Paramagnetic Resonance Study of Liquids during Photolysis. XII. Alloxan, Parabanic Acid, and Related Compounds[1,2]

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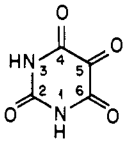
Abstract: Paramagnetic resonance spectra of the short-lived radicals made by photoreduction of aqueous solutions of alloxan containing small amounts of isopropyl alcohol and acetone have been studied. At pH >2, a radical giving rise to a seven-line spectrum (spectrum I) is seen. When the pH is made less than -0.4, this radical is protonated to form a 15-line spectrum (spectrum II). A rapid average of spectra I and II is observed at intermediate values of pH. The pK of the protonated species is 0.5. Similarly, radicals are made by the photoreduction of aqueous solutions of parabanic acid containing isopropyl alcohol. Spectrum III (15 lines) is obtained at a pH >3 and spectrum IV (15 lines) at pH <0. An average of spectra III and IV is obtained at intermediate values of pH. The pK is 1.17. Hyperfine couplings and g values are given and identification of the various radicals is discussed. Photolysis of solutions of alloxanic acid formed by rearrangement of alloxan gives the same radicals formed from parabanic acid. Photoreduction of parabanic acid in isopropyl alcohol gives a mixture of spectra III and IV. The exchange rate is slow. The effects of adding water and acid have been studied. Radicals have also been formed from N-methylparabanic acid.

We have been studying the paramagnetic spectra of short-lived free radicals made during the course of photolysis of liquids with ultraviolet light. The extension of this work to heterocyclic compounds and eventually to types having biological significance has been of special interest to us. This paper reports a study of free radicals made by the photolytic reduction in the presence of hydrogen donors[4] of alloxan (**1**), parabanic acid (**2**), and N-methylparabanic acid.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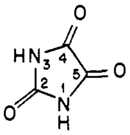
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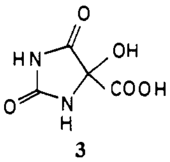
Several of the observed spectra serve as points of departure for the discussion of others. Those to be designated spectra I and II arise from different states of protonation of a radical formed by the reduction of alloxan, while spectra III and IV arise from the corresponding radicals from parabanic acid. The degree of protonation can be controlled by the acidity of the medium, and a variety of intermediate spectra as well as the measurement of the pK for the protonation in aqueous media will be presented.

Radicals made by the photoreduction of parabanic acid were also made by the photolysis of aqueous solutions of alloxanic acid (**3**).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A spectrum of seven lines that we will designate as spectrum I has already been reported. This spectrum has been ascribed to the anion radical of alloxan[5] and to the corresponding radical protonated on the oxygen attached to the 5 position of alloxan.[6] The radical was produced by chemically reducing alloxan with ascorbic acid or glutathione in aqueous solution[5] and with dithionate in a methanol–water solvent. A spectrum of 15 lines formed in a nonaqueous medium, dimethyl sulfoxide, has been ascribed to the anion radical of alloxan.[7] Hyperfine couplings but not g values were reported in the earlier papers.

Experimental Section

The spectrometer which operated at a nominal frequency of 9.5 GHz and employed 100-kHz field modulation, the sample handling operations, and the technique for making measurements of the couplings and g values have already been described.[8,9] The solutions were freed of dissolved oxygen by purging with argon and were photolyzed near room temperature as they slowly flowed through a flat silica cell positioned inside the cavity of the spectrometer. The ultraviolet source was a high-pressure mercury arc, Philips Type SP500W.

Acidities of solutions were adjusted with either hydrochloric acid or sodium hydroxide, and pH measurements were made with a Beckman Model G pH meter equipped with a glass electrode (Coleman 3-479). The alloxan monohydrate (Eastman) and the parabanic acid (Aldrich) were used as purchased. Anhydrous alloxan was prepared by sublimation of alloxan monohydrate at 250° in a vacuum of about 1 Torr. The N-methylparabanic acid as well as an initial small quantity of parabanic acid were synthesized[10] several years ago at this laboratory.

Results and Discussion

Radicals from Alloxan in Aqueous Solution at pH >2. Spectrum I, Figure 1, consisting of seven equally spaced hyperfine components with relative intensities of about 1:4:8:10:8:4:1 was observed upon uv irradiation at room temperature of a 6.25 mM aqueous solution of alloxan monohydrate containing 5% (by volume) isopropyl alcohol and 1% acetone when the pH was

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[1] Research sponsored by the U. S. Atomic Energy Commission under contract with Union Carbide Corp.

[2] Part XI: H. Zeldes and R. Livingston, *J. Amer. Chem. Soc.*, **93**, 1082 (1971).

[3] Fellowship support is gratefully acknowledged to the Deutsche Forschungsgemeinschaft.

[4] H. Zeldes and R. Livingston, *J. Chem. Phys.*, **45**, 1946 (1966).

[5] C. Lagercrantz and M. Yhland, *Acta Chem. Scand.*, **17**, 1677 (1963).

[6] J. C. Orr, *Nature (London)*, **201**, 816 (1964).

[7] G. A. Russell and M. C. Young, *J. Amer. Chem. Soc.*, **88**, 2007 (1966).

[8] R. Livingston and H. Zeldes, *J. Chem. Phys.*, **47**, 4173 (1967).

[9] R. Livingston and H. Zeldes, *ibid.*, **44**, 1245 (1966).

[10] H. Kwart, R. W. Spayd, and C. J. Collins, *J. Amer. Chem. Soc.*, **83**, 2579 (1961).

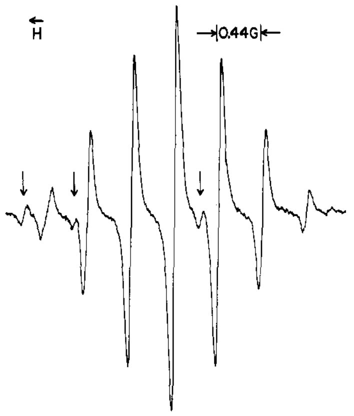
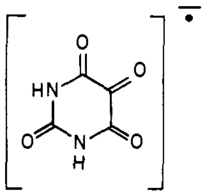


Figure 1. Spectrum during photolysis at 37° of aqueous 6.25 m*M* alloxan containing 5% isopropyl alcohol and 1% acetone. The pH was adjusted to 2.01 with hydrochloric acid. Excluding extraneous lines indicated by arrows, this is designated spectrum I in the text.

adjusted to values in the range of 2–9. The splitting between adjacent components, 0.44 G, and the *g* value, 2.00492, at 37° did not change over this pH range.

A weak spectrum I was seen in the absence of isopropyl alcohol and acetone. The addition of 5% isopropyl alcohol increased the signal strength by a factor of about 3. The isopropyl alcohol apparently serves as a good hydrogen donor to the optically excited alloxan in much the same manner as it does for excited acetone[4] or biacetyl.[11] Further addition of 1% acetone gave a further tenfold increase in signal strength. Moreover, the spectrum of $(CH_3)_2\dot{C}OH$ was not seen even though photolysis of acetone with isopropyl alcohol gives a strong spectrum of this radical.[4] The radical $(CH_3)_2\dot{C}OH$ is known to be an efficient electron donor.[12–14] The large enhancement in radical yield upon the addition of a small amount of acetone implies that the primary source of the radical from alloxan involves the initial formation of $(CH_3)_2\dot{C}OH$ from the acetone and isopropyl alcohol with subsequent electron transfer to alloxan. The radical could be either **4** or **5** which forms by protonation of **4**.



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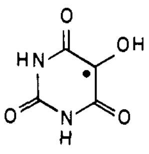

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Lagercrantz and Yhland[5] reported a seven-line spectrum similar to spectrum I with a splitting between adjacent hyperfine components of about 0.4 G in an aqueous solution at pH values between 4.6 and 8.6. These authors explained the observed hyperfine structure by the interaction of the unpaired electron with the pair of equivalent nitrogens and the pair of equivalent hydrogens of radical **4**, where the nitrogen coupling constant fortuitously equals the proton constant. This would give rise to a seven-line spectrum with relative intensities 1:4:8:10:8:4:1, in agreement with observation. They also prepared the radical in D_2O and found the spectrum altered in the manner expected with this interpretation when H is replaced by D. We have also prepared the radical in D_2O and corroborate their finding and adopt their analysis of the spectrum. We find $a(N_1) = a(N_3) = 0.44$ G and $a(H_1) = a(H_3) = 0.44$ G, where the subscripts refer to the numbering given for structure **1** of alloxan. A similar spectrum with hyperfine splittings of 0.48 G was studied by Orr[6] and was ascribed to radical **5**. Our coupling values and those quoted above are in good agreement. Russell and Young[7] prepared the radical by dissolving alloxantin in dimethyl sulfoxide containing potassium *tert*-butoxide. In this case the nitrogen coupling was altered, and a 15-line spectrum was seen. The species was ascribed to the anion **4** with couplings of $a(N_1) = a(N_3) = 0.587$ G and $a(H_1) = a(H_3) = 0.48$ G. It is conceivable that the use of a different solvent caused the moderate shift in coupling.

We cannot differentiate between structures **4** and **5**. Both satisfy the requirement of having a pair of equivalent nitrogens and a pair of equivalent hydrogens. Failure to see a hyperfine interaction from the hydroxyl hydrogen of **5** is reasonable. Rapid exchange of this hydrogen would prevent seeing the interaction. Even in the absence of rapid exchange hydroxyl hydrogen interactions are not always resolved.[9] We should further point out that our experiments would not differentiate between neutral forms of the radical where the hydrogen is attached at the 5 position as given in **5** or at the 2 position. Both structures would give equivalent nitrogens and equivalent protons. There is evidence giving preference to structure **5** for the neutral form. Theoretical calculations of Pullman[15] show that the anion should protonate at the 5 position.

We have ignored the possibility of hydration of the radicals. Alloxan readily forms a monohydrate, and it is known from a crystal structure determination[16] that hydration in the solid takes place at the 5 position with bending of that carbon out of the plane of the ring.

Radicals from Alloxan in Aqueous Solution at pH <2. Upon photolysis of aqueous solutions of alloxan monohydrate containing 5% isopropyl alcohol and 1% acetone at pH values below 1.75, a variety of symmetrical, sharp-lined spectra was observed. Two of these spectra are shown in Figures 2 and 3. The spectra could be analyzed in terms of a model having two equivalent nitrogens and two equivalent protons whose couplings depend on pH. The stick spectra given in Figures 2 and 3 are based on this analysis. The shift of the couplings was paralleled by a decrease of the *g* value from 2.00492 at pH >2 to 2.0045 at pH −0.4. The shift of the parameters was reversible; the same spectra were obtained by starting at pH 2 and adding hydrochloric acid and by starting at pH <0 and adding sodium hydroxide. Measured couplings as a function of pH are given in Figure 4. The pH value of −0.4 was calculated from the concentration of hydrochloric acid present.

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(11) H. Zeldes and R. Livingston, *J. Chem. Phys.*, 47, 1465 (1967).

(12) A. L. Buley and R. O. C. Norman, *Proc. Chem. Soc., London*, 225 (1964).

(13) R. O. C. Norman and R. J. Pritchett, *J. Chem. Soc. B*, 378 (1967).

(14) H. Zeldes and R. Livingston, *J. Phys. Chem.*, 74, 3336 (1970).

(15) A. Pullman, *J. Chim. Phys. Physicochim. Biol.*, 61, 1666 (1964).

(16) C. Singh, *Acta Crystallogr.*, 19, 759 (1965).

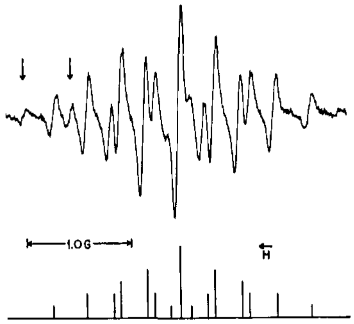

Figure 2. Spectrum from aqueous alloxan at a pH of 0.50 and other conditions the same as for Figure 1. Arrows indicate extraneous lines not included in the stick spectrum.

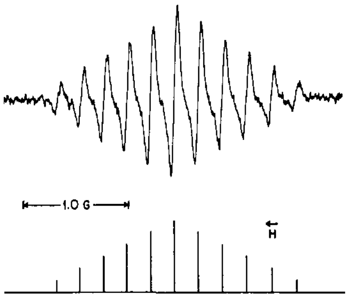

Figure 3. Spectrum from aqueous alloxan at a pH of about −0.4 and other conditions the same as for Figure 1. This was the lowest pH used and the closest approach to spectrum II attained in aqueous solution.

These results indicate an acid–base dissociation equilibrium

$$\mathrm{II}^{n+} \rightleftarrows \mathrm{I}^{(n-1)+} + \mathrm{H}^+ \tag{1}$$

between the radical (spectrum I) predominating at pH >2 and a protonated species (spectrum II) prevailing at pH <0. The reaction rates are sufficiently fast (on the time scale of the paramagnetic resonance experiment) that only a single, sharp-lined symmetric spectrum is obtained at all pH values corresponding to an appropriate average of spectra I and II. It was not possible to adjust the pH of an aqueous system to a low enough value to give essentially 100% of the protonated species. Thus spectrum II which consists of 15 lines could not be obtained in aqueous media, but, as shown later, it was observed in a different medium.

The solid curves of Figure 4 were calculated with the equation[17]

$$a(\mathrm{j}) = \frac{a(\mathrm{j})_{\mathrm{I}} + a(\mathrm{j})_{\mathrm{II}} \times 10^{(\mathrm{p}K-\mathrm{pH})}}{1 + 10^{(\mathrm{p}K-\mathrm{pH})}} \tag{2}$$

which is valid for the equilibrium given in eq 1. The hyperfine couplings are given by a(j), where j refers to hydrogen or nitrogen and the subscripts I and II refer to the radical species defined by eq 1. The values used for $a(\mathrm{H})_{\mathrm{I}}$ and $a(\mathrm{N})_{\mathrm{I}}$ were those given earlier for spectrum I. Values of $a(\mathrm{H})_{\mathrm{II}}$, $a(\mathrm{N})_{\mathrm{II}}$, and p$K$ where deduced by adjusting their values to give the best fit to the points defining the two curves of Figure 4. The couplings found in this way for the protonated species (spectrum II) are $a(\mathrm{N}_1) = a(\mathrm{N}_3) = 0.20$ G and $a(\mathrm{H}_1) = a(\mathrm{H}_3) = 0.71$ G. The pK is 0.5.

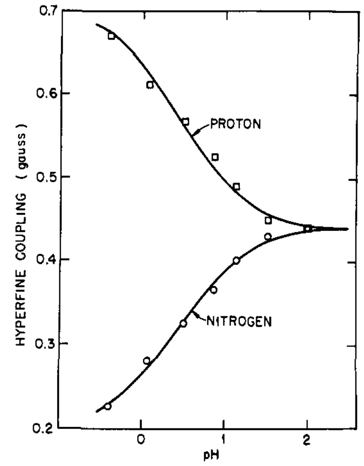

Figure 4. Measured values and calculated curves for the dependence of hyperfine couplings on pH in the alloxan system at 37°.

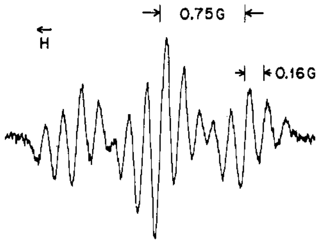

Figure 5. Spectrum during photolysis at 36.5° of 3.75 mM alloxan monohydrate in a solvent consisting of 656 ml of concentrated HCl, 75 ml of H_2O, 10 ml of acetone, and isopropyl alcohol to make 1000 ml.

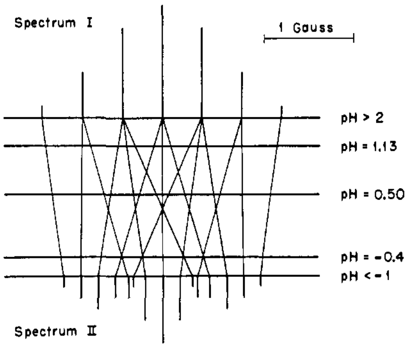

Figure 6. Correlation diagram for spectra I and II in the aqueous alloxan system. Spectra obtained at selected intermediate values of pH are indicated by horizontal lines. Two of these spectra are shown in Figures 2 and 3. The shift in g value has not been represented.

The pK depends on the composition of the solvent. Spectrum II shown in Figure 5 was obtained from a solution containing 3.75 mM alloxan monohydrate in a solvent consisting of 75 ml of water, 656 ml of concentrated hydrochloric acid, 10 ml of acetone, and enough isopropyl alcohol to give 1000 ml. The measured parameters at 36.5° are $a(\mathrm{N}_1) = a(\mathrm{N}_3) = 0.16$ G, $a(\mathrm{H}_1) = a(\mathrm{H}_3) = 0.75$ G, and $g = 2.0045$, which compare well with those deduced above in a more completely aqueous solvent.

Figure 6 is a correlation diagram showing spectra I and II and all intermediate spectra. The shift of the g value is not represented. The spectra corresponding to Figures 2 and 3 are indicated by the appropriate

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[17] H. Fischer, K.-H. Hellwege, and M. Lehnig, *Ber. Bunsenges. Phys. Chem.*, 72, 1166 (1968).

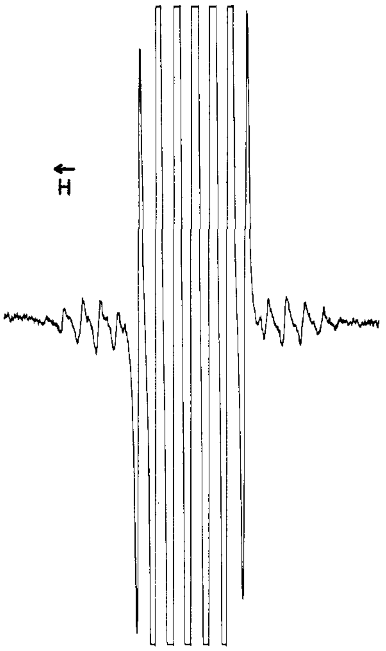

Figure 7. The spectrum of the radical from aqueous alloxan at a pH of 8.20 (spectrum I) showing satellite lines from ^{13}C.

value of the pH. Note that the lowest pH used in this solvent (−0.4) was not quite adequate to arrive at spectrum II.

Some information can be obtained on the rate of conversion of one form of the radical to the other form, since it is known that the rates of conversion are fast enough to always give a rapidly averaged spectrum. The rate must be faster than the largest displacement, $\Delta\omega$, of corresponding hyperfine lines of spectra I and II shown in Figure 6 (and properly taking into account the shift in g value). This displacement is 1.65 G, corresponding to a value of $\Delta\omega$ of 2.9×10^7 radians sec^{-1}. In the limit of fast exchange the exchange frequency, ω_e, can be estimated[18] from the line width due to exchange, $\delta\omega$, and from $\Delta\omega$ by means of the relation $\omega_e \approx (\Delta\omega)^2/\delta\omega$. For $\delta\omega \approx 1 \times 10^6$ radians sec^{-1} (0.07 G), one obtains $\omega_e \approx 8 \times 10^8$ radians sec^{-1} or a mean lifetime, τ, of about 7×10^{-9} sec for both radicals if the pH is equal to the pK of 0.5. The second-order rate constant, k, for the protonation step, is $k = 1/\tau[\mathrm{H}^+] \approx 5 \times 10^8$ l. mol^{-1} sec^{-1}. This value represents the lower limit of k since it was based on the experimental line width which certainly contains broadening mechanisms other than chemical exchange.

The observed decrease in g value upon protonation is consistent with the view that protonation of an oxygen lowers the spin density on the oxygen with a corresponding decrease in g value.[19,20]

The experiments do not give a value for n in eq 1. The only reasonable values are $n = 0$, which corresponds to dissociation of the neutral radical **5** to give the anion **4**, and $n = 1$, which corresponds to the loss of a proton by a cation radical to give **5**. Under the experimental conditions used, the requirement of equivalence of nitrogen nuclei and of protons would be met regardless of which oxygen of **5** became protonated to form the cation radical. For example, protonation of **5** at the 4 position would lack the required symmetry; however, protonation at the 6 position would occur with equal probability, and under the conditions of rapid dynamic exchange an average spectrum corresponding to the required symmetry would be seen.

Although assignment of spectrum II to a cation radical is not completely ruled out, we have preference for assignment of spectrum II to the neutral radical **5** and spectrum I to the anion **4**. The neutral radical is a remarkably strong acid when compared to $CH_3CO\dot{C}(OH)CH_3$ and $CH_3OCO\dot{C}(OH)CH_3$ which have pK values respectively[13,14] of 4 and a value in the range of 3–5. On the other hand, comparison with semiquinone radicals[21] suggests that the neutral radical **5** would be a strong acid. The pK for neutral p-benzoquinone is 3.7. However, if the pair of keto groups are ortho to each other the pK drops to 1.7. Alloxan has three rather than a pair of adjacent keto groups and a further drop in pK to 0.5 does not seem unreasonable. Our finding that the parameters did not change over the pH range of 2–9 is evidence that the neutral radical is either a strong acid or an implausibly weak acid. This is also shown (see later) by the nearly constant value of a ^{13}C coupling constant over the pH range of 1.75–8.20.

^{13}C Hyperfine Interaction in the Alloxan System. Spectrum I was sufficiently strong to give splittings from ^{13}C in natural abundance. At 38° and a pH of 1.75 an aqueous solution containing 5% isopropyl alcohol and 1% acetone gave a ^{13}C hyperfine coupling of 4.78 G. From the strength of the ^{13}C satellites, it was clear that two equivalent carbon sites of the radical were involved. Accordingly, we assign the coupling to carbons at positions 4 and 6. Splittings from the remaining carbon sites were not seen. Their hyperfine couplings are probably much smaller than the value reported here.

A very strong spectrum I was also obtained upon photolysis of an aqueous solution 6.25 mM in alloxan and 6.94 mM in dialuric acid (5-hydroxybarbituric acid). The solution was prepared under an atmosphere of argon, and the pH was adjusted with either hydrochloric acid or sodium hydroxide. Spectrum I was seen up to a pH of 9.5. In an attempt to establish experimental evidence for a second equilibrium, eq 1, the ^{13}C coupling, which is a sensitive probe into the spin density distribution, was measured at various pH values from 2.30 to 8.20. The following values (G) were obtained: 4.74 (pH 2.30), 4.70 (pH 3.20), 4.68 (pH 4.78), 4.67 (pH 6.00), 4.66 (pH 6.65), and 4.65 (pH 8.20). Figure 7 shows the satellites from ^{13}C at pH 8.20. The slight increase in coupling may, in part, be due to the equilibrium characterized by a pK of 0.5. We do not detect a trend characteristic of a second equilibrium with a pK in this higher range.

Radicals from Alloxan in Aqueous Solution at pH >11.5. A strong spectrum I was also seen upon photolysis of a solution prepared by dissolving dialuric acid in water in the presence of air. Oxidation of dialuric acid by oxygen produces alloxan which is then reduced photolytically to give the observed spectrum. An excess of dialuric acid is the hydrogen donor.

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(18) A. Carrington and A. D. McLachlan, "Introduction to Magnetic Resonance," Harper and Row, New York, N. Y., 1967, p 204.

(19) A. J. Stone, *Mol. Phys.*, **6**, 509 (1963).

(20) P. D. Sullivan, J. R. Bolton, and W. E. Geiger, Jr., *J. Amer. Chem. Soc.*, **92**, 4176 (1970).

(21) I. C. P. Smith and A. Carrington, *Mol. Phys.*, **12**, 439 (1967).

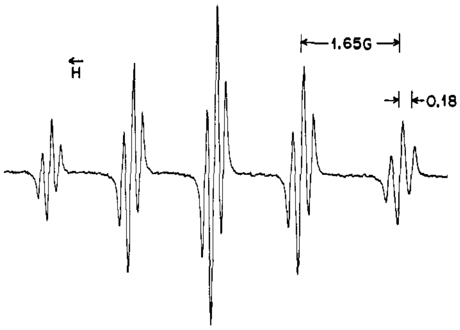
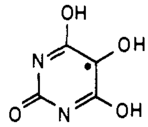


Figure 8. Spectrum during photolysis at 32.5° of aqueous 8.77 mM parabanic acid containing 5% isopropyl alcohol. The pH was 3.90. This is designated spectrum III in the text.

At very high pH values (>11.5) we observed a spectrum of five rather broad lines from two equivalent nitrogens with a coupling of about 0.35 G. The g value at 37° and a pH of 12.17 was 2.00477. Orr[6] reported a five-line spectrum from reduced alloxan in 8 N KOH with a splitting of 0.24 G. He suggested that the radical was the lactim form (**6**) of radical **5**. We would



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expect this radical to be in one of its anion forms at the high pH necessary for its formation.

Alloxan is known to undergo a benzilic acid type of rearrangement[10] in alkaline solution to give the anion of alloxanic acid (**3**). Solutions of alloxan maintained at a high pH upon photolysis give a spectrum of a radical derived from parabanic acid. This spectrum was not seen in the experiments described above with dialuric acid.

Radicals from Parabanic Acid in Aqueous Solution. Photolysis of an 8.77 mM aqueous solution of parabanic acid (**2**) containing 5% isopropyl alcohol and at pH values between 3 and 8 gave a strong spectrum (spectrum III) exhibiting hyperfine interactions from two equivalent nitrogens and two equivalent hydrogens (Figure 8). At 32.5° the hyperfine couplings are $a(\mathrm{N_1}) = a(\mathrm{N_3}) = 1.65$ G and $a(\mathrm{H_1}) = a(\mathrm{H_3}) = 0.18$ G, and the g value is 2.00417. The subscripts refer to the numbering given for parabanic acid (**2**).

A weak spectrum III was also obtained for just parabanic acid in water, but the addition of isopropyl alcohol greatly enhanced the radical yield. In the absence of isopropyl alcohol the spectrum of a second, unidentified radical was also present. This radical had a slightly lower g value and consisted of one rather broad line with some indication of structure, but attempts to resolve this structure were unsuccessful. This spectrum was not seen after the addition of isopropyl alcohol. As with alloxan the spectrum from $(\mathrm{CH_3})_2\dot{\mathrm{C}}\mathrm{OH}$ was not present, although, contrary to the finding with alloxan, the addition of small amounts of acetone did not enhance the strength of spectrum III.

The strength of spectrum III gradually decreased with increasing pH, and beyond a pH of 8 the solution of aqueous parabanic acid containing isopropyl alcohol appeared unstable. No spectrum was obtained at a pH

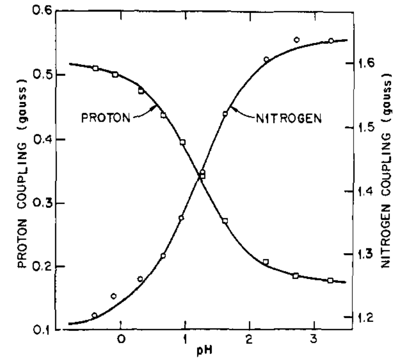


Figure 9. Measured values and calculated curves for the dependence of hyperfine couplings on pH in the parabanic acid system at 33°.

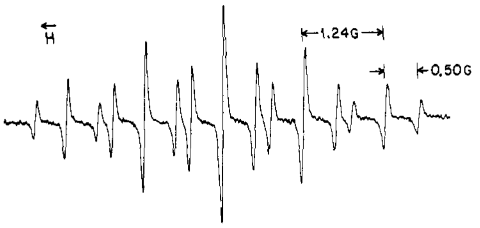


Figure 10. Spectrum during photolysis at 33° of aqueous 8.77 mM parabanic acid containing 5% isopropyl alcohol. The pH was adjusted to −0.1 with hydrochloric acid.

of about 11, and when this solution was acidified the spectrum did not reappear; there was an irreversible chemical change in the solution.

At low values of pH obtained by the addition of hydrochloric acid the hyperfine couplings changed in qualitatively the same manner as already described for alloxan. The nitrogen and proton hyperfine couplings as a function of pH are shown in Figure 9. We call the spectrum for the completely protonated species spectrum IV. This spectrum was almost attained at the lowest pH, −0.10, shown in Figure 9, where the couplings at 33° are $a(\mathrm{N_1}) = a(\mathrm{N_3}) = 1.24$ G and $a(\mathrm{H_1}) = a(\mathrm{H_3}) = 0.50$ G and the g value is 2.00402. This spectrum is shown in Figure 10 and is essentially spectrum IV. The g values for spectra III and IV are almost identical with those for anions and neutral radicals, respectively, made by photoreduction of oxalic acid and certain of its diesters.[14] As in the case of alloxan the radicals giving spectra III and IV are related by an acid–base dissociation equilibrium analogous to eq 1. The curves of Figure 9 were calculated using an equation like eq 2, using the measured hyperfine couplings for spectrum III and adjusting pK and the hyperfine couplings for spectrum IV to give the best fit of the experimental data. The hyperfine couplings deduced for the completely protonated species, spectrum IV, are $a(\mathrm{N_1}) = a(\mathrm{N_3}) = 1.19$ G and $a(\mathrm{H_1}) = a(\mathrm{H_3}) = 0.52$ G. These values are close to those given above for pH −0.10. The pK is 1.17.

In parallel manner to the discussion for alloxan, the experiments do not reveal the charge, n, of the protonated radical (eq 1). For $n = 0$, spectrum III would be assigned to the anion radical **7** and spectrum IV to the neutral radical **8**. Although we feel this is the more reasonable choice, we cannot completely rule out the equilibrium between **8** and a cation radical formed by

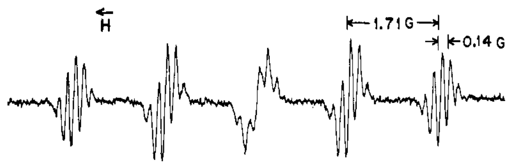
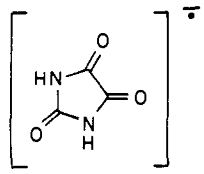
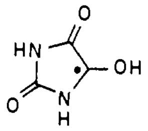


Figure 11. Spectrum during photolysis at 34.5° of aqueous 7.87 m*M* *N*-methylparabanic acid containing 5% isopropyl alcohol. The pH was 4.05.

protonating **8** at one of the oxygens. Moreover, we have no evidence from paramagnetic resonance to rule out the formation of the neutral radical by protonation



7



8

at the 2 position. We have chosen the 5 position as shown in **8** by analogy with that given for the neutral radical from alloxan, **5**. The 4 and 5 positions are equivalent, and rapid intra- or intermolecular exchange of hydrogen would assure a spectrum with equivalent nitrogens and equivalent hydrogens.

Taking the view that the equilibrium involves **7** and **8**, the p*K* of 1.17 indicates that the neutral species, **8**, is a very strong acid. The value, however, is higher than that for the radical from alloxan, 0.5, and consistent with the earlier comparisons with the semiquinone radicals. Also parallel to the discussion for alloxan, the *g* value and hyperfine couplings for spectrum III did not change over the pH range of 3–8, thus failing to give evidence for a second acid–base equilibrium. This supports the view that the observed equilibrium involves the anion and neutral species. Also, from the fact that the lines of the spectra remained sharp in the transition region the rate of exchange between the two radical species is fast, just as it was for the alloxan system. Finally, the decrease in *g* value of spectrum III upon protonation is similar to that observed in the alloxan system.

Radicals from Alloxanic Acid (3). A 62.5 m*M* aqueous solution of alloxan with the pH adjusted to 9 by addition of sodium hydroxide was allowed to stand at room temperature for 30 min. These conditions cause practically complete rearrangement of the alloxan to the anion of alloxanic acid.[10] Upon acidifying with hydrochloric acid and photolyzing, the same radicals appeared that were observed in the parabanic acid system, and their dependence on pH was identical. The most reasonable explanation is that the alloxanic acid formed in the rearrangement looses its carboxyl group by the action of the uv light. This type of reaction is known to occur with α-hydroxy carboxylic acids.[22]

Radicals from *N*-Methylparabanic Acid. A 7.87 m*M* aqueous solution of *N*-methylparabanic acid containing 5% isopropyl alcohol upon photolysis gives a spectrum showing hyperfine structure from two nitrogens and four protons, Figure 11. The couplings of the two nitrogens are slightly different, thus giving rise to a broadening of the inner groups of lines. The coupling of the methyl protons accidentally equals the coupling of the unique hydrogen attached to one of the nitrogens. The measured parameters at 34.5° and a pH of 4.05 are $a(\mathrm{N}_1) \approx 1.71$ G, $a(\mathrm{N}_3) \approx 1.71$ G, $a(\mathrm{CH}_3) \approx 0.14$ G, $a(\mathrm{H}) \approx 0.14$ G, and $g = 2.00417$. These parameters are very close to those of spectrum III from parabanic acid. The addition of hydrochloric acid to the solution produced a shift of the parameters similar to the findings in the parabanic acid system. The spectrum at a pH of -0.1 was rather weak. It consisted of 40 lines from two nitrogens with an average splitting of $a(\mathrm{N}_1) \approx a(\mathrm{N}_3) \approx 1.28$ G, from three equivalent protons with $a(\mathrm{CH}_3) = 0.40$ G, and from the unique hydrogen with $a(\mathrm{H}) = 0.51$ G. These couplings are very close to those obtained in the parabanic acid system in the presence of strong acid, spectrum IV. It is clear that the radicals made from *N*-methylparabanic acid are the analogs of those made from parabanic acid. The two nitrogen couplings (for both radicals) from N-methylparabanic acid being approximately equal and also approximately equal to those found from parabanic acid rules against a lactim structure as having a role in the observed radicals.

Radicals from Parabanic Acid in Isopropyl Alcohol. Spectra III and IV are observed upon photolysis of 8.77 m*M* parabanic acid in isopropyl alcohol. As described earlier, in aqueous solution at intermediate values of pH a single sharp-lined spectrum consisting of an average of spectra III and IV is seen. This is a consequence of rapid dynamic exchange. In isopropyl alcohol the exchange rate is much slower, and a mixture of the two sharp-lined spectra is seen with line widths of about 0.1 G. The splittings are a little different than seen in aqueous solution. At 34° in a solution of isopropyl alcohol containing 1% water the hyperfine couplings for spectrum III are $a(\mathrm{N}_1) = a(\mathrm{N}_3) = 1.60$ G and $a(\mathrm{H}_1) = a(\mathrm{H}_3) = 0.16$ G. Those for spectrum IV are $a(\mathrm{N}_1) = a(\mathrm{N}_3) = 1.03$ G and $a(\mathrm{H}_1) = a(\mathrm{H}_3) = 0.60$ G. The fact that the rate of exchange must be slow indicates that the hydrogen at the 5 position of radical **8** cannot be involved in rapid intermolecular dynamic exchange. Finding the two nitrogens and the two hydrogens equivalent requires that this hydrogen be shared equivalently by the oxygens at the 4 and 5 positions, say, by intramolecular exchange. An alternate possibility is that protonation does not occur at the 4 or 5 positions as shown in structure **8** but rather at the 2 position, which would give a symmetric radical.

For several additions of water to the parabanic acid solution in isopropyl alcohol, spectra III and IV remained sharp. During these successive additions spectrum IV became weaker and spectrum III became stronger, which means that the protonated radical was dissociating in the presence of water. By the time the water concentration was 15.5% (by volume) only spectrum III was seen; however, some of the lines were now noticeably broadened, indicating a contribution to the spectrum of a small amount of the protonated radical. Subsequent additions of concentrated hydrochloric acid produced the shift of the couplings as already described for aqueous solutions (Figure 9). However, during the course of increasing the concentration of acid a broadening and resharpening of certain hyperfine lines was observed; there were line-broadening effects due to in-

[22] R. Livingston and H. Zeldes, *J. Chem. Phys.*, **53**, 1406 (1970).

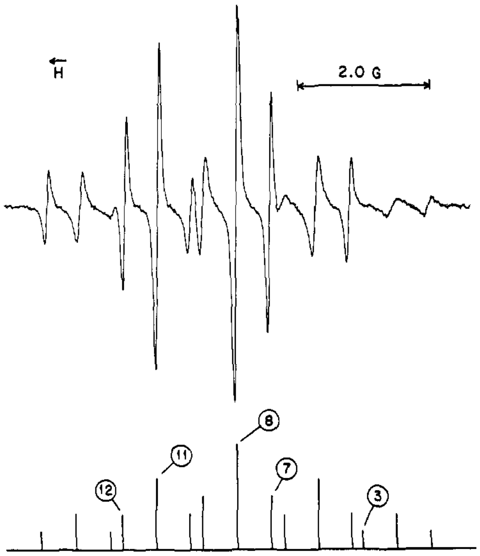


Figure 12. Spectrum during photolysis at 34° of 7.46 m*M* parabanic acid and 1.56 m*M* HCl in isopropyl alcohol containing 15.5% H_2O by volume. Numbered lines are discussed in the text.

termediate rates of exchange. Above 50 m*M* hydrochloric acid a sharp 11-line spectrum from the fully protonated radical (spectrum IV) was seen. At 34° in an isopropyl alcohol solution containing 15.5% water the couplings are $a(N_1) = a(N_3) = 1.12$ G and $a(H_1) = a(H_3) = 0.56$ G. Note that spectrum IV in aqueous solution consists of 15 lines, Figure 10. The small shift in hyperfine couplings in the above solution causes certain of the components to superimpose, giving a spectrum of 11 lines.

Figure 12 shows one of the spectra obtained at an intermediate acid concentration, 1.56 m*M* hydrochloric acid, where line-width effects are present. The correlation diagram, Figure 13, shows the manner in which spectra III and IV are averaged. These spectra are displaced by the observed shift in *g* values, $\Delta g = 1.6 \times 10^{-4}$, corresponding to 0.27 G. The horizontal line labeled a gives the admixture of spectra III and IV responsible for the spectrum shown in Figure 12. The numbering of certain lines in Figure 13 corresponds to that in Figure 12. In Figure 12 line 3 is so broad that it cannot be seen. It is apparent (Figure 13) that at an exchange rate appropriate to produce a maximum broadening of line 3 (an average of widely spaced lines) the lines numbered 7, 8, 11, and 12 are undergoing relatively fast exchange and remain sharp. All of the other lines show a more or less pronounced broadening which depends upon the spacing between the lines being averaged. The broadening is not symmetric about the center of the spectrum because of the shift in *g* value. The observed broadening effects in Figure 12 are in qualitative agreement with the prediction of the correlation diagram.

Additional Experiments. We have been unable to find spectrum I during the photolysis of solutions of alloxan monohydrate and of anhydrous alloxan in isopropyl alcohol at room temperature. We did see a weak 15-line spectrum with parameters identical with those measured for spectrum IV obtained with parabanic acid in isopropyl alcohol (no added water). Lines from $(CH_3)_2\dot{C}OH$ were not present. When the solution was made alkaline (0.75 m*M* in sodium hydroxide added as a solution in ethyl alcohol) a different 15-line spectrum appeared with parameters close to those measured for spectrum III obtained with parabanic acid in isopropyl alcohol.

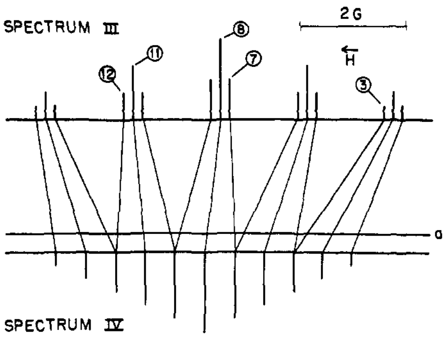


Figure 13. Correlation diagram for spectra III and IV from parabanic acid in isopropyl alcohol containing 15.5% H_2O by volume. The horizontal line, a, determines spectral line positions seen in Figure 12. Numbered lines are discussed in the text.

Spectrum I was obtained by adding water to the solution of alloxan in isopropyl alcohol. Its strength could be enhanced by the addition of small amounts of acetone. In a solution containing 10% water spectrum I showed a peculiar line-width effect in that the low-field lines were broader than the corresponding high-field lines. Closer inspection of the spacings between adjacent lines revealed a gradual decrease with increasing magnetic field, the spacing between the first two low-field lines being 0.45 G as compared with 0.42 G for the first two high-field lines. The only reasonable explanation is that two radicals with almost identical splittings and *g* values contribute to the spectrum which in fact is a superposition of two spectra. We have been unable to resolve the components. Further addition of water (20%) gave a normal spectrum I with parameters identical with those reported earlier.

If hydrochloric acid was added to a solution of alloxan in isopropyl alcohol containing 10% water, the same equilibrium phenomena already dealt with were observed. In a certain range of lower acid concentrations, however, line-width effects were seen, indicating that exchange between the species giving spectra I and II is slower than in an aqueous medium.

Solutions of alloxan in ethyl alcohol containing 10% water, 1% acetaldehyde (in order to generate $CH_3\dot{C}HOH$), and various amounts of hydrochloric acid were also studied. The results were in qualitative agreement with those obtained in isopropyl alcohol except that in the absence of hydrochloric acid a cleaner spectrum I with splittings of 0.44 G and $g = 2.00500$ at 32° was seen.