Substituent Effect On The Stability Of The Hydroxyl Radical Adduct Of α-Phenyl N-*tert*-Butyl Nitrone (PBN)

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Abstract: α -Hydroxy-(3- and 4-substituted)-benzyl tert-butyl aminoxyl radicals, the hydroxyl radical spin adducts of 3and 4-substituted α -phenyl N-tert-butyl nitrones (R-PBN's), decay rapidly (in seconds) by first order kinetics in acetonitrile (ACN). The Hammett equation is obeyed with $\rho = -1.36$ and r = 0.90. Polar solvents produce an increase in rate of decay: water (fast) > ACN (fast) > benzene (slow). Thus substantial charge separation in the transition state is indicated. The mechanism proposed is a unimolecular cleavage reaction producing the substituted benzaldehyde and tert butyl nitroso anion radical:

$$\begin{array}{c} & & & & & \\ RC_{6}H_{4}-CH(OH)-N-C_{4}H_{9} \rightarrow \left\{ \begin{array}{c} \oplus & & & & \\ RC_{6}H_{4}CH(OH) & NC_{4}H_{9} \\ \oplus \end{array} \right\} \rightarrow R-C_{6}H_{4}-CHO + H-NC_{4}H_{9} \\ \end{array}$$

Support for this mechanism is provided by the detection of tert-butyl hydroaminoxyl when the decay is studied in water.

Previously we have studied the substituent effect on the absolute rate constants of spin trapping benzoyloxyl¹ and *tert*-butoxyl² radicals in benzene by 3- and 4-substituted α -phenyl N*tert*-butyl nitrones (R-PBN's). Relatively small substituent effects were found, indicating that the rate of addition of oxyl radicals is not strongly affected by polar influences on the transient state.

Here we report on the first study of a substituent effect on the decay rate of a PBN spin adduct. The hydroxyl adduct was selected since the hydroxyl radical is considered to be perhaps the most damaging of all radicals in biological systems. Also, dependable hydroxyl radical detectors are still being sought^{3,4} and a knowledge of the mechanism of decay of spin adducts will allow better synthetic strategies to be formulated for new spin traps.





Figure 1(a) top: EPR spectrum of hydroxyl adduct of 3-CI PBN in ACN; (b) bottom: EPR mixture spectrum of the hydroxyl and hydroperoxyl adducts of 3-Me PBN in ACN.

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The decay kinetics of the hydroxyl adduct of PBN in aqueous phosphate buffer has been found to be 1st order.⁵ The half-life is approximately 1 minute and depends on pH.⁵ Since 3- and 4 substituted PBN's are almost insoluble in water we have selected acetonitrile (ACN) for a study of the substituent effect on the decay of the hydroxyl adduct of PBN. Thus brief photolysis (~1 second) of hydrogen peroxide (~1%) in ACN containing 0.04 M 3-Cl-PBN produces the EPR spectrum shown in Figure 1a. Conditions were selected so that only one spin adduct is observed. Based on its hyperfine splitting constants (hfsc's), the spectrum was assigned to the hydroxyl adduct of PBN.⁶ However, with many substituted PBN's a mixture of two spectra was observed (Figure 1b), assigned to the hydroxyl and hydroperoxyl adducts on the basis of their hfsc's.⁶ The hydroperoxyl adducts have smaller hfsc's than the hydroxyl adducts of PBN if recorded in the same solvent ⁶ (see Table 1). Both the N- and β -H hfsc's correlate well with Hammett's sigma constant ⁷ and this provides additional support

for the assignments. The detection of the hydroxyl hydrogen hfsc in the spectrum of the hydroxyl adduct is a more dependable basis for assignment⁵ but this feature could not be resolved for the hydroxyl adduct of PBN in ACN.

$$\begin{array}{cccc} & & & & & & \\ H_2O_2 & \rightarrow & 2HO \bullet & & \\ & & & & \\ & & & & \\ & & & & \\ R - C_6H_4 - CH - N - C_4H_9 + & HO \bullet \rightarrow R & C_6H_4 - CH(OH) - N - C_4H_9 \end{array}$$

$$HO_{1} + H_{2}O_{2} \rightarrow H_{2}O + HOO_{2}$$

$$\begin{array}{c} O \\ \uparrow \\ R - C_6H_4 - CH - N - C_4H_9 + HOO \rightarrow R - C_6H_4 - CH(OOH) - N - C_4H_9 \end{array}$$

Group	σ	HO.		HOO	
		a ^N	${}^{a}_{\beta}^{H}$	a ^N	a^{H}_{β}
4-MeO	-0.27	14.86	2.83	13.96†	2.341
4-Me	0.17	14.80	2.83	13.73	2.31
3-Me	-0.07	14.80	2.74	13.69	2.41
Н	0	14.76	2.75	13.69	2.30
3-MeO	0.12	14.75	2.73	13.66	2.25
4-Br	0.23	14.74	2.63	13.65	2.09
4-Cl	0.23	14.58	2.34	13.55	2.05
3-Cl	0.37	14.52	2.29	13.56	1.97
3-Br	0.39	14.68	2.43	13,52	2.04
4-CN	0.66	14.45	2.06	13.42	1.84
3-NO2	0.71	14.58	2.04	13.77†	2.08
4-NO2	0.78	14.41	2.06	13.41	1.79
	ρ	-0.025	-0.36	-0.026	-0.32
	r	0.90	0.95	0.97	0.97

Table 1. Hyperfine Splitting Constants (in Gauss)* for Hydroxyl and Hydroperoxyl Adducts of R-PBN's in Acetonitrile at Room Temperature Compared to Hammett's Sigma Substituent Constant**

*Error in the hfsc's is ± 0.05 Gauss.

** See reference 8.

 \dagger These values are not included in the calculations of ρ and τ as they are based on an insufficient number of data points. Hyperfine splitting constants could not be obtained for 4 CF₃-PBN due to the presence of di-*tert*-butyl nitroxide.



Figure 2. Decay plot of EPR peak from the hydroxyl adduct of 3 nitro-PBN; vertical scale is in arbitrary chart paper units and horizontal scale is in seconds (~84 seconds full scan).

The decay profile for the hydroxyl adduct of a substituted PBN is shown in Figure 2. At higher concentrations of spin adduct in aqueous systems decay is 2nd order indicating disproportionation⁵. However, at sufficiently low concentrations the decay is cleanly 1st order. Only first order decay was seen here. The half-life depends on the substituent in the meta or para position. Electron-donor groups increase the rate of decay while electron-withdrawing substituents decrease the rate of decay. A plot of ln k/k₀ (where k = first order rate constant for decay of substituted PBN/hydroxyl radical adduct, and k₀ = first order rate constant for



Figure 3. Hammett plot for decay rates of the hydroxyl adduct of substituted PBN's using EPR peak height in arbitrary units in acetonitrile at room temperature.

Group	σ	k*	in k/k _c
4-MeO	-0.27	13.88	0.78
4-Me	-0.17	7.14	0.11
3-Me	-0.07	5.50	-0.15
Н	0	6.37	0
3-MeO	0.12	8.07	0.25
4-Br	0.23	3.69	-0.55
4-Cl	0.23	4.58	-0.33
3-Cl	0.37	4.52	-0.34
3-Br	0.39	3.20	-0.69
4-CF3	0.54	3.58	-0.58
4-CN	0.66	2.09	-1.12
3-NO2	0.71	2.77	-0.84
4-NO ₂	0.78	2.25	-1.04

Table 2. Rates of Decay of Hydroxyl Radical Spin Adducts of Substituted PBN's at 25 °C Compared to Hammett's Sigma Substituent Constant

*x 10^{12} s⁻¹. Error in k is ± 0.6 s⁻¹. A linear regression gave $\rho = -1.36$ and r = 0.90.

decay of PBN/hydroxyl radical adduct) vs Hammett's sigma constant, shows considerable scatter (r = 0.90) but the trend is clear (Figure 3). The slope is $\rho = -1.36$ (see Table 2). On the basis of this data we conclude that the carbon-nitrogen bond is considerably weakened by the presence of a hydroxy group in the α -position of the aminoxyl radical and that in the transition state substantial polarity is developed with the benzylic carbon assuming a partial positive charge:

$$\begin{array}{c} O \\ R \\ - C_{6}H_{4} \\ - CH(OH) \\ - N \\ - C_{4}H_{9} \\ - C_{4}H_{9} \\ - C_{6}H_{4} \\ - C_{6}H_{4} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{4}H_{9} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{4}H_{9} \\ - C_{4}H_{9} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{4}H_{9} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{4}H_{9} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{4}H_{9} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{6}H_{9} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{6}H_{9} \\ - C_{6}H_{4} \\ - C_{1}O \\ - C_{6}H_{9} \\ - C_{6}H_{$$

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