An Experimental Study of Some NMR Methods for the Measurement of Slow Exchange Rates

P. Baine*
Department of Chemistry, California State University, Long Beach, California 90840 USA

J. T. Gerig* and **A. D. Stock**
Department of Chemistry, University of California, Santa Barbara, California 93106 USA

The rates of interconversion of the *cis* and *trans* rotational isomers of *N*-trifluoroacetyl-*N*-methylbenzylamine have been determined by a variety of NMR lineshape experiments and by several double resonance methods. Comparison of the results for the slow exchange region suggests that a Fourier transform version of the transfer of saturation method of Forsen and Hoffman, as well as transfer of magnetization after selective 180° inversion of the resonance of one rotamer, give rate constants nearly as reliable as lineshape methods. Use of the rate of signal recovery after saturation of the resonance of one rotamer gave results which were less accurate. The available data produced the following activation parameters for amide rotation in this compound: $\Delta G^{\ddagger}$, 18.9 kcal mol^{-1}; $\Delta H^{\ddagger}$, 22.3 kcal mol^{-1} and $\Delta S^{\ddagger}$, 9.8 e.u. when the solute was 2 M in 1,1,2,2-tetrachloroethane-d_2.

INTRODUCTION

NMR spectroscopy is by now a familiar and essential tool for the measurement of reaction rates. Slow exchange processes are those which take place at a leisurely enough rate that separate NMR signals are observed for each chemical species which interconverts in the system, and a variety of experiments for quantitating these rates are available. These include analysis of the NMR signal lineshape using several methods, especially simulation by computer,[1-2] and double resonance procedures.[3-5] The various double resonance methods offer the advantage that their utility does not depend on accurate knowledge of chemical shifts at all temperatures. However, the range of rates which can be determined by these methods is limited by the spin–lattice relaxation times for the nuclei under study. Some discussions of the experimental errors involved and the accuracy of NMR lineshape studies have been offered[1,6,7] but a complete analysis of the errors and limitations inherent in the double resonance experiments has not appeared. Our laboratory has become interested in using NMR methods to measure the rates of several slow biological processes and we wanted some idea of the reliability of double resonance experiments as compared to lineshape methods. Rather than attempt a mathematical analysis of the former methods, we decided first to approach this question empirically. A simple system involving rotation of the amide function in *N*-trifluoroacetyl-*N*-methylbenzylamine, which was amenable to many dynamic NMR experiments, was chosen and careful lineshape analyses were used to define the temperature dependence of the rate process; several types of double resonance experiments were then carried out and the results compared to those obtained by lineshape methods.

The results suggest that, with the possible exception of an experiment which measures the exchange rate by following recovery from saturation, the double resonance methods provide results which are probably as accurate as those obtained by lineshape analysis. While this paper was in preparation, a similar study comparing rates obtained by lineshape, spin–echo and double resonance methods appeared and a similar conclusion was reached.[7]

EXPERIMENTAL

N-Trifluoroacetyl-*N*-methylbenzylamine was prepared by treating *N*-methylbenzylamine (Columbia, 6.1 g, 0.05 moles), dissolved in 75 ml of chloroform and chilled in an ice bath, with 21 g (0.1 mol) of trifluoroacetic anhydride (Matheson). After 0.5 h, the solvent was removed by evaporation *in vacuo* and the residue distilled to afford a product (b.r. 74–75 °C at 0.1 mmHg) which was >99% pure by gas chromatography. The ^{1}H and ^{19}F NMR spectra of this material were completely consistent with the expected structure.

Samples for NMR spectroscopy were prepared using carbon tetrachloride or 1,1,2,2-tetrachloroethane-d_2 (Stohler) as the solvent; samples were subjected to at least three freeze-thaw degassing cycles before being sealed under vacuum.

A Varian Associates XL-100 spectrometer was

---

* Authors to whom correspondence should be addressed.

CCC-0030-4921/81/0017-0041$02.50

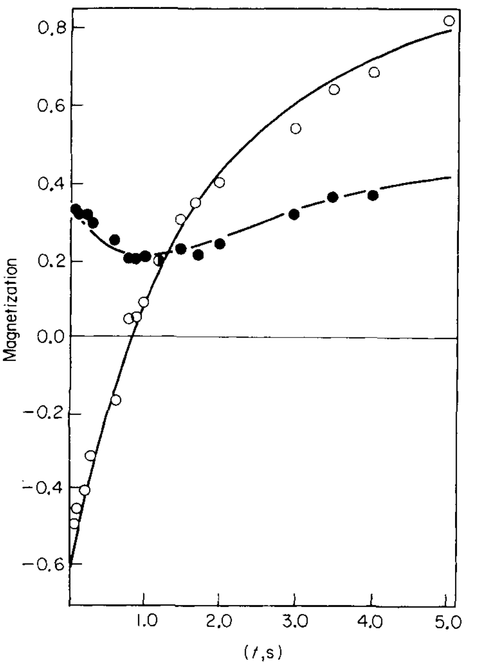


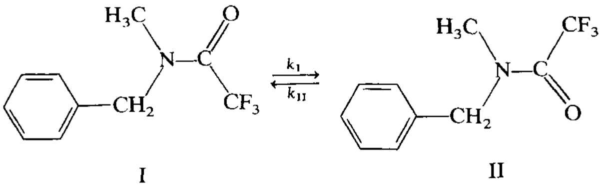
Figure 1. Selective inversion experiment using the fluorine resonances of *N*-trifluoroacetyl-*N*-methylbenzylamine at 42°. The signal from the major resonance is inverted and the recovery of both signals back to equilibrium followed (points). The best fits of Eqns (3) and (4) to the data are shown as solid lines. T_1 for both conformers was 2.4 s in these calculations.

used for both the CW and Fourier transform experiments. For homonuclear double resonance experiments irradiation at a 40.96 kHz field modulation sideband was used, while $^{19}F\{^1H\}$ and $^1H\{^{19}F\}$ experiments were carried out as described previously.[8] Sample temperatures were maintained with the Varian controller but were determined with a calibrated copper-constantan thermocouple inserted in a dummy sample tube at the level of the receiver coil. Care was taken that the H_1 power during the CW experiments was well below saturation.

Lineshape calculations used a locally modified version of Binsch's DNMR3 program, as supplied by the Quantum Chemistry Program Exchange, Bloomington, Indiana.

RESULTS

N-Trifluoroacetyl-*N*-methylbenzylamine exists in solution in two conformations (I and II), generated by rotation about the amide C—N bond, and both are clearly represented in the proton and fluorine spectra



I II (1)

of this molecule. At room temperature two quartets of signals are present for both the CH_3 and CF_3 groups; the ratio of the intensities of the two multiplets is 0.32:0.68. The more abundant species is assigned structure I based on the magnitude of $^5J(CH_3CF_3)$ (1.5 Hz for I compared to 0.7 Hz for II)[9] and the effects of aromatic solvents on chemical shifts.[10] (Details of the solvent effect studies are given in Ref. 11.) As the sample temperature is progressively raised the multiplets for each conformer broaden and collapse to broad singlets. Finally, the broad signals merge and evolve into a single broadened peak; at the highest temperature examined (110°, limited by reflux of the sample) complete averaging of the signals had not taken place. Three independent series of experiments were carried out to estimate the rate constant for the interconversion of I and II.

(1) The lineshape of the CH_3 signals, obtained by CW spectroscopy at various temperatures, was simulated by the DNMR3 program. The calculations involve the conversion of one A_3X_3 spin system into another. The linewidth of the signals and the chemical shift difference of the protons appeared to be independent of temperature up to ~40°, where significant exchange effects became apparent, and these parameters were assumed to be independent of temperature in making the lineshape analysis.

(2) The lineshape of the CH_3 group was obtained with complete fluorine decoupling and analyzed in a similar way. With decoupling the interconverting methyl spin systems are reduced to the classical two-site exchange system.[12]

(3) The lineshape of the CF_3 group, again obtained in the CW mode, was analyzed by DNMR3. The chemical shift difference between the two CF_3 multiplets (1.6 ppm) is nearly ten times larger than the shift difference between the methyl signals, and the CH_3—CF_3 spin coupling interaction has a proportionately smaller effect on the lineshape as coalescence of the signals takes place. A small temperature dependence of the chemical shift difference between the CF_3 resonances of I and II was noted at lower temperatures, and a correction for this temperature dependence was applied when fitting those spectra in the coalescence region by assuming that the effect observed at lower temperatures could be extrapolated to the higher temperatures.

The result of these three separate series of experiments was 21 rate constants for the exchange process for the temperature range 46–110 °C. The data were fitted to the Eyring equation (Eqn (2)) by means of an iterative least squares procedure based on the program of Conway, *et al.*[13] Here k_I is the rate

$$k_I=\kappa\frac{k_BT}{h}\exp(-\Delta H^{\ddagger}/RT)\exp(\Delta S^{\ddagger}/R) \qquad (2)$$

constant for conversion of I to II, κ is a transmission coefficient assumed here to be unity, k_B and h are Boltzmann and Planck's constants, respectively, and the other symbols have their usual meaning. In 1,1,2,2-tetrachloroethane-d_2, $\Delta H^{\ddagger}=22.3_1\pm0.04$ kcal mol^{-1} and $\Delta S^{\ddagger}=9.8\pm0.1$ e.u.; Eqn (2) with these parameters reproduced the experimental rate constants with an average error of ±7%. With the exception of errors in temperature determination, the common systematic errors for the three series of experiments used to determine $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ should be different, and the fact that the three sets of rate constants are well described by a single equation suggests that such errors have been minimized; Eqn (2) with these values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ was assumed to provide 'correct' rate constants for comparison with those obtained by other methods, as discussed below.

Selective inversion experiments

In this experiment a 'soft' selective pulse is used to invert the signals of one conformer. Re-establishment of the equilibrium value for these signals and transients effects noted in the magnetization of the corresponding resonances of the second conformer are related to the spin–lattice relaxation rates (T_{1A}, T_{1B}) and the lifetimes before interchange (τ_A, τ_B) by Eqns (3 and 4).

$$M_Z{}^A(t)=M_0{}^A+\frac{\left[\left(\frac{1}{\tau_{1A}}-\lambda_2\right)(M_Z{}^A(0)-M_0{}^A)-\frac{1}{\tau_B}(M_Z{}^B(0)-M_0{}^B)\right]}{\lambda_1-\lambda_2}\exp(-\lambda_1 t)$$
$$+\frac{\left[\frac{1}{\tau_B}(M_Z{}^B(0)-M_0{}^B)-\left(\frac{1}{\tau_{1A}}-\lambda_1\right)(M_Z{}^A(0)-M_0{}^A)\right]}{\lambda_1-\lambda_2}\exp(-\lambda_2 t) \tag{3}$$

$$M_Z{}^B(t)=M_0{}^B+\frac{\left[\tau_B\left(\frac{1}{\tau_{1A}}-\lambda_2\right)(M_Z{}^A(0)-M_0{}^A)-(M_Z{}^B(0)-M_0{}^B)\right]\left(\frac{1}{\tau_{1A}}-\lambda_1\right)}{\lambda_1-\lambda_2}\exp(-\lambda_1 t)$$
$$+\frac{\left[(M_Z{}^B(0)-M_0{}^B)-\tau_B\left(\frac{1}{\tau_{1A}}-\lambda_1\right)(M_Z{}^A(0)-M_0{}^A)\right]\left(\frac{1}{\tau_{1A}}-\lambda_2\right)}{\lambda_1-\lambda_2}\exp(-\lambda_2 t) \tag{4}$$

$$\lambda_{1,2}=0.5\left(\frac{1}{\tau_{1A}}+\frac{1}{\tau_{1B}}\right)\pm 0.5\sqrt{\left(\frac{1}{\tau_{1A}}-\frac{1}{\tau_{1B}}\right)^2+\frac{4}{\tau_A\tau_B}} \tag{5}$$

$$\frac{1}{\tau_{1A}}=\frac{1}{T_{1A}}+\frac{1}{\tau_A}\qquad \frac{1}{\tau_{1B}}=\frac{1}{T_{1B}}+\frac{1}{\tau_B}$$

The parameters λ_1 and λ_2 are given by Eqn (5); $M_Z{}^A(t)$ and $M_Z{}^B(t)$ are the longitudinal magnetizations of the two conformers at time t, $M_Z{}^A(0)$ and $M_Z{}^B(0)$ are these magnetizations immediately after the soft pulse and $M_0{}^A$ and $M_0{}^B$ are the magnetizations at equilibrium. The magnetizations at any time are determined by a sampling 90° pulse. For more complete discussions of this experiment see Refs 4 and 5. In the present work sets of values for $M_Z{}^A(t)$ and $M_Z{}^B(t)$ for the well separated fluorine signals were collected and the data then fitted to Eqns (3) and (4) by iterative adjustment of T_{1A}, T_{1B}, τ_A and τ_B until good agreement with the experimental data was achieved. Because of the larger dynamic range afforded, the resonance of the major conformer was the one inverted. Figure 1 is typical of the kinds of fits which could be achieved. T_1 values for the conformers were found to be equal and fall in the range 2.4–3.0 s between 40 and 65°. In this temperature range the theoretical curves given by Eqns (3) and (4) were found to be quite sensitive to the values of τ_A and τ_B and a few percent change in these reaction half-times made a detectable difference in the shapes of the curves.

Figure 1 shows in graphical form the results of studies of the exchange process in *N*-trifluoroacetyl-*N*-methylbenzylamine by selective inversion experiments using the fluorine resonance. The rate constants estimated in this way had an average deviation from those extrapolated from the lineshape results of 10%, suggesting that slow exchange rate data obtained by the selective inversion experiments are probably at least as accurate as those from the lineshape experiments.

Saturation transfer

When the resonance of one partner (say B) in a two-site exchange situation is instantaneously saturated, the signal from the other site (A) decays to a new equilibrium value which depends on the rate of interchange between the two.[14] The temporal response of the magnetization at site A is given by Eqn (6) where the symbols have the

$$M_Z{}^A(t)=M_0{}^A\left\{\frac{\tau_{1A}}{T_{1A}}+\frac{\tau_{1A}}{\tau_A}\exp\left(\frac{-t}{\tau_{1A}}\right)\right\} \tag{6}$$

same meanings as given before. Saturation transfer to the fluorine resonances of the major conformer of the amide when the quartet of the minor form is saturated was followed at several temperatures using the CW mode of operation. An auxiliary strip chart recorder was used to provide a record of the experiment. Semi-logarithmic plots of the data were used to evaluate τ_{1A} from the exponential term of Eqn (6). In our experience the first few data points in such plots were not on the line defined by the remaining data. This observation has not been explained, but the reproducibility of the slopes of such plots in replicate experiments was generally better than ±5% when the first points were ignored. Analysis of the data gave T_1 values for the major form of 2.2–2.4 s, in reasonable agreement with those obtained by fitting the selective inversion experiments. Rate constants for exchange from I to II obtained by the CW saturation transfer experiments are plotted in Fig. 2. These rate constants show an average deviation from the values predicted from the lineshape studies of 12%.

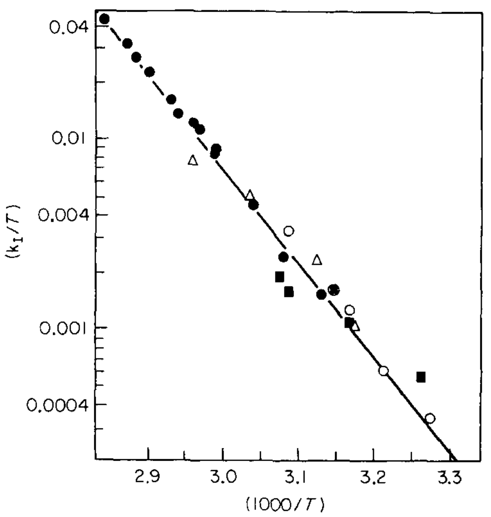


Figure 2. Plot of k_I/T vs $1/T$ for the available rate constants for conversion of the major form of *N*-trifluoroacetyl-*N*-methylbenzylamine into the minor form. The solid line represents the least squares best fit to the lineshape data as described in the text. The triangles correspond to data obtained by the selective inversion experiments while the circles represent data from the CW transfer of saturation experiments. The solid squares were obtained by the CW saturation recovery method. The dots correspond to values obtained from the lineshape analyses; more of such points lie to the left of the plot.

Recovery from saturation

When the saturating radiofrequency field is removed from the B resonance the recovery of the magnetizations of A and B to their equilibrium values is given by Eqns (7) and (8), where the symbols have the same meanings as defined above. The recovery of the fluorine magnetization of the major form (I) following the saturation of the minor form, as described in the previous section, was followed in a CW experiment. Since the recovery is bi-exponential a computer program was used to search through values of T_{1A} (assumed to be equal to T_{1B}) and τ_A until the best fit to the data, by the least squares criterion, was found. Values of T_{1A} determined in this way fell in the range 2.0–2.6 s but the values for τ_A which gave the best fit at each temperature were not very sensitive to the choice of T_{1A}. The rate data from five experiments are plotted in Fig. 2; it is seen there that the agreement with the rate constants for conformer interchange obtained by the lineshape methods is not particularly good; an average error of ~30% attended the rates obtained by saturation recovery if the lineshape data are assumed to be correct.

$$M_Z^A(t) = M_0^A\left\{1+\frac{\tau_{1A}\lambda_2}{\tau_A(\lambda_1-\lambda_2)}\exp(-\lambda_1 t) + \frac{\tau_{1A}\lambda_1}{\tau_A(\lambda_2-\lambda_1)}\exp(-\lambda_2 t)\right\} \quad (7)$$

$$M_Z^B(t) = M_0^B\left\{1+\frac{(1-\tau_{1A}\lambda_1)\lambda_2}{(\lambda_1-\lambda_2)}\exp(-\lambda_1 t) + \frac{(1-\tau_{1A}\lambda_2)\lambda_1\exp(-\lambda_2 t)}{(\lambda_2-\lambda_1)}\right\} \quad (8)$$

DISCUSSION

Rotation about the amide bond in *N*-trifluoroacetyl-*N*-methylbenzylamine has been followed by several NMR methods, including analysis of proton and fluorine lineshapes, recovery of the spin systems after selective inversion of a resonance from one conformer, transfer of saturation from one conformer to another and recovery of magnetization after selective saturation. Because a wider temperature range can be examined, the lineshape methods provided the largest body of data. Over the temperature range where comparable data could be obtained, the selective inversion experiment and the transfer of saturation experiment gave rate data which appeared to compare favorably in accuracy to those obtained by lineshape studies.

The CW recovery-from-saturation experiment seemed less accurate. This experiment starts with the observed signal reduced because of transfer of saturation into it by the exchange process, and observes the recovery of this signal to its equilibrium value. The signal, thus, can have a less favorable strength relative to noise in the initial part of the experiment and, in the CW mode of operation, it is difficult to signal average to overcome this. It is necessary to fit a bi-exponential function to this (noisy) data and it is, perhaps, not surprising that less than optimum results are obtained. Carrying out this experiment in an FT mode of operation would be likely to overcome some of these difficulties, but, given the easier data analysis possible with the saturation transfer experiment (Eqn (6)), there is little incentive to do this.

We have become satisfied that the double resonance methods explored here can provide reasonably reliable data; the fact that the often temperature-dependent chemical shift difference between exchanging nuclei need not be specified for the analysis makes them especially attractive for use with protein systems where large structural changes over small temperature ranges may be expected.

Acknowledgments

P. Baine acknowledges receipt of a sabbatical leave from the Department of Chemistry, California State University, Long Beach, California. This work was supported by grants GM-25975 and CA-11220 from the National Institutes of Health.

REFERENCES

1. G. Binsch, in *Dynamic NMR Spectroscopy*, ed. by L. M. Jackman and F. A. Cotton (1975). Chapt. 3. Academic Press, New York.
2. J. I. Kaplan and G. Fraenkel, *NMR of Chemically Exchanging Systems*, Academic Press, New York (1980).
3. R. A. Hoffman and S. Forsen, *J. Chem. Phys.* **45**, 2049

(1966).
4. J. R. Alger and J. H. Prestegard, *J. Magn. Reson.* **27**, 137 (1977).
5. I. D. Campbell, C. M. Dobson, R. G. Ratcliffe and R. J. P. Williams, *J. Magn. Reson.* **29**, 397 (1978).
6. R. R. Shoup, E. D. Becker and M. L. McNeel, *J. Phys. Chem.* **76**, 71 (1972).
7. W. M. M. J. Bovée, *Mol. Phys.* **37**, 1975 (1980).
8. J. T. Gerig, K. F. S. Luk and D. C. Roe, *J. Am. Chem. Soc.* **101**, 7698 (1979).
9. L. L. Graham, *Org. Magn. Reson.* **4**, 335 (1972).
10. W. E. Stewart and T. H. Siddall, III, *Chem. Rev.* **70**, 517 (1970).
11. A. D. Stock, PhD thesis, University of California, Santa Barbara (1974).
12. C. S. Johnson Jr, *Adv. Magn. Reson.* **1**, 33 (1965).
13. G. R. Conway, N. R. Glass and J. C. Wilcox, *Ecology* **51**, 503 (1970).
14. S. Forsén and R. A. Hoffman, *J. Chem. Phys.* **39**, 2892 (1963).

Received 30 December 1980; accepted 23 April 1981