>4</sup> Multiple-pulse line-narrowing experiments previously made in this laboratory<sup>5</sup> showed that the "high-resolution" nmr spectrum of solid perfluorocyclohexane has qualita-

(1) This work was supported in part by the National Science Foundation.

(2) For an introduction to the literature on this topic, see the review article by W. A. Thomas in "Annual Review of NMR Spectroscopy," Vol. 1, E. F. Mooney, Ed., Academic Press, New York, N. Y., 1968, p 44 ff.

(3) (a) G. V. D. Tiers, *Proc. Chem. Soc., London*, 389 (1960); (b) H. S. Gutowsky and F.-M. Chen, *J. Phys. Chem.*, **69**, 3216 (1965).

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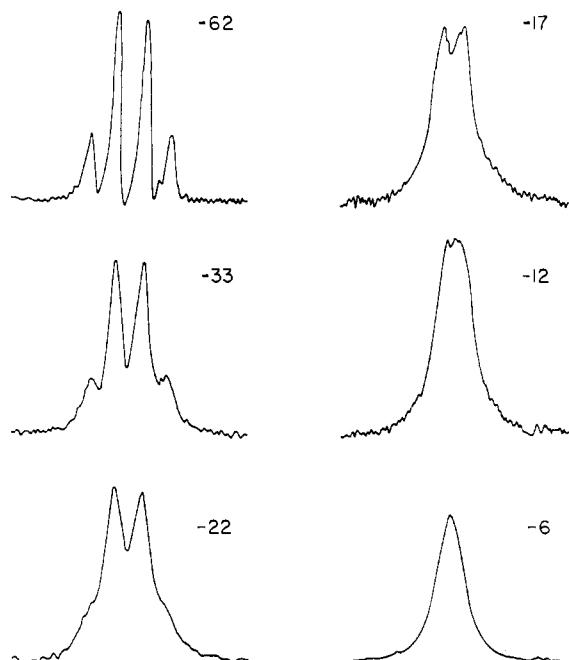


Figure 1. Line-narrowed  $^{19}\text{F}$  nmr spectra of solid perfluorocyclohexane for several temperatures.

tively the same temperature dependence as the spectrum of the compound in solution;<sup>3a</sup> at low temperatures an AB quartet is observed which at higher temperatures collapses to a single line. If the enthalpy and entropy of activation which characterize this process in the solid state differ from those found for the liquid state, the mechanism of interconversion in the two states must be different. In this paper we report the results of a quantitative study of the temperature dependence of the line-narrowed spectrum of a solid sample.

Also we report below an observation of the anisotropy of the  $^{19}\text{F}$  chemical shift in perfluorocyclohexane. In our previous work we did not observe the anisotropy because at the temperatures we used the perfluorocyclohexane molecules were tumbling isotropically about their lattice sites,<sup>6</sup> averaging out the anisotropy.<sup>7</sup> By cooling the sample to below approximately  $-157^\circ$ , it is possible to freeze out this tumbling and observe the characteristic line shape of a powder average of a chemical shift tensor.

### Experimental Section

**Sample Preparation.** The perfluorocyclohexane sample was purchased from Pierce Chemical Co.<sup>8</sup> It was purified and degassed by repeated trap-to-trap sublimations. Approximately 1 ml of the purified solid was sublimed into an evacuated 5-mm nmr sample tube and the tube was sealed.

**Spectra.** Line-narrowed spectra were obtained by the WAHUA multiple-pulse technique.<sup>9</sup> A detailed discussion of the spectrometer on which the data were taken and the procedure for finding the optimum instrumental settings can be found in ref 10. The spectrometer operated at 54.100 MHz. The chemical shift scale factor was 0.564, as measured directly by the procedure given on p 171 of ref 10.

**Temperature.** The sample was cooled by flowing nitrogen.

(6) A. Fratiello and D. C. Douglass, *J. Chem. Phys.*, **41**, 974 (1964).

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(8) Pierce Chemical Company, P. O. Box 117, Rockford, Ill. 61105.

(9) J. S. Waugh, L. M. Huber, and U. Haerberlen, *Phys. Rev. Lett.*, **20**, 180 (1968).

(10) J. D. Ellett, Jr., et al., "Advances in Magnetic Resonance," Vol. 5, J. S. Waugh, Ed., Academic Press, New York, N. Y., 1971, pp 117-175.

Table I. The Mean Time between Inversions for the Chair-to-Chair Inversion of Solid Perfluorocyclohexane Determined for Three Values of the Residual Line Width

Temp, $^\circ\text{C}$	Residual line width, Hz		
	90	100	110
	$\tau$ , msec		
8.5	0.204	0.185	0.165
-6.2	0.551	0.539	0.526
-12.5	0.80	0.82	0.82
-17.7	1.05	1.10	1.15
-22.3	1.60	1.70	1.80
-25.2	1.90	2.05	2.20
-33.3	3.2	3.6	4.2

The temperature of the gas stream was held constant with a temperature regulator<sup>11</sup> whose platinum resistance temperature sensor was placed approximately 15 cm upstream from the sample. Before and after each spectrum was taken, the temperature was measured with a copper-constantan thermocouple which was calibrated in a Dry Ice bath according to the procedure given by Scott.<sup>12</sup> The thermocouple was inserted in a sample tube containing  $\sim 1$  ml of heptane and placed at the position of the sample in the probe. The root-mean-square of the differences between temperature measurements made before and after the spectra was  $0.25^\circ$ .

**Data Manipulation.** The value of the "mean time between inversions"  $\tau$  corresponding to a particular partially collapsed spectrum was found by comparing the observed spectrum to a series of computed spectra. These spectra were calculated and plotted with a computer program, kindly supplied by William J. Horsley, which numerically solved the coupled equations for the elements of the density matrix describing a pair of chemically exchanging spin one-half nuclei.<sup>13</sup> The following values were used in the calculation: coupling constant  $J$ , 284 Hz; chemical shift difference  $\Delta\nu$ , 555 Hz; residual line width  $W$ , 90, 100, and 110 Hz. The values for  $J$  and  $\Delta\nu$  were taken from the high-resolution study of Tiers,<sup>3a</sup> except that the multiple-pulse scale factor of 0.564 was used to compute  $\Delta\nu$ . The residual line widths were estimated from a spectrum taken at  $-62^\circ$ . Below this temperature the lines broadened because of the anisotropic chemical shift. The line widths of the two central peaks of the  $-62^\circ$  spectrum were 93.6 and 111.8 Hz. Since it was impossible to determine the residual line width exactly, three values were used. For each temperature, three values of  $\tau$  were obtained by fitting the experimental spectrum to spectra calculated using the three residual line widths. Best fits were judged visually. No one value of the residual line width consistently gave better fits to the observed spectra.

### Results and Discussion

The collapse of the spectrum of perfluorocyclohexane from an AB quartet to a single line is illustrated in Figure 1. Values for the "mean time between inversions"  $\tau$ <sup>13</sup> corresponding to each partially collapsed spectrum were obtained by visually fitting the experimental spectrum to a calculated one. The value of  $\tau$  giving the best fit to a particular spectrum depended strongly on the value of the "residual line width" used for the calculated spectra, because the line-narrowing technique did not reduce the line widths to a sufficiently small fraction of the spacing of the lines in the quartet. The residual line width could only be estimated to be within  $\pm 10\%$  of 100 Hz. For this reason in Table I values of  $\tau$  are tabulated as a function of temperature for three different values of the natural line width: 90, 100, and 110 Hz.

The rate constant  $k$  for the chair-to-chair inversion equals  $1/\tau$ . Figure 2 shows Arrhenius plots of  $k$  vs. the inverse temperature for the three values of residual

(11) Model 87-E, Bayley Instrument Co., Danville, Calif.

(12) R. B. Scott in "Temperature: Measurement and Control in Science and Industry," Reinhold, New York, N. Y., 1941, pp 212-213.

(13) S. Alexander, *J. Chem. Phys.*, **37**, 967 (1962).

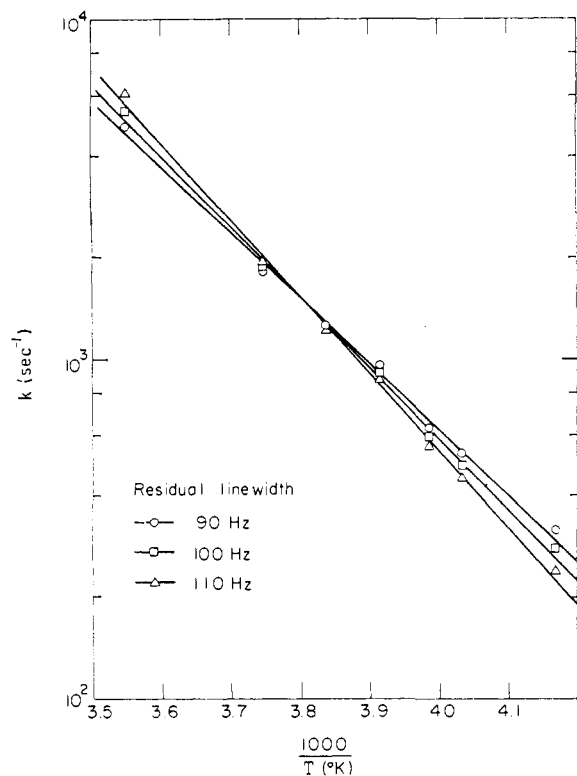


Figure 2. Arrhenius plots of the rates of chair-to-chair inversion of solid perfluorocyclohexane vs.  $1/T$  for three values of the residual line width.

Table II. Activation Parameters for the Chair-to-Chair Inversion of Perfluorocyclohexane

Experiment	Residual line width, Hz	$\Delta H_{cc}^*$ , kcal/mol	$\Delta S_{cc}^*$ , cal/(mol deg)	$\Delta G_{cc}^*/T$ (262°K), kcal/mol
Line narrowed	90	8.4	-12	11.5
Solid	100	9.1	-9	11.5
This work	110	9.9	-6	11.5
High-resolution liquid <sup>2</sup>		$7.5 \pm 0.3$	-10.2	10.2
Spin-echo liquid <sup>3</sup>		$9.9 \pm 0.2$	-4.4	11.1

line width used. It is customary to analyze an Arrhenius plot in terms of an activation enthalpy  $\Delta H^*$  and entropy  $\Delta S^*$ . The text by Bovey<sup>14</sup> gives the procedure we used for this analysis. First the Gibbs free energy of activation  $\Delta G_{cc}^*$  for each temperature was computed from the rate constants with Eyring's formula and a canonical set of approximations discussed by Bovey. Then  $\Delta H_{cc}^*$  and  $\Delta S_{cc}^*$  were determined by fitting  $\Delta H_{cc}^* - T\Delta S_{cc}^*$  to the  $\Delta G_{cc}^*$  data by the standard least-squares technique. The results are given in Table II, along with the activation parameters for the ring inversion in the liquid state found by previous workers.

The large variation of  $\Delta H_{cc}^*$  and  $\Delta S_{cc}^*$  for different line width values makes a detailed comparison of the solid and liquid state inversion processes difficult. The activation enthalpy and entropy for the solid sample are similar in magnitude to their values in the liquid

(14) F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N. Y., 1969, pp 188-195. Note that  $\tau$  as defined by Bovey and ref 3 is one-half the value of  $\tau$  used in ref 13 and this paper.

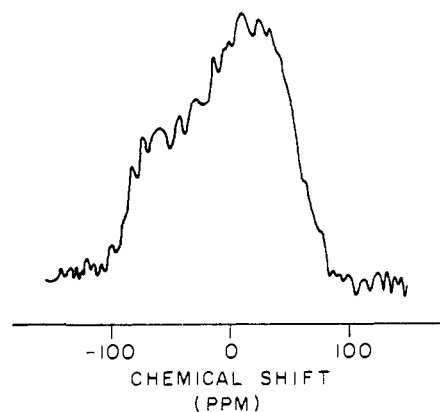


Figure 3. Line-narrowed  $^{19}\text{F}$  nmr spectrum of solid perfluorocyclohexane at  $-157^\circ$  showing the characteristic powder pattern at an anisotropic chemical shift. The chemical shift scale factor of the WAHUA experiment has been taken into account in the horizontal scale.

state and the activation entropy is still negative in the solid. The similarity between the activation parameters in the solid and liquid states is not surprising in view of the fluid-like properties of solid perfluorocyclohexane above  $210^\circ\text{K}$ . It is a soft, plastic material composed of molecules which tumble rapidly<sup>6</sup> and jump frequently from site to site.<sup>15</sup>

Notice that the three Arrhenius plots in Figure 2 cross at a single point. At this temperature,  $262^\circ\text{K}$ , the spectrum has just collapsed to a single line, so this point can be taken to define the coalescence temperature. Because  $\tau$  and therefore  $\Delta G_{cc}^*$  are relatively independent of the residual line width at this temperature, one can perhaps make a meaningful comparison of the solid and liquid state measurements. Table II shows that the Gibbs free energy of activation is roughly 1 kcal/mol higher in the solid state than in the liquid at  $262^\circ\text{K}$ .

Below about  $-62^\circ$ , the lines of the quartet begin to broaden asymmetrically and at  $-157^\circ$  the spectrum assumes the characteristic shape produced by the powder average of an anisotropic chemical shift tensor. This spectrum is shown in Figure 3. The spectrum corresponds roughly to an axially symmetric tensor with  $\Delta\sigma = 126.6$  ppm, the  $\sigma_{\parallel}$  and  $\sigma_{\perp}$  components lying at  $-84.4$  and  $+42.2$  ppm relative to the mean chemical shift of  $\text{C}_6\text{F}_{12}$ . The low-temperature spectrum of perfluorocyclohexane is, of course, the superposition of two powder patterns from the nonequivalent axial and equatorial fluorine nuclei, and the spectrum is further complicated by the fact that the two nuclei are coupled. Nonetheless, one can see that the spectrum resembles that of another fluorinated aliphatic compound, Teflon.<sup>16</sup> The width of the Teflon powder pattern at  $-192^\circ$  is 139 ppm and the  $\sigma_{\perp}$  component is the more shielded. In perfluorinated aromatic compounds the  $\sigma_{\perp}$  component of the powder pattern is usually the less shielded component.<sup>16</sup>

**Acknowledgment.** We wish to thank William J. Horsley for letting us use his computer program for calculating spectra of chemically exchanging systems.

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