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Spin Trapping with 5,5-Dimethylpyrroline-N-oxide in Aqueous Solution

YUTAKA KIRINO,*,[a] TAKA'AKI OHKUMA,[a] and TAKAO KWAN[b]

Faculty of Pharmaceutical Sciences, The University of Tokyo,[a] Hongo, Bunkyo-ku, Tokyo 113, Japan and Faculty of Pharmaceutical Sciences, Teikyo University,[b] Sagamiko-cho, Tsukui-gun, Kanagawa 199-01, Japan

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Spin trapping with 5,5-dimethylpyrroline-N-oxide (DMPO) of various radicals was carried out in aqueous solutions and the ESR parameters for the spin adduct radicals were determined. Comparison of hyperfine coupling constants obtained in aqueous solutions and in benzene revealed that considerable solvent effect exists. For the radical obtained by the addition of a hydroxyl radical to DMPO, the hyperfine coupling constant was found to depend on the basicity of the solution. This phenomenon was analyzed in terms of the acid dissociation equilibrium of the hydroxyl proton of the adduct radical, and the pK value was determined to be 12.96.

Keywords——ESR; spin trapping; 5,5-dimethylpyrroline-N-oxide; *in situ* photolysis; acid dissociation

Application of the spin trapping technique to aqueous systems is becoming of greater importance in the field of free radical biology. For example, spin trapping has provided considerable support for the participation of oxygen radicals in the degradation of DNA under the influence of bleomycin and has contributed to elucidation of the mechanism of the antitumor action of the antibiotic.[1)] Direct evidence for the involvement of radicals in lipid peroxidation[2)] and photodegradation of chlorophylls[3)] was also obtained by this method. In the preceding paper[4)] we examined some physico-chemical properties of three nitrones and a nitroso compound in aqueous solutions, and found that 5,5-dimethylpyrroline-N-oxide (DMPO) is suitable for use in aqueous solutions. In organic solvent systems, spin trapping reactions employing this compound have been widely investigated and the ESR parameters of the spin adducts are well-documented.[5)] In aqueous media, however, only a limited number of radicals have been trapped[2,3,6)] and the documentation of the ESR parameters, which depend on solvent composition in general, is poor. In the present study, spin trapping with DMPO of known radicals derived from well-established sources has been carried out in aqueous solutions, and the ESR spectral parameters and some properties of the spin adducts are presented here.

Experimental

DMPO was prepared and purified as described previously.[4)] Other chemicals used were of the highest grade commercially available. ESR spectra were obtained with a JEOL PE-1X (X band) spectrometer equipped with a cylindrical TE_{011} mode cavity and 100 kHz field modulation. Hyperfine coupling constants were determined by comparison with that (86.9 G) of Mn^{2+} ions diffused in solid MgO. 1,1-Diphenyl-2-picrylhydrazyl (DPPH, $g=2.00359$) was used as a standard for g factor.

The primary radical-producing reaction utilized was photolysis of photo-labile compounds such as peroxydisulfate, sulfite, and hydrogen peroxide. Secondary radicals were produced by reaction of the primary radical, $SO_4^{\overline{\cdot}}$, with appropriate compounds. All experiments were carried out by *in situ* photolysis with a flow system. A neutral or alkaline solution containing 2 mM spin trap and 5—30 mM $K_2S_2O_8$ (or 40 mM Na_2SO_3 or 0.29 M H_2O_2) with or without the addition of a suitable compound to produce a secondary radical was deoxygenated by bubbling N_2 gas through it. The solution was passed at a rate of 0.1—100 ml/min through a flat quartz cell (JEOL LC-11) mounted in the ESR cavity. The solution in the cell was irradiated with UV light through a quartz window of the cavity. The light source used was a 500 W ultra-high pressure mercury lamp. In order to absorb heat, a quartz cell filled with water (100 mm optical path length) was placed between the lamp and the cavity. The temperature of a sample solution was measured with a copper-constantan thermocouple 10 mm downstream from the illumination point, and the temperature increase caused by UV irradiation was found to be less than 4°.

For experiments at strongly alkaline pH, potassium hydroxide was used as a base. For solutions of pH <13.3, pH measurement was carried out with a Hitachi-Horiba model F-7 pH meter equipped with a Horiba 6028-10T combination electrode. For more concentrated KOH solutions, the concentration of the base was determined by titration. There are few basicity scales available above the pH scale for concentrated KOH solutions. In the present study, we employed the H_- function of Yagil,[7] which is based on the ionization of indoles. Below 8 M KOH, it is in good accord with the scale of Schwarzenbach and Sulzberger,[8] who used indigo dyes as indicators.

Results and Discussion

Radical Production

Established reactions were utilized to produce radicals to be trapped. Primary radicals were generated by the photolysis of photo-labile compounds such as peroxydisulfate, sulfite and hydrogen peroxide, as shown by equations 1—3.

$$S_2O_8^{2-} \longrightarrow 2\,SO_4^{\dot{-}}$$ (1)[9]

$$(SO_3^{2-}) \longrightarrow SO_3^{\dot{-}} + e_{aq}^{-}$$ (2)[10]

$$H_2O_2 \longrightarrow 2\cdot OH$$ (3)

where e_{aq}^{-} represents a hydrated electron. The hydroxyl radical can also be produced by oxidation of the hydroxide anion with the sulfate anion radical in alkaline solutions.[11]

$$OH^- + SO_4^{\dot{-}} \longrightarrow \cdot OH + SO_4^{2-}$$ (4)

Some radicals were produced secondarily by utilizing the hydrogen abstraction reaction of the sulfate radical, as shown below.

$$CH_3OH + SO_4^{\dot{-}} \longrightarrow \cdot CH_2OH + HSO_4^{-}$$ (5)

$$CH_3CH_2OH + SO_4^{\dot{-}} \longrightarrow \cdot CH_2CH_2OH + HSO_4^{-}$$ (6)[12]

$$(CH_3)_2CHOH + SO_4^{\dot{-}} \longrightarrow (CH_3)_2\dot{C}OH + HSO_4^{-}$$ (7)[10]

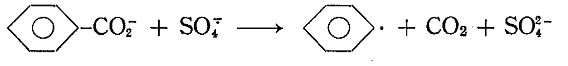
$$NH_3 + SO_4^{\dot{-}} \longrightarrow \cdot NH_2 + HSO_4^{-}$$ (8)[11]

$$HCO_2^- + SO_4^{\dot{-}} \longrightarrow CO_2^{\dot{-}} + HSO_4^{-}$$ (9)[11]

Other radicals were produced by one-electron oxidation with the sulfate anion radical, followed by decarboxylation.

$$^-O_2C\text{-}CO_2^- + SO_4^{\dot{-}} \longrightarrow CO_2^{\dot{-}} + CO_2 + SO_4^{-}$$ (10)[12]

$$CH_3CO_2^- + SO_4^{\dot{-}} \longrightarrow \cdot CH_3 + CO_2 + SO_4^{2-}$$ (11)[13]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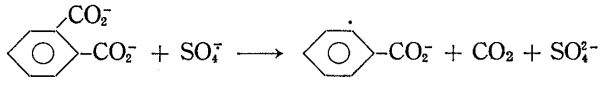
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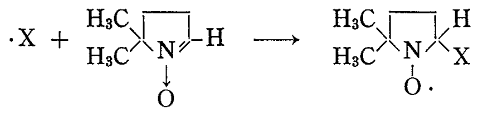
ESR Parameters of Spin Adducts

Spin trapping with DMPO is expressed by reaction 14.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The *in situ* photolysis of a solution containing 2 mM DMPO and 30 mM $K_2S_2O_8$ (pH 12.1, adjusted with KOH) gave the ESR spectrum shown in Fig. 1. The main component of the spectrum can be assigned to the $SO_4^{\dot{-}}$ adduct to DMPO ($X=SO_4^{-}$). ESR parameters are $a^N=13.82$ G, $a_\beta^H=10.10$ G, $a_\gamma^H=1.42$ G, $a_\gamma^H=0.83$ G, and $g=2.0059$. An interesting finding is that the hyperfine splittings due to two γ-protons are resolvable, and they are non-equivalent. The minor component of the spectrum is ascribable to the •OH adduct (X=•OH) where •OH has been produced by reaction 4.

Fig. 2 shows a spectrum obtained by UV-irradiation of a solution of 2 mM DMPO and 40 mM Na_2SO_3. Two component signals are recognized. One is that of the SO_3^- adduct ($X=SO_3^-$) and the other is nominally the H adduct (X=H), which is formed by the addition of e_{aq}^- to DMPO followed by protonation.

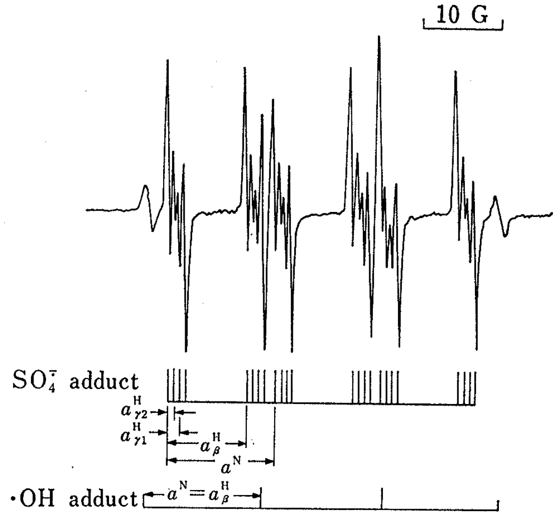


Fig. 1. ESR Spectrum obtained by UV-Irradiation of a Solution containing 2 mM DMPO and 30 mM $K_2S_2O_8$ at pH 12.1

The spectrum is displayed with the magnetic field increasing from left to right.

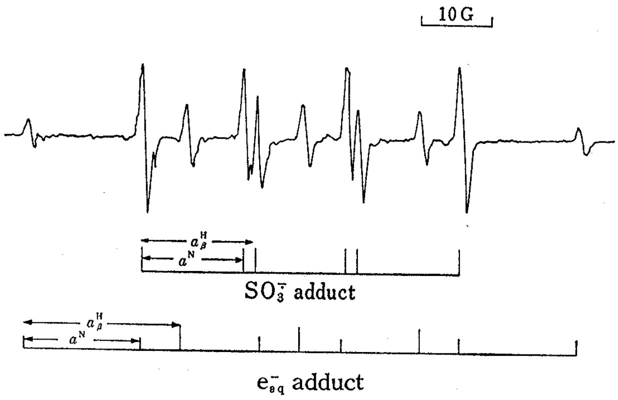
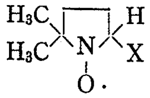
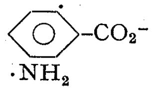


Fig. 2. ESR Spectrum observed during the UV-Irradiation of a Solution of 2 mM DMPO and 40 mM Na_2SO_3

TABLE I. ESR Parameters for Spin Adducts with DMPO



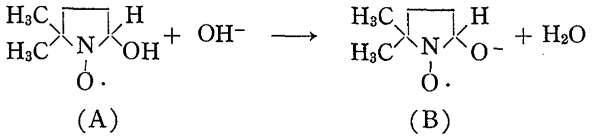
No.	X	Source reaction[a]	hf coupling const. (G)[b] N	hf coupling const. (G)[b] β-H	hf coupling const. (G)[b] Others	g factor[c]
1	SO_4^-	1	13.82	10.10	1.42(γ-H) 0.83(γ-H)	2.0059
2	SO_4^-	2	14.55	16.16		2.0055
3	$e_{aq}^- + H^+$	2	16.58	22.50[d]		2.0054
4	•OH (O^-	3, 4 4	14.90 16.20	14.90 16.20		2.0057 2.0057)
5	$\cdot CH_2OH$	5	15.87	22.57		2.0053
6	$\cdot CH_2CH_2OH$	6	15.98	22.83		2.0057
7	$(CH_3)_2\dot{C}OH$	7	15.92	23.66		2.0054
8	CO_2^-	9, 10	15.79	18.97		2.0054
9	$\cdot CH_3$	11	16.33	23.24		2.0052
10		12	15.97	24.30		2.0053
11		13	15.95	23.54		2.0053
12	$\cdot NH_2$	8	15.85	19.03	1.71(amino N)	2.0054

a) Reactions producing X according to the numbering in the text.
b) Accurate within ±0.08 G.
c) Accurate within ±0.0001.
d) Two protons.

By using a solution (neutral pH) of 2 mM DMPO and 0.29 M (1%) H_2O_2, the spectrum of the OH adduct (X=OH) was obtained. However, the hyperfine coupling constants are not equal to those of the same radical in a strongly alkaline solution obtained by reaction 5. The pH-dependence of the hyperfine splittings for the OH adduct radical will be discussed in the next section. The spectra of the spin adducts obtained by the addition to DMPO of the radicals produced *via* reactions 5—15 were measured and their ESR parameters determined. The results are shown in Table I. It is worth noting that the NH_2 adduct gives rise to a hyperfine splitting due to the amino nitrogen nucleus. The variety in the hyperfine coupling constants of the spin adducts is shown as a "scatter plot" in Fig. 3, where the ordinate is a^H_β and the abscissa is a^N. The fact that the points are well scattered implies that DMPO is a good enough spin trap to distinguish trapped radicals based on the coupling constants of the corresponding spin adducts. The hyperfine coupling constants for adduct radicals in benzene solution have been reported to be $a^N=14.66$ G and $a^H=20.52$ G for X=hydroxymethyl, $a^N=14.31$ G and $a^H=20.52$ G for X=methyl, and $a^N=13.76$ G and $a^H_\beta=19.22$ G for X=phenyl.[5a)] On comparison of these values with those for radicals No. 5,9, and 10 in Table I, the solvent effect is such that a^N is 1—2 G larger and a^H_β is 2—4 G larger in the aqueous system than in the organic system.

Acid Dissociation of OH Adduct

The hyperfine coupling constants for the OH adduct radical have been found to depend on the basicity of the solution, while the g factor was independent of it. For this radical, a^H_β is fortuitously equal to a^N and the relationship has been found to hold under all the basicity conditions examined. The coupling constants changed from 14.95 G in acidic and neutral solutions to 16.20 G in strongly basic solutions. The phenomenon was analyzed in terms of the dissociation equilibrium of the hydroxyl protons (eq. 15). If the interchange between



(15)

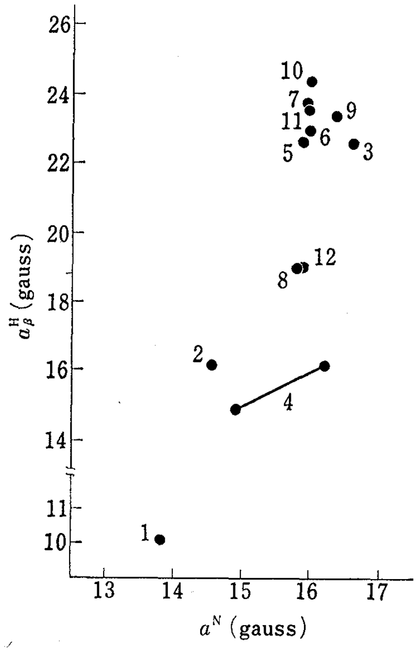


Fig. 3. A Plot of β-Hydrogen against Nitrogen Hyperfine Coupling Constants for Various DMPO Spin Adducts

See Table I for radical structures.

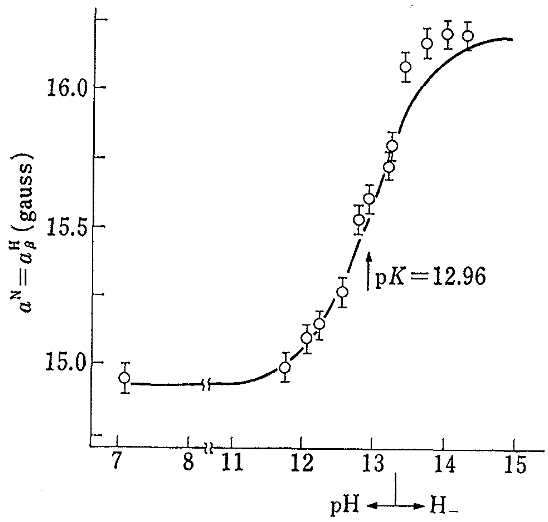


Fig. 4. The Hyperfine Coupling Constants ($a^N=a_\beta{}^H$) as a Function of Basicity for the Radical Produced by the Addition of OH to DMPO

The solid curve was calculated by using $pK=12.96$, $a_A=14.95$ G, and $a_B=16.20$ G based upon the nature of the acid dissociation equilibrium (eq. 17).

the acid form (A) and the base form (B) is rapid, the observed coupling constant, a, represents the weighted average of the concentrations of the two forms,

$$a = a_A f_A + a_B f_B \tag{16}$$

where a_A and a_B are the limiting values corresponding to the acid (14.95 G) and base (16.20 G) forms, respectively, and f_A and f_B represent the fractions of A and B. For a given solution the dissociation constant can be calculated by means of eq. 17.

$$\mathrm{p}K = \mathrm{pH} + \log(f_A/f_B) = \mathrm{pH} + \log[(a_B - a)/(a - a_A)] \tag{17}$$

where pH should be replaced by an appropriate acidity function if pH>13.3. An appropriate acidity function means that the activity coefficient behavior of the compound in question must be the same as that of the indicators used to establish the acidity function.[15] Actually, however, there is little choice of acidity functions because only a few are known for concentrated potassium hydroxide solutions.[7,8] Yagil's H_- function[7] has been adopted in the present study.

Figure 4 shows the coupling constant ($a^N = a^H_\beta$) for the radical in aqueous KOH as a function of pH (pH<13.3) and of Yagil's H_- function (pH>13.3). Obviously there is a discontinuity between the two acidity scales, implying that the H_- function is inappropriate for this radical. Therefore, we concluded that the use of only the experimental points in the pH range and the two limiting values would give a more accurate pK value than the use of all the experimental points. Using the seven points between pH 11.76 and 13.27, eq. 17 gave a pK value of 12.96 with a standard deviation of 0.06. The solid curve in Fig. 4 is a calculated curve using the pK value thus determined.

On comparison with the pK values for aliphatic alcohols (pK=15—16),[16] the hydroxyl group of the OH adduct radical is seen to be highly acidic. The enhanced acidity may arise from the electron-attracting character of the nitroxide group at the position β to the hydroxyl group. A similar effect has previously been observed in the case of β-hydroxyalkyl radicals.[17]

Decay of the Spin Adduct

The lifetime of a spin adduct is a very important factor determining the success of a spin trapping experiment. A radical formed in a low concentration can be detected by spin trapping if the spin adduct has a sufficiently long lifetime. The time course of the decay reaction was followed for some of the spin adduct radicals described above. A decay curve was obtained as follows: the magnetic field was fixed at the peak of the signal, a strip of chart paper was moved at 5—10 cm/sec and a sample solution not flowing but residing in the ESR cell was irradiated for several seconds with UV light.

The lifetime of the OH adduct was found to be too short to measure. It decayed immediately when the UV-irradiation was turned off. Decay curves were obtained for the spin adducts of $SO_4^{\overline{\cdot}}$, $SO_3^{\overline{\cdot}}$, e_{aq}^- ($+H^+$), $\cdot CH_2CH_2OH$, and phenyl radicals. Except for the $SO_4^{\overline{\cdot}}$ adduct, these radicals decayed in a second-order manner with respect to the radical concentra-

Table II. Half-Lives of DMPO Spin Adduct Radicals
Reaction numbering is the same as in Table I

Reaction No.	X	Half-life
1	$SO_4^{\overline{\cdot}}$	0.35 min
2	$SO_3^{\overline{\cdot}}$	1.2 min
3	$e_{aq}^- + H^+$	0.60 min
4	$\cdot OH$	Less than 5 sec
6	$\cdot CH_2CH_2OH$	4.8 min
10		21 min

tion. The decay route may be disproportionation, since it has been shown that nitroxide radicals which have a hydrogen attached to the β-carbon can decay by disproportionation in organic solvents.[18] The decay curve for the $SO_4^{\cdot}$ adduct fitted neither a second-order nor a first-order process. We did not attempt to determine the decay rate constant because the reactions may be complicated and in any case it is difficult to determine the absolute concentration of radicals, which is required to calculate the rate constant for a second-order reaction. Instead, we determined the half-life, which serves as a practical measure of the decay rate of the spin adduct radicals. The results are summarized in Table II. A radical which has a neutral bulky group as X appears to have a longer lifetime.

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