Paramagnetic Resonance of the Free Radical Intermediates in the Reaction of OH with Oximes

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Abstract: The reaction of the OH radical, produced by the chemical reaction of H_2O_2 with $TiCl_3$ in dilute sulfuric acid solution, with alkyl oximes was studied with epr to determine the effect of nitrogen lone-pair electrons adjacent to a double bond. In every case the β-hydroxy nitroxide was formed by the addition of OH to the oxime double bond. The hyperfine coupling constants of the β protons in the series of β-hydroxy nitroxides indicated hindered internal rotation about the C–N bond.

Both chemical[1] and photolytic[2] methods have been used to generate high concentrations of OH radicals from H_2O_2 for reaction with other molecules in flow systems passing through electron paramagnetic resonance (epr) cavities. The low pH required for the existence of H_2O_2 in solution results in protonation of most nitrogen-containing molecules. The resulting positive charge in the molecule profoundly affects the site of proton abstraction by the OH radical.[3]

The reaction of OH with oximes is interesting because oximes have readily protonated, nitrogen lone-pair electrons adjacent to a double bond. Several different reaction paths could be hypothesized on the basis of the epr studies of other OH reactions: the abstraction of a β proton[4] in the aldoximes, as with alcohols;[2] the abstraction of the hydroxyl proton to form an iminoxy radical;[5] or the addition to the double bond, as with olefins.[1] Protonation of the nitrogen lone-pair electrons would be expected to inhibit bonding of OH to the nitrogen atom, if the OH added to the double bond.

The epr spectra of the free radical intermediates formed in the OH–oxime reaction in acid solution indicate that β-hydroxy nitroxides, which have not been previously observed, are formed in every case. The characteristic quartet of the -NH–O nitroxide group with 13-gauss hyperfine splitting and 1:2:2:1 intensity ratio, previously observed in alkyl nitroxides,[6] is found in all the OH–oxime spectra. The hyperfine coupling constants of the β protons in the series of β-hydroxy nitroxides suggest hindered internal rotation about the C–N bond.

Experimental Section

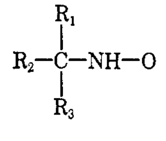
Radical Production. The radicals were generated in a two-stream, fast-mixing flow system described previously.[7] The OH radicals, produced by the chemical reaction of H_2O_2 with $TiCl_3$ in dilute sulfuric acid solution,[1] were allowed to react with formaldoxime, acetaldoxime, or acetone oxime.

Materials. The formaldoxime hydrochloride, acetaldoxime, and acetone oxime were obtained from K and K Laboratories, Columbia Organic Chemicals, and Eastman Organic Chemicals, respectively, and were used without further purification. The 20% $TiCl_3$ was obtained from Matheson Coleman and Bell and the 3% H_2O_2 from J. T. Baker Chemical Co.

Epr Measurements. The epr measurements were made with a standard Varian X-band epr spectrometer using potassium peroxylamine disulfonate in aqueous solution as a reference standard for the hyperfine splitting intervals.[7] Computer simulations of the epr spectra were performed by a FORTRAN IV program on an IBM 360/65. The epr spectra were adequately represented by the usual isotropic high-field spin-Hamiltonian.

Results

Figures 1–3 are the first-derivative epr spectra obtained from the reaction of OH with acetone oxime, acetaldoxime, and formaldoxime, respectively. The radicals can be definitely identified as nitroxides of the type



$$R_2\text{—}\overset{\overset{\displaystyle R_1}{|}}{\underset{\underset{\displaystyle R_3}{|}}{C}}\text{—NH—O}$$

on the basis of previous epr studies of simple iminoxy and nitroxide radicals.[5,6] The radicals cannot be iminoxy radicals, formed by abstracting the proton from the -NOH, since the iminoxy ^{14}N hyperfine coupling constants, 29–33 gauss,[5] are far greater than any observed in this study. The radical species are very likely $(CH_3)_2C(OH)NHO$ from acetone oxime, $CH_3CH(OH)NHO$ from acetaldoxime, and $CH_2(OH)NHO$ from formaldoxime, where the OH has bonded to the carbon atom by adding to the double bond.

Acetone Oxime. The spectrum of the radical from acetone oxime (Figure 1) shows a characteristic 13.4-gauss -NH–O hyperfine splitting, found previously in CH_3NHO,[6] in which both the nitrogen and hydrogen hyperfine couplings are identical. A quartet pattern with intensity ratios 1:2:2:1 is produced. The hyperfine splitting of the γ protons should not be greater than 0.5 gauss[8] and could not be resolved in the experimental spectra. The γ-proton hyperfine splitting could not be resolved in the alkyl nitroxides previously studied.[6] The half-life of this radical was, roughly, 1 sec in 0.5 M H_2SO_4 when the flow through the reaction cell was cut off.

Acetaldoxime. The spectrum of the radical from acetaldoxime (Figure 2) consists of two 1:2:2:1 -NHO

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(1) W. T. Dixon and R. O. C. Norman, *J. Chem. Soc.*, 3119 (1963).

(2) R. Livingston and H. Zeldes, *J. Chem. Phys.*, **44**, 1245 (1966).

(3) W. T. Dixon and R. O. C. Norman, *J. Chem. Soc.*, 4850 (1964).

(4) The proton position nomenclature is the usual one for nitrogen radicals, namely

$$\text{—}\underset{\gamma}{CH_2}\text{—}\underset{\beta}{CH_2}\text{—}\underset{\alpha}{NH}\text{—O}$$

(5) J. R. Thomas, *J. Am. Chem. Soc.*, **86**, 1446 (1964).

(6) J. Q. Adams, S. W. Nicksic, and J. R. Thomas, *J. Chem. Phys.*, **45**, 654 (1966).

(7) J. Q. Adams and J. R. Thomas, *ibid.*, **39**, 1904 (1963).

(8) R. W. Fessenden and R. H. Schuler, *ibid.*, **39**, 2147 (1963).

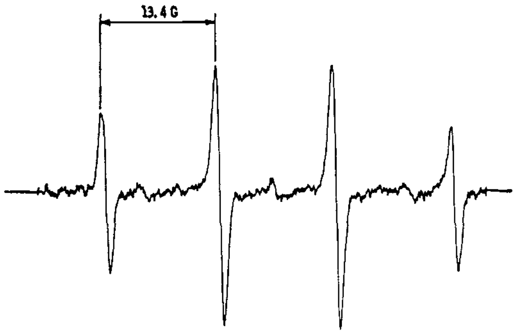


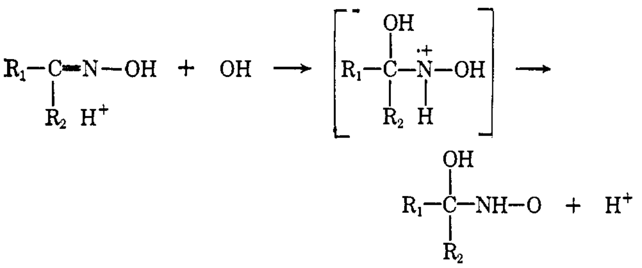
Figure 1. First-derivative epr spectrum of the free radical formed in the reaction of OH with acetone oxime: $(CH_3)_2C(OH)NHO$.

quartets (13.2-gauss hyperfine splitting), which must be produced by an additional 8.8-gauss hyperfine coupling to a nucleus of spin $^1/_2$, the β proton. The γ-proton coupling could not be resolved. This radical also had, roughly, a 1-sec half-life in 0.5 M H_2SO_4.

Formaldoxime. The spectrum of the radical from formaldoxime (Figure 3A) can be resolved into three 1:2:2:1 -NHO quartets (13.0-gauss hyperfine splitting) with relative intensity 1:2:1, which must be produced by an additional 7.8-gauss hyperfine coupling to two equivalent nuclei of spin $^1/_2$, the two β protons. Since this spectrum could not be completely resolved, it was synthesized by computer from the coupling constants and the observed line width. The agreement between the computed spectrum (Figure 3B) and the experimental spectrum is satisfactory. This radical was very unstable in 0.5 M H_2SO_4, having a half-life less than 0.1 sec. The epr spectrum of the radical could be resolved satisfactorily only after the H_2SO_4 concentration was lowered to 0.05 M. Then the radical half-life was, roughly, 1 sec.

Discussion

Reaction Mechanism. The postulated structures for the radicals from the oximes follow directly from a very simple reaction mechanism. In acid solution the nitrogen lone-pair electrons would be protonated, leading to the mechanism



$$R_1-\underset{R_2\ \ H^+}{C}=N-OH + OH \longrightarrow \left[R_1-\overset{OH}{\underset{R_2}{C}}-\overset{\cdot+}{\underset{H}{N}}-OH \right] \longrightarrow R_1-\overset{OH}{\underset{R_2}{C}}-NH-O + H^+$$

where protonation has blocked the bonding of the ·OH to the nitrogen atom. The path of the reaction in the absence of protonation would be very interesting. Unfortunately, presently known techniques for producing high concentrations of OH radicals preclude such a study.

Hindered Internal Rotation. The observed hyperfine coupling constants for β protons have been successfully explained by the McConnell relation[9]

$$a_\beta{}^H = Q(\theta)\rho_N{}^\pi \qquad (1)$$

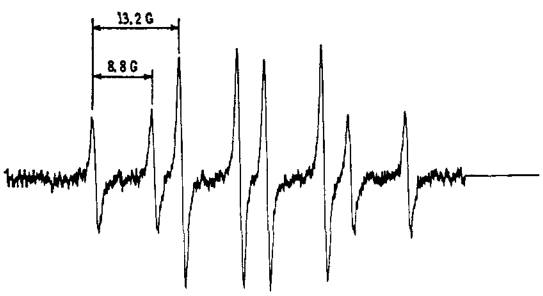


Figure 2. First-derivative epr spectrum of the free radical formed in the reaction of OH with acetaldoxime: $CH_3CH(OH)NHO$.

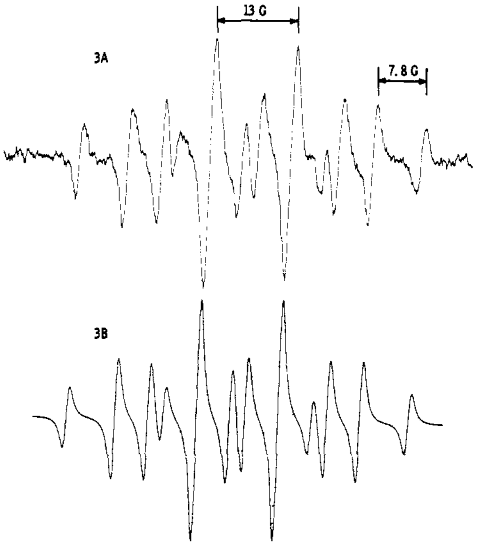


Figure 3. First-derivative epr spectra of the free radical formed in the reaction of OH with formaldoxime: $CH_2(OH)NHO$: (3A) experimental spectrum; (3B) computed spectrum.

where $\rho_N{}^\pi$ is the spin density in the p_z orbital of the nitrogen atom, and $Q(\theta)$ is given by the Heller and McConnell relation[10]

$$Q(\theta) = B_0 + B_2 \cos^2 \theta \qquad (2)$$

In (2) θ is the angle between the axis of the p_z orbital and the C–H bond, both projected onto a plane perpendicular to the C–N bond. Current theory suggests that the value of B_0 is very small compared to the value of B_2.[11] Consequently, (2) can be simplified to

$$Q(\theta) = B \cos^2 \theta \qquad (3)$$

If internal rotation occurs in the free radical, (1) must be modified to

$$a_\beta{}^H = \langle Q(\theta)\rangle \rho_N{}^\pi \qquad (4)$$

where $\langle Q(\theta)\rangle$ is the quantum-mechanical average over the statistically weighted internal rotational states of the system. In this circumstance clearly, from (3) and (4)

$$a_\beta{}^H = B\langle \cos^2 \theta\rangle \rho_N{}^\pi \qquad (5)$$

In the case of free rotation $\langle\cos^2 \theta\rangle = {}^1/_2$. In the case of hindered rotation, the value of $\langle\cos^2 \theta\rangle$ can be greater or less than $^1/_2$, depending upon the internal rotational states of the system and their statistical weights.

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(9) H. M. McConnell, *J. Chem. Phys.*, **24**, 632, 764 (1956).

(10) C. Heller and H. M. McConnell, *ibid.*, **32**, 1535 (1960).

(11) J. P. Colpa and E. DeBoer, *Mol. Phys.*, 7, 333 (1964).

The change in the hyperfine coupling constants of the β protons in the series CH_3NHO (I), $CH_2(OH)NHO$ (II), and $CH_3CH(OH)NHO$ (III) is characteristic of hindered internal rotation. The value of a_α^H, the hyperfine coupling constant of the α protons, in these radicals should be a good measure of ρ_N^π, since a McConnell relation like (1) without a θ dependence also apparently holds for a_α^H.[6] In methanol solution the hyperfine coupling constants of all protons and the nitrogen atom in CH_3NHO are 13.8 gauss. The change in a_β^H in the series I → II → III → is not linearly proportional to the change in a_α^H. Consequently, the simplest explanation is that $\langle\cos^2\theta\rangle$ changes in the series I → II → III. Similar effects in alkyl nitro anion radicals have been ascribed to hindered internal rotation.[12]

The low symmetry of II and III prevents their treatment after the fashion of Stone and Maki, who were able to use $1 - \cos 2\theta$ internal rotation potential barriers for the alkyl nitro anion radicals.[12] Radicals II and III should probably be assigned a threefold potential barrier with unequal potential barrier maxima.

Acknowledgments. The author acknowledges with pleasure the many helpful discussions with Dr. J. R. Thomas and the continued encouragement of Dr. R. L LeTourneau and Dr. L. P. Lindeman.

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[12] E. W. Stone and A. H. Maki, *J. Chem. Phys.*, 37, 1326 (1962).

Slow Hydrogen–Deuterium Exchange in a Non-α-helical Polyamide

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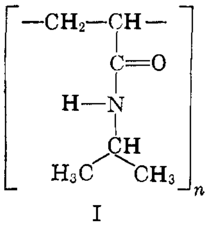
Abstract: The kinetics of hydrogen–deuterium exchange was measured in a polymeric amide, poly-N-isopropylacrylamide, in D_2O solutions. The intrinsic viscosity of the polymer corresponds to that of a swollen, unordered macromolecule. Infrared spectra indicate about one-third of the pendant N–H groups are not hydrogen bonded to carbonyls. Despite the presence of two states of the N–H group only one first-order exchange process is seen. Furthermore the rate constant k is the same whether computed from disappearance of free N–H or bonded N–H $\cdots$O=C, or from appearance of O–H. Exchange behavior of the polymer is similar to that of comparable small amides in regard to catalysis by D^+ and OD^-, activation energy (20 kcal/mole), and pD_{min} of minimum exchange rate. However, the minimum rate constant, k_{min}, is $^1/_{100}$th that of small amides. This markedly reduced exchange rate of an amide group attached to this macromolecule can be interpreted in terms of a change in K_w, the self-dissociation constant of water, in the neighborhood of the pendant residues of the polymer.

One of the techniques that has been widely used to examine protein conformations in solution is hydrogen–deuterium exchange. In native proteins, at least some peptide hydrogens exchange slowly with the aqueous solvent,[1,2] and the rates of exchange are markedly affected by the solvent environment.[3,4] Nevertheless, it is still not clear how much of the decrease in exchange rates is due to locking in of the exchangeable hydrogens in N—H$\cdots$O=C bonds and how much to interactions of side chains with each other or with solvent.[4]

One method of trying to assess the relative importance of different factors in slowing down hydrogen–deuterium exchange is to examine this reaction in a polymeric amide unable to form α-helical conformations but containing fairly large pendant apolar groups. Detailed studies have now been carried out with one such polymer, polyisopropylacrylamide (I), in which the C=O and N–H groups are *not* part of the backbone of the macromolecule. The behavior of this polymer shows some remarkable similarities to that of α-helical polypeptides.



I

Experimental Section

Materials and Methods. N-Isopropylacrylamide was obtained from American Cyanamid Co. and was recrystallized repeatedly from a toluene–hexane mixed solvent. Dioxane was purified by the method of Fieser[5] and was stored over sodium. It was always freshly distilled before use. Chloroform was first washed with water to remove the ethanol impurity and was refluxed over P_2O_5 before distillation. It was kept in a black bottle and used without delay in order to avoid formation of phosgene. Heavy water was purchased from Bio-Rad Laboratories and was warranted to be 99.84% D_2O.

N-Isopropylpropionamide was prepared from N-isopropylacrylamide by dissolving the latter in 95% ethanol and hydrogenating over 10% platinum oxide. When hydrogenation was complete the catalyst was filtered off, the ethanol was removed by rotary evaporation, and the residue was dissolved in ethyl acetate.

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[1] H. Lenormant and E. R. Blout, *Nature*, 172, 770 (1953).

[2] A. Hvidt and K. Linderstrom-Lang, *Biochim. Biophys. Acta*, 14, 574 (1954).

[3] A. Hvidt and S. O. Nielsen, *Advan. Protein Chem.*, 21, 287 (1966).

[4] B. H. Leichtling and I. M. Klotz, *Biochemistry*, 5, 4026 (1966).

[5] L. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1957, p 284.