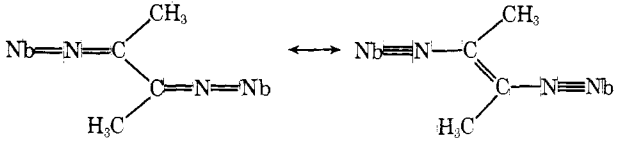
1348-1366 (s), 1097-1103 (m), 1019-1025 (w), 919-929 (w), and 652-662 (s) cm^{-1}.

Crystalline material suitable for X-ray diffraction work was obtained with the $[(C_6H_5)_3P]_2N^+$ salt of the $[Nb_2Cl_8(NCCH_3)_2L]^{2-}$ anion. A mixture containing $[(C_6H_5)_3P]_2N^+Cl^-$ and $[Nb_2Cl_6(NCCH_3)_4L]$ in a 2:1 mole ratio was dissolved in 30 ml of acetonitrile to provide a green solution. After addition of 60 ml of chlorobenzene chunky green crystals were deposited. These were filtered under nitrogen, washed with chlorobenzene, and dried *in vacuo*. A suitable crystal was selected, mounted in a sealed Lindeman capillary, and subjected to X-ray structure determination.

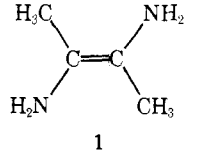
The monoclinic space group was determined to be $P2_1/n$, with the unit cell parameters a = 14.95 (1) Å, b = 20.96 (1) Å, c = 15.09 (1) Å, and β = 105.9 (1)°. The structure was determined using 4754 reflections with intensities $I \geq 3\sigma(I)$ out of the 7300 measured, and phased by the heavy atom procedure. After several electron density syntheses, all 57 nonhydrogen atoms in the asymmetric unit were located; subsequent full-matrix least-squares refinements and electron density difference syntheses revealed the locations of 40 of the 41 hydrogen atoms in the asymmetric unit. Further anisotropic refinement converged to a final R value (defined as $\Sigma||F_o| - |F_d||/\Sigma|F_o|$) of 0.066. The successful refinement of the structure determined the previously unknown composition as $\{[(C_6H_5)_3P]_2N\}_2[Nb_2Cl_8(CH_3CN)_2(C_4H_6N_2)]\cdot 2C_6H_5Cl$, and for the unit cell Z = 2, V = 4547.09 Å^3, and d(calcd) = 1.414 g cm^{-3}, with only one-half the formula unit per asymmetric unit.

The essential feature of the structure concerning the anion is depicted by the computer-generated drawing shown in Figure 1. Of special interest is the bridging $CH_3CNCNCH_3$ ligand derived from the reductive coupling of two acetonitrile molecules. In the anion there is an inversion center at the midpoint between C(N1) and C′(N1); thus, the four atoms in the bridging chain N(L1)-C(N1)-C′(N1)-N′(L1) are rigorously required by symmetry to lie in the same plane. The two niobium atoms are only slightly displaced from this plane (0.01 Å), and the two C atoms of the methyl groups similarly are displaced by only 0.03 Å. Some key bond distances and angles within the anion are listed in Table I.

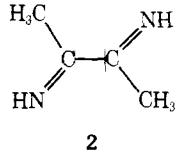
Bond distances worthy of special comment are Nb-N(L1) (1.752 (6) Å), which is indicative of double or triple bond character,[4] N(L1)-C(N1) (1.378 (8) Å), which is intermediate between those expected for a single or double bond, and C(N1)-C′(N1) (1.35 (1) Å), which is near that expected for olefins. Since the bond angle Nb-N(L1)-C(N1) is very nearly 180°, the lone pair density on N(L1) must be small. These data strongly suggest that the π bonding is extensively delocalized over the metal atoms and bridging ligand chain, as indicated by the resonance



At this point insufficient evidence has been obtained to determine if the bridging ligand is better regarded as the tetraanion of the *trans*-diaminoolefin (1) or the dianion of



1



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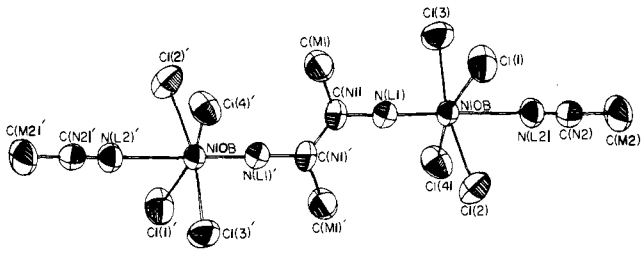

Figure 1. Structure and labeling scheme of the anion $[Nb_2Cl_8(CH_3CN)_2C_4H_6N_2]^{2-}$.

the diimine (2). However, the coplanarity of the C-N skeleton, trans configuration, and bond distances are possibly more supportive of the tetraanion of **1.**

The relation is uncertain between the $[Ta_2Cl_6(NCCH_3)_4(C_4H_6N_2)]$ reported here and the compound prepared by Blight, *et al.*,[5] by reaction of $TaCl_4$ with acetonitrile. The latter was reported as a metal-metal bonded dimeric complex of Ta(III), $Ta_2Cl_6(NCCH_3)_4$.[5] It seems likely that the compounds are actually identical although in the latter case no mention was made of the characteristic bands of the bridging ligand $C_4H_6N_2$ in the inrared spectra. More recently, Gert and Perron[3] noticed that a green compound formulated as $TaCl_3(NCCH_3)_3$ was formed when $TaCl_5$ was reduced with Sn, Zn, or Al in acetonitrile. Work done in this laboratory subsequently has shown that reduction of $TaCl_5$ with zinc, as reported by Gert and Perron, provides the same compound reported here as $[Ta_2Cl_6(NCCH_3)_4(C_4H_6N_2)]$. Further work on the properties, mode of formation, and reactions of compounds containing these interesting bridging ligands is in progress.

References and Notes

(1) M. A. Schaefer, M.S. Thesis, Iowa State University of Science and Technology, Ames, Iowa, 1969.

(2) For example both the tantalum(IV) halides TaX_4 (X = Cl, Br, I) and their pyridine adducts $TaX_4(C_5H_5N)_2$ hydrolyze in water to produce tantalum(IV) oxide and readily reduce Fe(III) to Fe(II) in aqueous solution.

(3) R. Gert and W. Perron, *J. Less-Common Met.*, **26**, 369 (1972).

(4) This Nb-N distance may be compared with the Re-N distances found in the nitridorhenium(V) compound $ReNCl_2(PEt_2Ph)_3$, d(Re-N) = 1.78 Å (J. A. Ibers, *et al.*, *Inorg. Chem.*, **6**, 197 (1967), in the iminorhenium(V) compounds $ReCl_3(NC_6H_4OCH_3)(PEt_2Ph)_2$, d(Re-N) = 1.69 Å, and $ReCl_3(NC_6H_4COCH_3)(PEt_2Ph)_2$, d(Re-N) = 1.71 Å (D. Bright and J. A. Ibers, *ibid.*, 7, 1099 (1968)). Similarly in the tungsten(VI)imino derivative, $WCl_4(NC_2Cl_5)(NCCCl_3)$, a bond distance d(W-N) = 1.70 Å was found (M. G. B. Drew, K. C. Moss, and N. Rolfe, *Inorg. Nucl. Chem. Lett.*, 7, 1219 (1971)).

(5) D. G. Blight, R. L. Deutscher, and D. L. Kepert, *J. Chem. Soc. Dalton Trans.*, 87 (1972).

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Received February 11, 1974

Identification of Tryptophan Resonances in Natural Abundance Carbon-13 Nuclear Magnetic Resonance Spectra of Proteins. Application of Partially Relaxed Fourier Transform Spectroscopy

Sir:

In recent years, numerous ^{13}C nmr studies of proteins have been reported.[1-8] Some of the reports have covered general features in the natural abundance ^{13}C nmr spectra of aqueous proteins in various states.[1,4] Others have dealt with ^{13}C nmr signals of incorporated ^{13}C-enriched amino

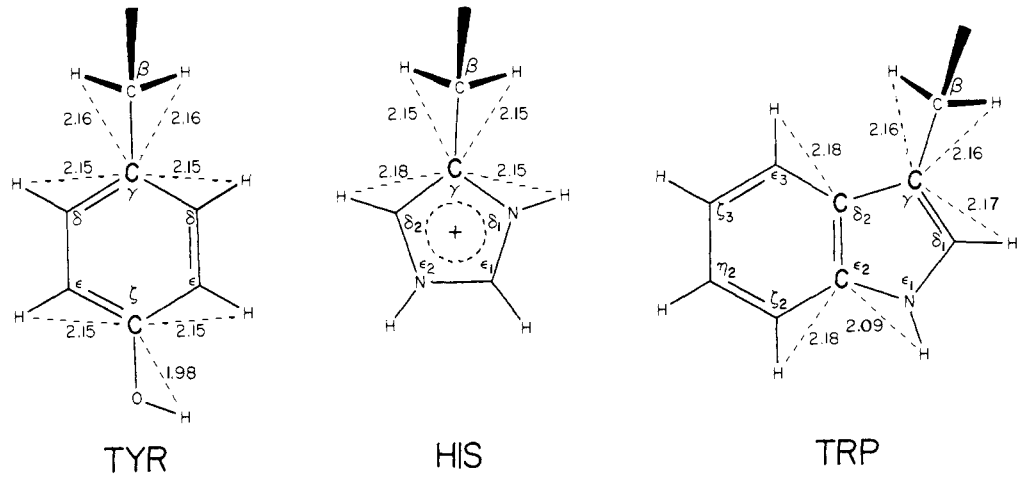

Figure 1. Estimated distances of nonprotonated aromatic carbons in Tyr, His, and Trp to hydrogens two bonds removed. These distances were computed with the use of known bond lengths and angles in some crystalline amino acids and small peptides[17] and on known CH, OH, and NH bond lengths in smaller molecules.[17] The calculated two-bond CH distances for C^{γ} of Phe are about the same as those shown for Tyr.

acid residues[2] and adducts.[3] We have chosen to concentrate our efforts on the observation of single-carbon resonances in the aromatic region of the *natural abundance* ^{13}C nmr spectra of native proteins.[5-8] The development of a 20-mm nmr probe has facilitated the detection of these resonances.[8]

We have reported the observation of numerous narrow resonances in the aromatic region of ^{13}C nmr spectra of native proteins.[5-7] We have shown that only nonprotonated aromatic carbons (Figure 1) give rise to these narrow signals. Theoretical considerations and experimental results indicate that protonated (methine) aromatic carbons of native proteins give rise to broad features.[5-7] Many of the observed narrow signals are actually resolved resonances of single nonprotonated aromatic carbons.[5-7] An important first step in the use of these resonances for studying the properties of individual atomic sites of proteins in solution is the assignment of each resonance to a specific amino acid residue. This difficult task would be facilitated if one could first identify which type of residue (Phe, Tyr, His, or Trp) gives rise to each resonance. Some resonances, most notably those of C^{γ} of Trp residues, are readily identified because they do not normally overlap with any other type of nonprotonated carbon peaks.[5,6] On the other hand, resonances of $C^{\delta 2}$ of Trp residues are not easily identified because they are found in the same range of chemical shifts as resonances of C^{γ} of Tyr and some His residues.[5,6] Resonances of $C^{\epsilon 2}$ of Trp residues are found in the same range of chemical shifts as those of C^{γ} of Phe and some His residues.[5,9] We show here that partially relaxed Fourier transform (PRFT) spectra[10,11] can be used to identify the resonances of $C^{\delta 2}$ and $C^{\epsilon 2}$ of Trp residues. We have tested this procedure on horse heart cytochrome *c* and hen egg white lysozyme, which contain one and six Trp residues, respectively. In the case of hen egg white lysozyme, some resonances of $C^{\delta 2}$ have already been identified by less general methods.[12]

Natural abundance ^{13}C nmr spectra were recorded at 14.2 kG on our home-built Fourier transform nmr spectrometer with the use of 20-mm probe,[8] under conditions of noise-modulated off-resonance proton decoupling,[13] as described previously.[5] The broad envelopes of protonated carbon resonances were removed digitally by a slight variation[14] of the convolution-difference method described by Campbell, *et al.*[15] The resulting convolution-difference spectra (Figures 2A and 3A) are particularly suitable for a detailed examination of the narrow nonprotonated carbon resonances.[14]

We have shown that the ^{13}C relaxation of hydrogen-bearing carbons in large diamagnetic molecules is overwhelmingly dominated by the ^{13}C-^{1}H dipolar relaxation mechanism.[11] The contribution of this relaxation mechanism is proportional to the inverse sixth power of the C-H distance. Therefore, ^{13}C-^{1}H relaxation is much less effective for nonhydrogen-bearing carbons than for hydrogen-bearing ones.[11] Nevertheless, we have shown that, in general, ^{13}C-^{1}H dipolar interactions dominate the relaxation of nonprotonated aromatic carbons of native proteins.[14]

The most important contributions to ^{13}C-^{1}H dipolar relaxation of nonprotonated aromatic carbons are those from hydrogens two bonds away (Figure 1). The δ_2-carbon of a Trp residue has only one such hydrogen, while C^{γ} of a Tyr has four and C^{γ} of a His has three or four (see Figure 1). The T_1 of $C^{\delta 2}$ of a Trp residue should be much longer than that of C^{γ} of a Tyr or His residue, if the ^{13}C-^{1}H dipolar relaxation mechanism is dominant. Whenever two classes of carbons have measurably different T_1 values, their resonances can be distinguished by means of PRFT spectra.[16] The PRFT method should also be applicable for distinguishing resonances of $C^{\epsilon 2}$ of Trp residues from those of C^{γ} of Phe and His residues, especially if D_2O is used as solvent. In H_2O, there are two hydrogens two bonds removed from $C^{\epsilon 2}$ of a Trp residue (Figure 1). However, one of these hydrogens is bonded to $N^{\epsilon 1}$. In D_2O solution, there will be only one hydrogen two bonds removed from $C^{\epsilon 2}$ after the hydrogen attached to $N^{\epsilon 1}$ has exchanged with deuterium. Because of the relatively small gyromagnetic ratio of ^{2}H, ^{13}C-^{2}H dipolar relaxation can be neglected here. Therefore, we expect that the T_1 value of $C^{\epsilon 2}$ of a Trp residue will be longer in D_2O than in H_2O, if the ^{13}C-^{1}H dipolar relaxation mechanism is dominant. Experimental results confirm this prediction (see below). If we consider the rather low signal-to-noise ratios of single-carbon resonances of proteins (Figures 2A and 3A), it is likely that the PRFT method will identify resonances of $C^{\epsilon 2}$ of Trp residues when D_2O is used as solvent but not when H_2O is used. This is indeed the case (see below).

We have calculated T_1 values for nonprotonated aromatic carbons of a native protein, assuming that the molecule rotates isotropically and that any internal rotation of aromatic amino acid residues can be neglected.[14] Contributions from two-bond and three-bond ^{13}C-^{1}H dipole-dipole interactions were considered (three-bond interactions contribute appreciably in the case of $C^{\delta 2}$ of Trp). The rotational correlation time that was used in the calculations was obtained from α-carbon T_1 measurements.[4,14] The calculated T_1

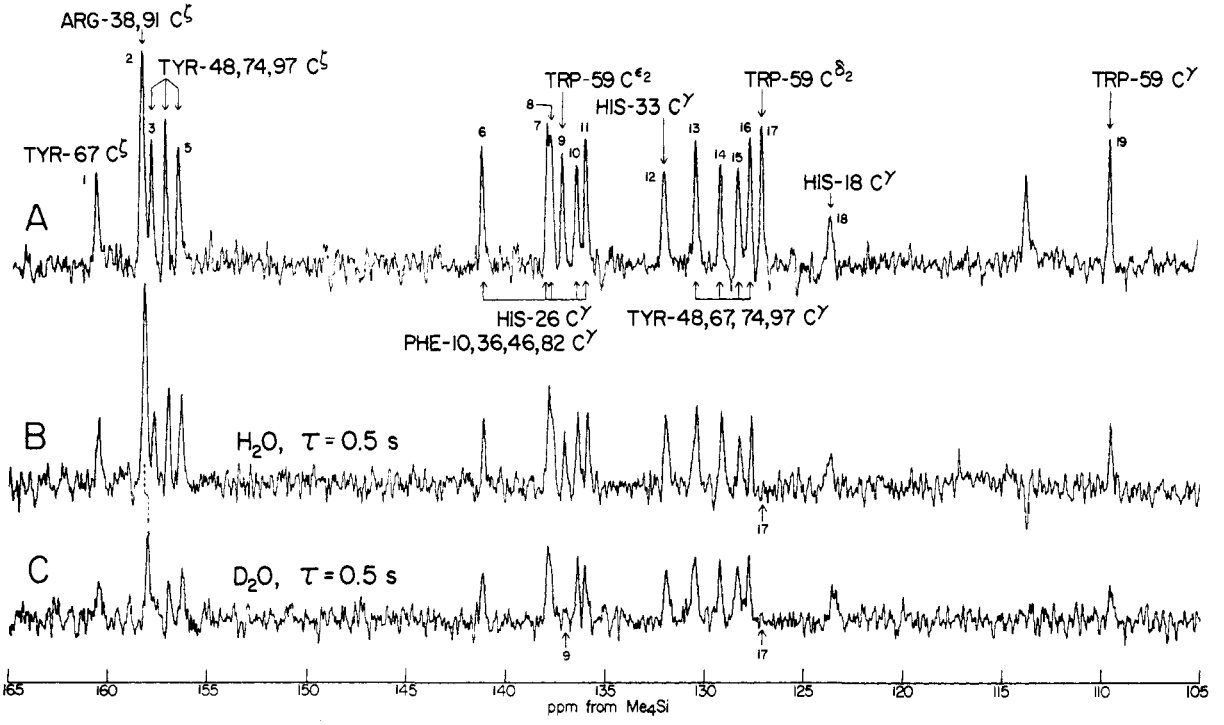

Figure 2. Aromatic region in the convolution-difference noise-modulated off-resonance proton-decoupled ^{13}C Fourier transform nmr spectra of 15 m*M* horse heart cyanoferricytochrome *c* (in 0.1 *M* NaCl, 0.05 *M* phosphate buffer, pH 6.7), recorded at 15.18 MHz and 36°, using 32,768 accumulations per spectrum: (A) normal spectrum (90° radiofrequency pulses only) of solution in H_2O, recycle time was 2.105 sec, total accumulation time 19 hr; (B) PRFT spectrum of same sample as in A, with a τ value of 0.5 sec, recycle time of 2.605 sec, total accumulation time 24 hr; (C) as B, but after deuterium exchange, in 0.1 *M* NaCl–0.05 *M* phosphate buffer in D_2O, pH (meter reading) 6.7. The peak at about 114 ppm arises from free HCN which is in fast exchange with about 0.5% free CN^-.

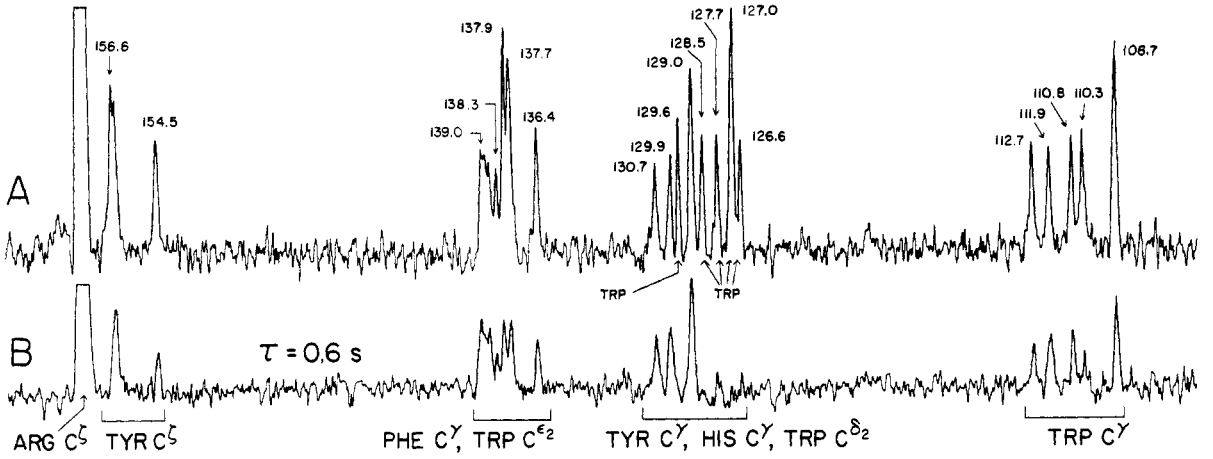

Figure 3. Aromatic region in the convolution-difference noise-modulated off-resonance proton-decoupled ^{13}C Fourier transform nmr spectra of 15 m*M* hen egg white lysozyme (in 0.1 *M* NaCl, pH 3.3) recorded at 15.18 MHz and 36°: (A) normal spectrum (90° radiofrequency pulses only) after 16,384 accumulations with a recycle time of 2.105 sec, 9.6 hr total accumulation time, chemical shifts are given in ppm downfield from tetramethylsilane; (B) as spectrum A, except that this is a PRFT spectrum recorded with τ = 0.605 sec, 32,768 accumulations, recycle time of 2.715 sec, 25 hr total time. Vertical gain is one-half that of spectrum A, to compensate for the twofold increase in the number of accumulations.

values are in good agreement with experimental ones.[14] Measured values for $C^{\delta 2}$ of Trp residues are at least twice as long as those of C^{γ} of Tyr and His residues and remain unchanged when going from H_2O to D_2O.[14] If H_2O is the solvent, the T_1 values of $C^{\epsilon 2}$ of Trp residues are not easily distinguished from those of C^{γ} of Phe and His residues. However, theoretical considerations and experimental results indicate that, after replacement of $H^{\epsilon 1}$ of a Trp residue by deuterium, the T_1 of $C^{\epsilon 2}$ is measurably longer than the T_1 values of C^{γ} of Phe and His residues.[14]

Figure 2A shows the aromatic region of the normal convolution-difference ^{13}C nmr spectrum of horse heart cyanoferricytochrome *c*. Peak 2 is the two-carbon resonance of C^{ζ} of the two Arg residues.[14] The other 18 peaks are the resonances of the 18 nonprotonated aromatic carbons of amino acid residues.[14] Peaks 1 and 3–5 are the resonances of C^{ζ} of the four Tyr residues.[14] Peaks 6–11 are the resonances of $C^{\epsilon 2}$ of the lone Trp, C^{γ} of His-26, and C^{γ} of the four Phe residues.[14] Peaks 12–17 are the resonances of $C^{\delta 2}$ of Trp-59, C^{γ} of His-33, and C^{γ} of the four Tyr residues.[14] Peaks 18 and 19 have been assigned to C^{γ} of His-18 and Trp-59, respectively.[14] The 16 nonprotonated aromatic carbons of the heme yield narrow resonances in the ^{13}C spectrum of the diamagnetic ferrocytochrome *c* but not in the spectra of the paramagnetic ferricytochrome *c* and cyanoferricytochrome *c*.[6,14]

In Figure 2B we show a ^{13}C PRFT nmr spectrum of horse heart cyanoferricytochrome *c* in H_2O, recorded using an interval (τ) of 0.5 sec between each 180° radiofrequency pulse and the following 90° pulse. In a PRFT nmr spectrum, a resonance will appear negative if $\tau < T_1 \ln 2$, nulled if $\tau = T_1 \ln 2$, and positive if $\tau > T_1 \ln 2$.[10] Peaks 12–16 are positive in the PRFT nmr spectrum of Figure 2B, while peak 17 is nulled. On this basis, peak 17 is assigned to $C^{\delta 2}$ of Trp-59.

The PRFT nmr spectrum of Figure 2B does not identify the resonance of $C^{\epsilon 2}$ of Trp-59 (one of peaks 6–11). In Figure 2C we show a PRFT nmr spectrum of horse heart cyanoferricytochrome *c* in D_2O, recorded using the same τ value as with the sample in H_2O. Note that peak 9 is nulled while peaks 6–8, 10, and 11 are positive. On this basis peak 9 is assigned to $C^{\epsilon 2}$ of Trp-59. We recorded the normal ^{13}C nmr spectrum of the sample in D_2O, in order to make sure that it did not show anomalies such as a missing peak 9. It is of interest that we noticed deuterium isotope effects on the chemical shifts of some resonances. For example, the two-carbon resonance of C^{ζ} of the two Arg residues (peak 2) moves upfield by about 0.2 ppm when going from H_2O to D_2O solution. Details will be given elsewhere.[14]

In Figure 3A we show the aromatic region of the convolution-difference ^{13}C nmr spectrum of hen egg white lysozyme at pH 3.3. The region 126–131 ppm downfield from tetramethylsilane contains two two-carbon resonances (at 127.0 and 129.0 ppm) and six single-carbon resonances, for a total of ten carbons: C^{γ} of the lone His (residue 15), C^{γ} of the three Tyr residues, and $C^{\delta 2}$ of the six Trp residues.[5] In the PRFT nmr spectrum of Figure 3B five of these peaks (representing a total of six carbons) are nulled, while three peaks (four carbons) are positive. We assign the resonances that are nulled to $C^{\delta 2}$ of the six Trp residues, a result that is consistent with assignments based on other methods.[12]

The γ-carbons of the three Phe residues and the ϵ_2-carbons of the six Trp residues of hen egg white lysozyme give rise to the single-carbon resonance at 136.4 ppm and the cluster of peaks in the range 137.7–139.0 ppm[5] (Figure 3A). The identification of the resonances of $C^{\epsilon 2}$ of the Trp residues will be presented elsewhere.[14]

Acknowledgment. This work was supported by the National Science Foundation (Grant GP-40688X), the United States Public Health Service (Grant NS-10977-02), and by Eli Lilly and Company.

References and Notes

(1) P. C. Lauterbur, *Appl. Spectrosc.*, **24**, 450 (1970); A. Allerhand, D. W. Cochran, and D. Doddrell, *Proc. Nat. Acad. Sci. U. S.*, **67**, 1093 (1970); J. C. W. Chien and J. F. Brandts, *Nature (London), New Biol.*, **230**, 209 (1971); F. Conti and M. Paci, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **17**, 149 (1971); V. Glushko, P. J. Lawson, and F. R. N. Gurd, *J. Biol. Chem.*, **247**, 3176 (1972); R. B. Moon and J. H. Richards, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2193 (1972); A. M. Nigen, P. Keim, R. C. Marshall, V. Glushko, P. J. Lawson, and F. R. N. Gurd, *J. Biol. Chem.*, **248**, 3716 (1973); J. C. W. Chien and W. B. Wise, *Biochemistry*, **12**, 3418 (1973); D. A. Torchia and K. A. Piez, *J. Mol. Biol.*, **76**, 419 (1973); M. H. Freedman, J. R. Lyerla, Jr., I. M. Chaiken, and J. S. Cohen, *Eur. J. Biochem.*, **32**, 215 (1973); N. A. Matwiyoff and B. F. Burnham, *Ann. N. Y. Acad. Sci.*, **206**, 365 (1973); J. H. Bradbury and R. S. Norton, *Biochim. Biophys. Acta*, **328**, 10 (1973); R. B. Visscher and F. R. N. Gurd, *J. Biol. Chem.*, in press.

(2) E. L. Packer, H. Sternlicht, and J. C. Rabinowitz, *Proc. Nat. Acad. Sci. U. S.*, **69**, 3278 (1972); *Ann. N. Y. Acad. Sci.*, **222**, 824 (1973); D. T. Browne, G. L. Kenyon, E. L. Packer, D. M. Wilson, and H. Sternlicht, *Biochem. Biophys. Res. Commun.*, **50**, 42 (1973); D. T. Browne, G. L. Kenyon, E. L. Packer, H. Sternlicht, and D. M. Wilson, *J. Amer. Chem. Soc.*, **95**, 1316 (1973); M. W. Hunkapiller, S. H. Smallcombe, D. R. Whitaker, and J. H. Richards, *J. Biol. Chem.*, **248**, 8306 (1973); M. W. Hunkapiller, S. H. Smallcombe, D. R. Whitaker, and J. H. Richards, *Biochemistry*, **12**, 4732 (1973).

(3) R. B. Moon and J. H. Richards, *J. Amer. Chem. Soc.*, **94**, 5093 (1972); A. M. Nigen, P. Keim, R. C. Marshall, J. S. Morrow, and F. R. N. Gurd, *J. Biol. Chem.*, **247**, 4100 (1972); D. J. Saunders and R. E. Offord, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **26**, 286 (1972); N. A. Matwiyoff, P. J. Vergamini, T. E. Needham, C. T. Gregg, J. A. Volpe, and W. S. Caughey, *J. Amer. Chem. Soc.*, **95**, 4429 (1973); C. H. Fung, A. S. Mildvan, A. Allerhand, R. Komoroski, and M. C. Scrutton, *Biochemistry*, **12**, 620 (1973); A. M. Nigen, P. Keim, R. C. Marshall, J. S. Morrow, R. A. Vigna, and F. R. N. Gurd, *J. Biol. Chem.*, **248**, 3724 (1973); J. S. Morrow, P. Keim, R. B. Visscher, R. C. Marshall, and F. R. N. Gurd, *Proc. Nat. Acad. Sci. U. S.*, **70**, 1414 (1973); P. J. Vergamini, N. A. Matwiyoff, R. C. Wohl, and T. Bradley, *Biochem. Biophys. Res. Commun.*, **55**, 453 (1973); E. Antonini, M. Brunori, F. Conti, and G. Geraci, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **34**, 69 (1973); R. G. Shulman, S. Ogawa, A. Mayer, and C. L. Castillo, *Ann. N. Y. Acad. Sci.*, **222**, 9 (1973); S. H. Koenig, R. D. Brown, T. E. Needham, and N. A. Matwiyoff, *Biochem. Biophys. Res. Commun.*, **53**, 624 (1973); I. M. Chaiken, J. S. Cohen, and E. A. Sokoloski, *J. Amer. Chem. Soc.*, **96**, 4703 (1974); I. M. Chaiken, *J. Biol. Chem.*, **249**, 1247 (1974); R. B. Moon and J. H. Richards, *Biochemistry*, **13**, 3437 (1974); R. B. Moon, M. J. Nelson, J. H. Richards, and D. F. Powars, *Physiol. Chem. Phys.*, **6**, 31 (1974); G. Robillard, E. Shaw, and R. G. Shulman, *Proc. Nat. Acad. Sci. U. S.*, **71**, 2623 (1974); H. M. Miziorko and A. S. Mildvan, *J. Biol. Chem.*, **249**, 2743 (1974); D. C. Harris, G. A. Gray, and P. Aisen, *J. Biol. Chem.*, **249**, 5261 (1974); C. F. Brewer, H. Sternlicht, D. M. Marcus, and A. P. Grollman, *Biochemistry*, **12**, 4448 (1973).

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

(5) A. Allerhand, R. F. Childers, and E. Oldfield, *Biochemistry*, **12**, 1335 (1973).

(6) E. Oldfield and A. Allerhand, *Proc. Nat. Acad. Sci. U. S.*, **70**, 3531 (1973).

(7) A. Allerhand, R. F. Childers, and E. Oldfield, *Ann. N. Y. Acad. Sci.*, **222**, 764 (1973).

(8) A. Allerhand, R. F. Childers, and E. Oldfield, *J. Magn. Resonance*, **11**, 272 (1973).

(9) V. G. Glushko, Ph.D. Thesis, Indiana University, 1972.

(10) R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, **48**, 3831 (1968).

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

(12) R. S. Norton, R. F. Childers, and A. Allerhand, unpublished results.

(13) E. Wenkert, A. O. Clouse, D. W. Cochran, and D. Doddrell, *J. Amer. Chem. Soc.*, **91**, 6879 (1969).

(14) E. Oldfield, R. S. Norton, and A. Allerhand, to be submitted for publication.

(15) I. D. Campbell, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J. Magn. Resonance*, **11**, 172 (1973); *Ann. N. Y. Acad. Sci.*, **222**, 163 (1973).

(16) A. Allerhand and D. Doddrell, *J. Amer. Chem. Soc.*, **93**, 2777 (1971); D. Doddrell and A. Allerhand, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1083 (1971).

(17) "Tables of Interatomic Distances and Configuration in Molecules and Ions," *Chem. Soc., Spec. Publ.*, **No. 11** (1958); Suppl., **No. 18** (1965); R. W. G. Wyckoff, "Crystal Structures," 2nd ed, Vol. 6, Part 1, Interscience, New York, N.Y., 1969.

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A Convenient Preparation of Solutions of Superoxide Anion and the Reaction of Superoxide Anion with a Copper(II) Complex

Sir:

In recent years, metalloenzymes containing copper and zinc,[1,2] manganese,[3-5] and iron[5,6] have been discovered which catalyze the disproportionation of superoxide anion, O_2^-, to molecular oxygen and hydrogen peroxide.[7,8] In attempting to understand the mechanism of these superoxide dismutase enzymes, it would be useful to know how superoxide anion reacts with transition metal complexes but there is little information about this chemistry, probably because of the inconvenience of the electrochemical preparation used previously.[1,9-16]

We wish to report a convenient preparation of solutions of superoxide in aprotic solvents and a reaction of dissolved superoxide with a complex of copper(II) which illustrates the effect of protons in changing the relative redox potentials of oxygen and superoxide. This effect may be important in determining enzymatic mechanisms.

Potassium superoxide, KO_2, is sparingly soluble in dry dimethyl sulfoxide (DMSO).[10,17] A 0.30 *M* solution of dicy-