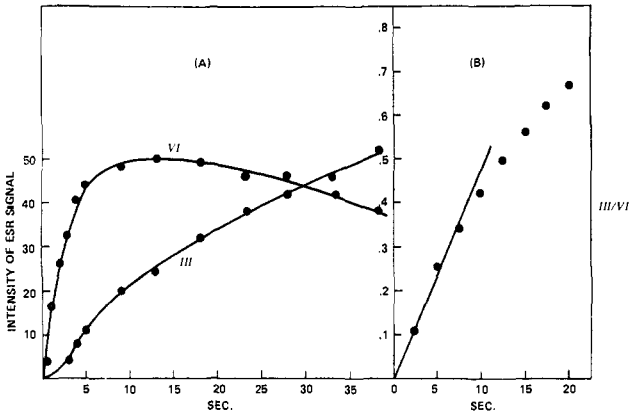

Figure 3. (a) The intensities of esr signals from 1,4-dinitrenobenzzene (III) and 1-azido-4-nitrenobenzene (VI) in methylcyclohexane at 77°K as a function of irradiation time (313 nm). (b) The ratio of concentration of 1,4-dinitrenobenzene to 1-azido-4-nitrenobenzene (III/VI) extrapolated to zero time.

The esr spectrum of the latter species is shown in Figure 2 (solid curve). The corresponding optical spectrum (Figure 1, curve 4) is in agreement with that reported for species IV.[4] It should be noted that the esr lines observed for this species are essentially those reported by Trozzolo, *et al.*,[1] for the species A obtained in the photolysis of *p*-diazidobenzene (*vide supra*). Furthermore, the ratio of the intensities of esr signals measured for solutions 2 and 4 (Figure 1) is nearly the same as the ratio of the observed optical densities.

The esr and optical spectral data presented above lead to the conclusion that a common dinitrene triplet ground-state species A is observed in the photolysis of the diazides I and V. For I, two species, A and B (other than the mononitrene), are observed characterized by $D^* = 0.059\ cm^{-1}$ and $D^* = 0.172\ cm^{-1}$, respectively, while for V only one ground-state triplet species A with $D^* = 0.059\ cm^{-1}$ is observed.

It is unlikely that radiation of 313 nm would result in the cleavage of the relatively stable azo linkage in IV. This, and the absence of species B ($D^* = 0.172\ cm^{-1}$) in the photolysis of 4,4′-diazidoazobenzene, lead us to assign the 4,4′-dinitrenoazobenzene structure IV to the species A ($D^* = 0.059\ cm^{-1}$). The low value of the zero-field splitting parameter ($D^* = 0.059\ cm^{-1}$) further suggests that the two unpaired electrons are considerably delocalized, as indeed would be expected from the presently assigned structure IV.[6] As a consequence of the above discussion we propose structure III to the species B ($D^* = 0.172\ cm^{-1}$). A value of $D = 0.181\ cm^{-1}$ for the species III has been calculated[7] which is in excellent agreement with the value reported here.[8] Further support for this assignment is obtained from the measurement of the irradiation time dependence of the esr signals from 1-azido-4-nitrenobenzene (VI) and *p*-dinitrenobenzene (III). This is shown in Figure 3a and is reminiscent of the kinetics of consecutive reactions. If III is formed from VI by absorption of radiation of energy which is identical with that which produces VI from I, then a square dependence on irradiation time is expected. If this were the case, the ratio of the concentration of III/IV extrapolated to zero irradiation time should be linear, which is indeed observed (Figure 3b). Furthermore, this species is the major product of prolonged photolysis (~30–60 sec) in all the solvent systems in which this photolysis was examined.

An additional feature of considerable interest was observed when frozen solutions containing the ground-state triplet species VI were allowed to stand in stoppered (but nondegassed) tubes for several hours at 77°K. Under these conditions a new ground-state triplet species with $D^* = 0.110\ cm^{-1}$ was formed. This corresponds to the line at 1473 G reported by Trozzolo, *et al.*[1] The structure of this species and its optical and esr spectra will be the subject of a subsequent communication.[10]

Acknowledgment. We wish to acknowledge several stimulating discussions with Professor A. C. Albrecht.

(6) The formation of the dinitrenoazobenzene IV in the photolysis of diazidobenzene I is not too surprising particularly since azo compounds are invariably formed in the photolysis of aromatic azides.

(7) J. Serre and F. Schneider, *J. Chem. Phys.*, **61**, 1655 (1964).

(8) The formation of the species A (IV) in the photolysis of I may occur by dimerization of the species B or by its reaction with the unreacted diazide I followed by photochemical decomposition of the resulting mononitrene to the species A. When solutions of the diazide are frozen there may be a tendency for the molecules of I to associate in pairs or groups. This may especially be true in more concentrated solutions ($>10^{-3}$ *M*) in MCH, 3MP, and HC where room temperature solubilities are poor to begin with. It should be noted that this species is not observed in MTHF which is a far better solvent. The heat of formation of an azide group is 205–208 kcal/mol and consequently considerable energy is liberated when such molecules decompose.[9] This could result in local melting of the matrix facilitating the dimerization process to form the azo linkage.

(9) P. A. S. Smith, "The Chemistry of Open-Chain Organic Nitrogen Compounds," Vol. II, W. A. Benjamin, New York, N. Y., 1966, p 214.

(10) J. S. Brinen and B. Singh, submitted for publication.

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Evidence Supporting a Common Transition State for Rotation and Inversion in *tert*-Butylbenzylmethylamine

Sir:

In recent communications, evidence from variable-temperature nmr spectroscopy has been presented for nitrogen *inversion* in dibenzylmethylamine[1] and C–N bond *rotation* in *tert*-butyldimethylamine.[2] In these two cases, the barriers ($\Delta G^{\pm}$) for two apparently different processes are very similar, *i.e.*, 6.2 ± 0.2 kcal/mol at −142° for inversion in dibenzylmethylamine and 6.0 ± 0.1 kcal/mol at −153° for *tert*-butyl–nitrogen bond rotation in *tert*-butyldimethylamine. The similarity in these barriers may be fortuitous or may indicate a common transition state for rotation–inversion in trialkylamines.[3] Indeed, there have been no reports to date concerning the observation of multiple nmr coalescence phenomena in acyclic amines indicating unequivocal evidence for both processes. Examination of the temperature dependence of the nmr spectrum of

(1) C. H. Bushweller and J. W. O'Neil, *J. Amer. Chem. Soc.*, **92**, 2159 (1970); M. J. S. Dewar and W. B. Jennings, *Tetrahedron Lett.*, 339 (1970). See also: M. Saunders and F. Yamada, *J. Amer. Chem. Soc.*, **85**, 1882 (1963).

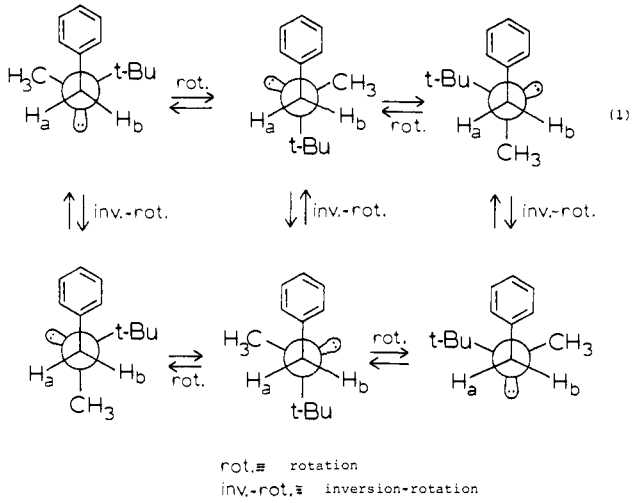
(2) C. H. Bushweller, J. W. O'Neil, and H. S. Bilofsky, *ibid.*, **92**, 6349 (1970).

(3) A. Rauk, L. C. Allen, and K. Mislow, *Angew. Chem., Int. Ed. Engl.*, **9**, 400 (1970).

an amine having a structure amenable to the observation of both inversion and rotation should resolve the dilemma.

This report concerns the observation of two coalescence phenomena in *tert*-butylbenzylmethylamine (I) providing strong evidence for a *common transition state for rotation and inversion.*

Examination of the pmr spectrum (60 MHz) of I (10% v/v in CD_2CDCl) at $-22°$ revealed three singlet resonances for the N-CH_2 (δ 3.49), N-CH_3 (δ 2.04), and N-$C(CH_3)_3$ (δ 1.09) hydrogens. Upon lowering the temperature, the N-CH_2 resonance broadened in a manner characteristic of a decreasing rate of exchange on the pmr time scale, finally sharpening into an AB spectrum ($\Delta\nu_{AB}$ = 69.6 Hz; J_{AB} = 13.4 Hz) at low temperatures (Figure 1a). Such spectral behavior can be rationalized on the basis of rapid *net inversion* at higher temperatures causing equivalence of the benzylic hydrogens with slow inversion at low temperatures generating an asymmetric center at the benzylic carbon and nonequivalent benzylic protons.[1] It would be expected that even with rapid N-CH_2 bond rotation and slow inversion, the two benzylic hydrogens would never experience the same time-averaged environment and should be nonequivalent (eq 1). However, the inversion–



rotation process (eq 1) can effectively exchange the environments of H_a and H_b causing equivalence at a high enough exchange rate. Thus, the spectral changes observed for the N-CH_2 resonance reveal a process involving net *nitrogen inversion.* A total line-shape analysis for the AB system at $-138°$ gives $\Delta G^{\ddagger}$ = 6.2 ± 0.2 kcal/mol for the inversion process.

The $C(CH_3)_3$ resonance also changed dramatically, going from a sharp singlet to a broad resonance at low temperatures with at least two peaks resolved at $-151°$ (Figure 1a). The best fit of a calculated spectrum of the $C(CH_3)_3$ peaks at $-151°$ (slow exchange conditions) gave three overlapping singlets of equal area (3 H) at δ 1.00, 1.11, and 1.28 all of the same width at half-height ($W_{1/2}$ = 16.0 Hz). A total calculated spectrum for the N-CH_2, N-CH_3, and $C(CH_3)_3$ resonances at $-151°$ is illustrated in Figure 1b. The only process which will account for the spectral changes observed for the $C(CH_3)_3$ resonance is *net rotation* about the N-$C(CH_3)_3$ bond (eq 2). From a total nmr line-shape analysis of the $C(CH_3)_3$ resonance at $-138°$, the barrier

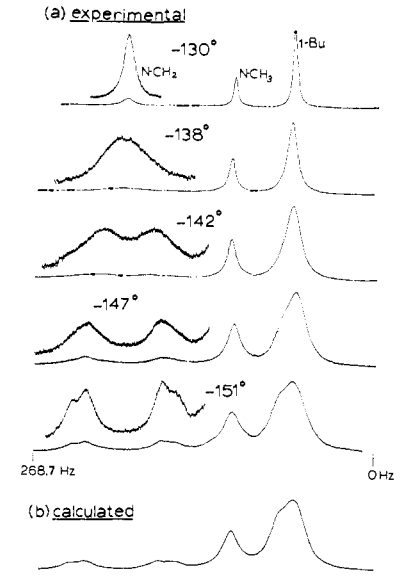
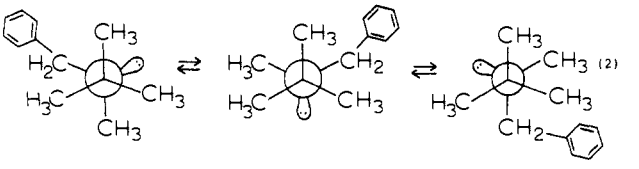
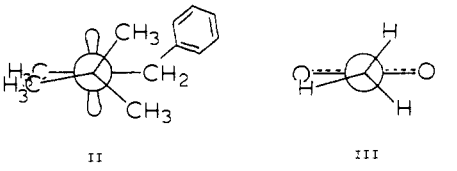


Figure 1. (a) The temperature dependence of the pmr spectrum (60 MHz) of the N-CH_2, N-CH_3, and N-$C(CH_3)_3$ protons in I. (b) The total calculated static pmr spectrum for the N-CH_2, N-CH_3, and N-$C(CH_3)_3$ protons in I at $-151°$.

($\Delta G^{\ddagger}$) to N-$C(CH_3)_3$ rotation is calculated to be 6.2 ± 0.2 kcal/mol.



The essentially identical barriers to nitrogen inversion as revealed by the N-CH_2 resonance and to N-$C(CH_3)_3$ rotation as revealed by the $C(CH_3)_3$ resonance suggest strongly a *common transition state* for rotation and inversion in I. The most likely common transition state is that involving planar nitrogen (sp^2 hybridized; lone pair in a p orbital) and one vicinal eclipsing interaction (*e.g.*, II), strictly analogous to the transition state for the sixfold barrier in nitromethane (III)[4] and methyl-



II III

difluoroborane.[5] Of course, a *combination* of rotation and inversion leads to this transition state II and the two processes occur in concert. By analogy with the very small sixfold rotational barrier in nitromethane (0.006 kcal/mol)[4] as compared to that in methylamine (2.0 kcal/mol),[6] a rate process invoking II as the transition state would be expected to lower the inherent barrier to the separate *tert*-butyl rotation process as compared to the situation involving fixed pyramidal (sp^3) nitrogen. Indeed, this rationale leads directly to the conclusion that *tert*-butyl rotation in I, as an independent process with no nitrogen inversion, is *slower* than nitrogen in-

(4) E. Tannenbaum, R. J. Myers, and W. D. Gwinn, *J. Chem. Phys.*, **25**, 42 (1956).
(5) R. E. Naylor and E. B. Wilson, *ibid.*, **26**, 1057 (1957).
(6) T. Nishikawa, T. Itoh, and K. Shimoda, *ibid.*, **23**, 1735 (1955).

version. The situation may be different in other amines in which vicinal nonbonded repulsions are less severe, *e.g.*, methylamine.

Theoretical calculations for species electronically analogous to amines, *e.g.*, $^{-}CH_2SH$, $^{-}CH_2S(O)H$, $^{-}CH_2S(O_2)H$, indicate a common transition state for rotation and inversion.[3]

Thus, it is not surprising that separate changes at different temperatures in the dynamic nmr spectrum of trialkylamines corresponding to rotation and inversion have not been reported. It is apparent that the two rate processes share the same transition state.

We are continuing our investigations in this area, especially with respect to rotational phenomena in those amines in which inversion is significantly slowed by substitution of electronegative groups on nitrogen.[3]

Acknowledgment. We thank the referees for helpful suggestions and the National Science Foundation for support (GP-18197). J. W. O. thanks the National Aeronautics and Space Administration for a traineeship, 1969–1970.

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Conformation and Segmental Motion of Native and Denatured Ribonuclease A in Solution. Application of Natural-Abundance Carbon-13 Partially Relaxed Fourier Transform Nuclear Magnetic Resonance[1]

Sir:

The high sensitivity of the Fourier transform nmr technique[3] makes it practical to study proton-decoupled natural abundance carbon-13 spectra of proteins.[2] Even at the relatively low magnetic field strength of 14.1 kG, a proton-decoupled carbon-13 spectrum of a protein is well separated into groups of resonances arising, in order of increasing shielding, from carbonyl groups, unsaturated side chains, α-carbons, and saturated side-chain residues.[2,4] By comparison, proton nmr spectra of proteins consist mainly of featureless envelopes resulting from a small range of chemical shifts and complicated spin–spin splittings.

We wish to demonstrate the value of carbon-13 spin–lattice relaxation time (T_1) measurements of individual resonances in protein spectra by means of partially relaxed Fourier transform (PRFT) nmr.[5,6] In this technique, the magnetization is first inverted by means of a 180° rf pulse. After an interval τ, a 90° pulse is applied and the digital signal averager is triggered. The sequence is repeated after a waiting period of several multiples of T_1. The resulting time-domain signal following an appropriate number of accumulations is Fourier transformed to yield a partially relaxed frequency-domain spectrum. If τ is much shorter than the T_1 of a particular resonance, a "negative" peak will appear, with an amplitude equal to that in the normal spectrum. As τ increases, this negative peak decreases in amplitude, goes through a null (at $\tau = T_1 \ln 2$), becomes positive, and for $\tau \gg T_1$ becomes equal to the normal resonance. Each amplitude A is given by[7]

$$A = A_0[1 - 2\exp(-\tau/T_1)] \quad (1)$$

where A_0 is the equilibrium amplitude.

The experiments were carried out on a high-resolution carbon-13 Fourier transform nmr spectrometer consisting mainly of a Varian high-resolution 14.1-kG electromagnet, a "home-built" nmr apparatus operating at 15.08 MHz, a Fabri-Tek 1074 signal averager, and a PDP-8/I computer. The apparatus included an external ^{19}F lock and noise-modulated proton decoupling.

A set of partially relaxed carbon-13 spectra of 0.019 *M* bovine pancreatic ribonuclease A at pH 6.51 (45°) is shown in Figures 1B–1F, with τ values ranging from 336.9 to 7.96 msec. Figure 1A shows the normal Fourier transform spectrum. Carbonyl signals are in the range 10–25 ppm "upfield" from CS_2. Unsaturated side chains are at 35–80 ppm. The β-carbon signal of the threonine residues is at 126 ppm. The α-carbon region is at 130–150 ppm. It excludes glycine but contains the β-carbon signals of the serine residues. The region above 150 ppm contains the α-carbon of the glycine residues and the remaining saturated side-chain carbons. More detailed assignments have been given by Allerhand, Cochran, and Doddrell.[2]

Least-squares analysis of the data in Figure 1 yields spin–lattice relaxation times for each resolved resonance. Representative results are given in Table I. We defer the interpretation of the T_1 values for nonprotonated carbons to a later publication.[8] If the ^{13}C

Table I. Some Carbon-13 Spin–Lattice Relaxation Times and Rotational Correlation Times in Aqueous Ribonuclease A[a,b]

Type of carbon	Native protein[c] T_1, msec	Native protein[c] τ_R, nsec	Denatured protein[d] T_1, msec	Denatured protein[d] τ_R, nsec
Carbonyls	416		539	
α-Carbons	42	30[e]	120	0.40
β-Carbons, Thr	~40	~30	99	0.48
Rigid side chains[f]	~30			
ϵ-Carbons, Lys	330	0.070	306	0.076

[a] Obtained for 0.019 *M* protein at 45° and 15.08 MHz. The T_1 and τ_R values have an estimated maximum error of ±30%. Bovine pancreatic ribonuclease A was obtained from Worthington Biochemical Corp., Freehold, N. J., and from Miles Laboratories, Inc., Elkhart, Ind. Samples from the two sources showed no measurable differences in behavior. [b] The temperature and pH dependence of the conformational transition are given by J. F. Brandts, *J. Amer. Chem. Soc.*, **87**, 2759 (1965). [c] pH 6.51. [d] pH 1.64. [e] Equation 2 yielded a second solution, 1.4 nsec, which gave α-carbon line widths of 8 Hz, in major disagreement with the observed envelope in Figure 1A, which was well simulated using τ_R = 30 nsec (45-Hz line width). [f] Broad signal component at 150–185 ppm in the spectrum of the native protein. Difficulties in separating these resonances from the narrow ones make their T_1 only an estimate of an average value.

(1) Carbon-13 Fourier Transform Nuclear Magnetic Resonance. III. Part II: ref 2.
(2) A. Allerhand, D. W. Cochran, and D. Doddrell, *Proc. Nat. Acad. Sci. U. S.*, 67, 1093 (1970).
(3) R. R. Ernst and W. A. Anderson, *Rev. Sci. Instrum.*, 37, 93 (1966).
(4) P. C Lauterbur, *Appl. Spectrosc.*, 24, 450 (1970).
(5) We propose the name partially relaxed Fourier transform (PRFT) nmr for the technique first described in ref 6.
(6) R. L Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, 48, 3831 (1968).
(7) A. Abragam, "The Principles of Nuclear Magnetism," Oxford University Press, London, 1961, p 64.
(8) A. Allerhand, D. Doddrell, V. Glushko, D. W. Cochran, E. Wenkert, P. J. Lawson, and F. R. N. Gurd, manuscript in preparation.