# The Electron Spin Resonance Spectra of Radical Intermediates in the Oxidation of Ascorbic Acid and Related Substances[1]

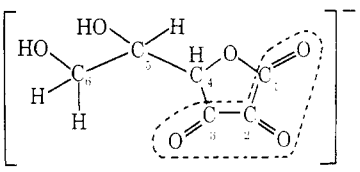
**Gary P. Laroff, Richard W. Fessenden, and Robert H. Schuler***

*Contribution from the Radiation Research Laboratories and Department of Chemistry, Mellon Institute of Science, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213.* *Received June 1, 1972*

**Abstract:** The radicals produced in the radiolysis of aqueous solutions of ascorbic, araboascorbic, reductic, and α-hydroxytetronic acids have been examined by the *in situ* radiolysis–esr method. Spectra of the $^{13}C$-containing radicals have been observed at the natural abundance level. These spectra show that in neutral and basic solutions oxidation produces radical anions in which the electron is spread over a highly conjugated tricarbonyl system. The radicals, which in these studies result mainly from the reaction of OH radicals, are identical with those previously found in other oxidation processes. For the radical from ascorbic acid the proton hyperfine structure consists of a 1.76 G doublet of 0.19 G triplets. In addition we have been able to resolve a further splitting of 0.07 G. The spectra in $H_2O$ and $D_2O$ are identical showing that each of these splittings is attributable to a CH proton. Examination of ascorbic-*4*-$d_1$ acid shows conclusively that the 1.76 G proton hyperfine constant is ascribable to the proton at the $C_4$ position. The splittings of 0.19 and 0.07 G are assigned to the protons at the $C_6$ and $C_5$ positions, respectively. The diastereomeric radicals produced from ascorbic and araboascorbic acids have observably different esr spectra as a result of slightly different $^1H$ and $^{13}C$ hyperfine constants. In acidic solutions the radical produced from ascorbic acid persists in the anionic form down to a pH of 0. The p$K$ for its protonation has been determined to be −0.45 so that protonation cannot be involved in the pronounced increase in rate constant for radical disappearance observed in pulse radiolysis experiments in the pH region between 7 and 4. At pH values below 6 a second radical is also present in this system. This second radical, which appears to be formed specifically by reaction of OH radicals with the neutral form of ascorbic acid, has a protonation equilibrium with a p$K$ of 2.0.



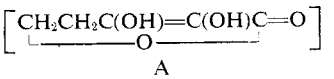
In 1960 Yamazaki, Mason, and Piette,[2] in one of the first studies of transient radicals in solution, reported the observation of a 1.7 G esr doublet during the enzymatic oxidation of ascorbic acid.[3] Because of its importance in biochemical processes, the radical responsible for this spectrum has been the subject of a number of subsequent esr investigations using a variety of chemical production methods.[4–6] Very recently the results of a pulse radiolysis study have also appeared.[7,8] While Yamazaki, *et al.*, in their original work,[2,3] indicated that a radical anion is responsible for the observed esr spectrum, recent workers[5,6] appear to have been confused as to the exact structure of the radical, particularly with respect to its state of protonation, and have discussed the spectrum in terms of a neutral species. In an attempt to clarify this situation we have examined ascorbic acid solutions by the *in situ* radiolysis–esr method.[9] It has been found that the same radical observed by Yamazaki, *et al.*, in neutral solutions is produced both in very acidic (pH 0) and in very basic (pH 13) solutions, presumably as a result of oxidation of ascorbic acid by radiation produced OH radicals. This work clearly shows that the radical exists as the anion and has the structure indicated by I with the unpaired electron spread over a



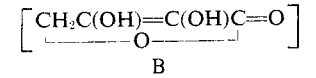
I

highly conjugated tricarbonyl system. Studies of the pH dependence of the esr parameters of this radical show that its p$K$ is −0.45 and that I is the predominant form even in very acidic solutions. Recently Kirino and Kwan,[6] in a very important study, also observed this radical at pH 1 in chemical flow experiments employing the Fenton reagents as oxidants.

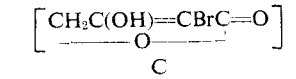
We wish to report our esr observations on the radicals present during the continuous electron irradiation of ascorbic acid (L-xyloascorbic acid) solutions and also related work on ascorbic-*4*-$d_1$ acid, araboascorbic acid (D-araboascorbic acid, the diastereomer of ascorbic acid) and on the model compounds reductic acid (A), α-hydroxytetronic acid (B), and α-bromotetronic acid (C). Ascorbic acid has been examined in



A



B



C

(1) Supported in part by the U. S. Atomic Energy Commission.
(2) I. Yamazaki, H. S. Mason, and L. H. Piette, *J. Biol. Chem.*, **235**, 2444 (1960).
(3) L. H. Piette, I. Yamazaki, and H. S. Mason, "Free Radicals in Biological Systems," M. S. Blois, Jr., H. W. Brown, R. W. Lemmon, R. O. Hindblom, and M. Weissbluth, Ed., Academic Press, New York, N. Y., 1961, Chapter 14, p 195.
(4) C. Lagercrantz, *Acta Chem. Scand.*, **18**, 562 (1964).
(5) G. V. Förster, W. Weis, and H. Staudinger, *Justus Liebigs Ann. Chem.*, **690**, 166 (1965).
(6) Y. Kirino and T. Kwan, *Chem. Pharm. Bull.*, **19**, 718 (1971).
(7) B. H. J. Bielski and A. O. Allen, *J. Amer. Chem. Soc.*, **92**, 3793 (1970).
(8) B. H. J. Bielski, D. A. Comstock, and R. A. Bowen, *ibid.*, **93**, 5624 (1971).
(9) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963); K. Eiben and R. W. Fessenden, *J. Phys. Chem.*, **75**, 1186 (1971).

considerable detail over a wide pH range (from 4 $N$ $HClO_4$ to 0.1 $N$ KOH). In the pH region of 0–6 a second radical in addition to I is also produced. The spectrum of this radical is pH dependent but at pH 1 is identical with one already described by Kirino and Kwan.[6] The acid–base properties of this radical are detailed here and it is shown not to be related to I by a simple protonation equilibrium. This second radical appears to be important with respect to the high rate of disappearance of radicals in the pulse radiolysis experiments at low pH.[8]

## Experimental Section

Ascorbic acid was obtained from Calbiochem, araboascorbic acid from Eastman Organic Chemicals, reductic acid from Pierce Chemical Co., and α-bromotetronic acid from Alfred Bader Chemicals Co. α-Hydroxytetronic acid was synthesized by the method of Micheel and Jung[10] which was also used by Kirino and Kwan[6] in their study. A small amount of a rather impure sample was obtained but this sample was sufficient to demonstrate that the radical observed by Kirino and Kwan[6] is also produced in the radiolysis. Subsequent to our initial studies on this compound Professors Kirino and Kwan supplied us with 500 mg of the sample used in their studies and we are extremely indebted to them for this. Ascorbic acid deuterated at the 4 position was very kindly furnished by Professor B. Tolbert of the University of Colorado. Bell, *et al.*,[11] have previously described the preparation and the nmr spectrum of this sample and the latter unequivocally identifies the position of the label. We estimate from our esr experiments that the sample is 98% deuterated at the 4 position.

The samples were dissolved in neutral triply distilled water which had previously been deoxygenated by bubbling with nitrogen. The pH was then adjusted to the desired value by addition of either KOH or $HClO_4$. In certain cases $KH_2PO_4$ was also added. Solutions were saturated with nitrous oxide before irradiation in order to convert the hydrated electrons to OH radicals and were used within 1–2 hr of preparation. At solute concentrations below $10^{-3}$ $M$ the observed signal intensities were somewhat dependent on concentration so that most experiments were carried out at concentrations of $10^{-2}$ $M$ in order to avoid, as far as possible, problems associated with secondary reactions. Because only limited quantities of the samples were available, deuterated ascorbic acid was examined at $10^{-4}$ $M$ and reductic acid at $10^{-3}$ $M$. One series of experiments on $10^{-2}$ $M$ ascorbic acid was carried out in 99% $D_2O$.

The radicals of interest were generated inside the esr cavity by the reaction of the solutes with the OH radicals produced in the irradiation of the solution with 2.8 MeV electrons. The *in situ* radiolysis–esr arrangement used has previously been described in detail.[6] The improved cavity design described very recently[12] minimizes field inhomogeneities and was necessary for resolution of certain of the smaller splittings. Both 100-kHz and 200-Hz modulation were used and the spectra obtained as the second derivative of the absorption. Magnetic field and microwave frequency were continuously monitored during the scan of each spectrum. Where the lines are narrow and well resolved, coupling constants are normally measurable to better than ±0.02 G and $g$ factors independently determined to better than ±0.00003. In many cases internal comparisons can be made even more accurately than this. The electron beam collected from the cell was ~1 μA or less. A solution flow rate of ~0.5 cc/sec was usually used so that the residence time of the sample within the irradiation zone (~0.025 cc) was ~50 msec. For solutions above pH 7 the radicals produced from ascorbic acid are relatively long lived[8] and little decay occurred within the cavity. At the highest dose rates employed radical concentrations of several times $10^{-4}$ $M$ were attained.

Above pH 7 sufficient intensity was available from each of the solutes so that the radicals containing carbon-13 at the natural abundance level could be detected. As large or larger intensities have been observed for the anion radicals of a number of carboxylic acids produced under similar conditions.[13] Unfortunately, however, the maximum signal intensities were obtained only under operating conditions where some of the spectrometer resolution required for the present study had been sacrificed and it will be seen that as a result uncertainties exist in the determination of the smaller carbon-13 hyperfine constants. For this reason, and in order to remove ambiguities in the assignment of the constants, it is desirable that these studies be extended when specifically labeled $^{13}C$ substances become available.

## Spectra Observed in Basic Solutions

**Ascorbic and Araboascorbic Acids. Proton Hyperfine Structure.** In 1964 Lagercrantz[4] showed that each line of the doublet reported by Yamazaki, *et al.*,[2] for the ascorbic acid radical could be resolved into a 0.17 G triplet. Under conditions of moderate resolution the radicals present during the irradiation of neutral or alkaline nitrous oxide saturated solutions of either ascorbic or araboascorbic acids show six line esr spectra (two 0.19 G triplets separated by 1.8 G) similar to that observed by Lagercrantz.[4] No change in this spectrum was observed up to a pH of 13 (or in fact down to a pH of 1 as will be discussed below). The independently measured $g$ factors are respectively 2.00518 and 2.00519 (which are identical within the measurement accuracy). These $g$ factors are unusually high so that the center of the spectrum is substantially below that of most other simple radicals (~4 G below that for alkyl radicals where $g \approx 2.0025$). The signal-to-noise ratio available is excellent (~500:1) and makes many detailed experiments possible.

At the modulation amplitude usually employed to maximize the signals, the spectra of the radicals from these two diastereomers appear to be identical. Upon lowering the modulation, however, an additional splitting of 0.07 G was partially resolved in the case of ascorbic acid at beam currents of ~0.2 μA or less.[14] Under identical irradiation and spectrometer conditions no analogous splitting was apparent in the case of araboascorbic acid although the width of the individual lines appears to be very similar to that found with ascorbic acid. The spectra obtained under conditions of best resolution are illustrated in Figures 1a and 1b, respectively. The spectrum of Figure 1b was obtained in $D_2O$ (see below) but the spectrum observed in $H_2O$ was identical. A spectrum essentially identical with that of Figure 1b was synthesized by summing second derivatives of Lorentzian curves of line width 0.082 G centered at the positions indicated by the stick spectrum of Figure 1c.[15] One sees very clearly in comparing Figures 1a and 1b that slightly different spectra are found for two radicals which are optical isomers of each other. No difference is, of course, expected for radicals which have only one optical center but a difference is possible in the case of diastereomeric radicals. The difference between Figures 1a and 1b clearly means that the asymmetry of the two optical centers at the $C_4$ and $C_5$ positions of the parent substances is maintained in the radicals.

For the radical from araboascorbic acid a proton

(10) F. Micheel and F. Jung, *Chem. Ber.*, **66**, 1291 (1933).
(11) E. M. Bell, B. M. Tolbert, J. V. Mengenhauser, and E. M. Baker, *J. Phys. Chem.*, in press.
(12) D. Behar and R. W. Fessenden, *J. Phys. Chem.*, **76**, 1710 (1972).
(13) G. P. Laroff and R. W. Fessenden, *J. Chem. Phys.*, **55**, 5000 (1971).
(14) At beam currents higher than 0.2 μA a sufficient radical concentration is present that line broadening occurs as a result of spin exchange between radicals and the 0.07 G doublet structure of Figure 1b is no longer resolvable.
(15) These spectra were conveniently synthesized on a Hewlett-Packard 9100-A calculator equipped with a plotter and an extended memory. The plotting routines were developed by G. P. Laroff and are available from Hewlett-Packard Co. as Program No. 09100-73208.

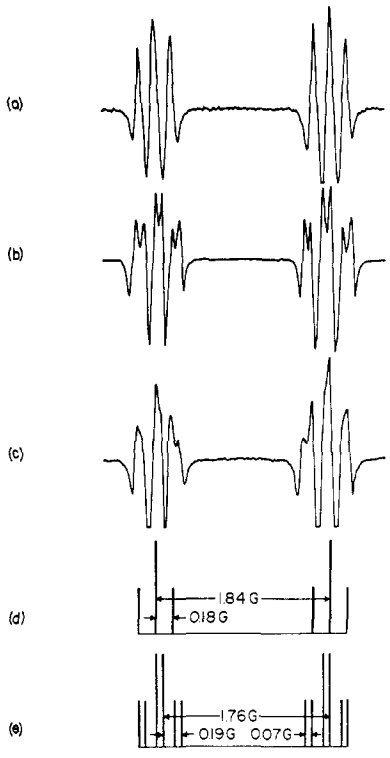



Figure 1. Second-derivative esr spectra of the radicals present during the irradiation of $10^{-2}$ *M* solutions of (a) araboascorbic acid, (b) ascorbic acid, and (c) a 50:50 mixture of ascorbic and araboascorbic acids at pH ~10. Spectrum (b) was taken in $D_2O$ but the spectrum in $H_2O$ is identical. Observations were made at low beam currents and at low modulation with the irradiation and spectrometer conditions identical in all three cases. Each group of lines has the appearance of a simple triplet under higher modulation conditions. Stick spectra for the araboascorbic and ascorbic acid radicals are given in (d) and (e), respectively. All spectra are displayed with the magnetic field increasing from left to right.

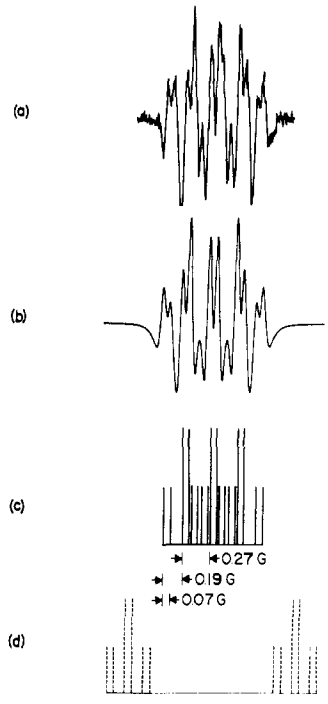



Figure 2. Esr spectra (a) observed during the irradiation of a $10^{-4}$ *M* solution of ascorbic acid deuterated at the 4 position under spectrometer conditions identical with those in Figure 1, (b) spectrum synthesized from the hyperfine pattern given in (c) with Lorentzian lines of width 0.082 G. The stick spectrum of (c) assumes that the 0.07 and 0.19 G splittings are identical with those in the completely protonated radical and that the 1.76 G doublet of the proton on $C_4$ is replaced by a three-line deuterium pattern with a splitting of 1.76/6.514 = 0.27 G. Stick spectrum (d) is that of the protonated radical. With higher modulation small absorptions are observed at these latter positions and correspond to ~2% isotopic impurity in the sample. The *g* factor measured from spectrum (a) was 2.00519. Irradiation of a sample spiked with 10% of normal ascorbic acid shows, as expected, that this *g* factor is identical (*i.e.*, within 0.00001) with that of the protonated radical.

analogous to the one responsible for the 0.07 G doublet in the case of ascorbic acid must also be present. The outermost lines for each of the triplets for the radical from araboascorbic acid appear to have a line width similar to that discussed above (~0.08 G) so that the coupling constant for this proton must be less than 0.04 G. The stick spectrum of Figure 1d also treats the pattern for this radical as a simple triplet. It is noted, however, that the intensities of the central lines of each of the triplets are only ~35% greater than those of the outer lines. This situation implies that the two protons responsible for the triplet structure are not quite equivalent on the time scale of the esr experiment. An effective difference of 0.038 G was estimated by synthesis of the spectrum of 1a. If any similar nonequivalence exists in the case of ascorbic acid, it is lost in the complications resulting from the other features of the spectrum. The spectral data for both of these radicals are recorded in Table I.

Because of the great similarity of the spectra illustrated in Figures 1a and 1b it was decided to examine a solution containing both solutes in order to eliminate the possibility that the difference between Figures 1a and 1b might be caused by some uncontrolled experimental variable. The resulting spectrum is reported in Figure 1c. It is obvious that radicals from both solutes are present. From the symmetry of the superimposed spectra it is evident that, as expected, the *g*

**Table I.** Esr Spectral Parameters

| | Ascorbic acid | Arabo-ascorbic acid | Hydroxy-tetronic acid | Reductic acid |
|---|---|---|---|---|
| *g* factor | 2.00518 | 2.00519 | 2.00519 | 2.00519 |
| $a_{H_4}$ | 1.76 | 1.84 | 2.32 | 6.00 |
| $a_{H_5}$ | 0.07 | <0.04[a] | | |
| $a_{H_6}$ | 0.19 | 0.17[b] | | |
| | 0.19 | 0.21[b] | | |
| $a^{C_1}$[c] | 5.74 | 5.70 | 5.72 | 4.11 (2) |
| $a^{C_3}$ | 3.62 | 3.72 | 3.65 | |
| $a^{C_4}$ | 2.78[d] | 2.62 | 2.85 | 2.43 (2)[e] |
| $a^{C_5}$ | 2.30[d] | 2.34[d] | | |
| $a^{C_2}$ | 0.96[f] | 0.92[g] | 1.03 | 0.88 |
| $a^{C_6}$ | *h* | *h* | | |

[a] Upper limit from observed line width (see text). [b] Difference of 0.038 G between the hyperfine constants of the 2 protons at the $C_6$ position determined from the reduced intensity of the central line of the triplets in Figure 1a (see text). [c] Assignment of the $^{13}C$ hyperfine constants to specific carbon atoms is tentative. The assignment to the $C_2$, $C_4$, and $C_5$ atoms appears to be reasonably certain. Assignment to $C_1$ and $C_3$ atoms may be inverted (see text). [d] Value obtained from detailed fitting of spectral pattern and subject to considerable uncertainty (see text). [e] This value is assigned to the equivalent $C_4$ and $C_5$ carbon atoms. Note that the $C_5$ position in the radical from reductic acid is not analogous structurally to the $C_5$ position in the radicals from the ascorbic acid. [f] An alternative value of 1.34 G is possible. [g] An alternative value of 1.30 G is possible. [h] Not observed. It is assumed that a carbon atoms at this $C_6$ position would have a very small coupling constant.

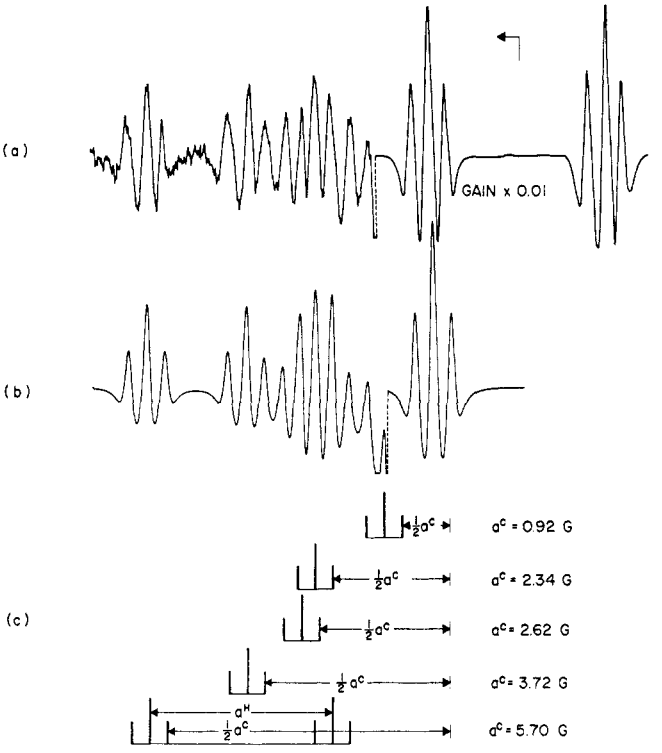



Figure 3. Low field portion of the esr spectrum of the $^{13}C$-containing radicals produced in the irradiation of araboascorbic acid: (a) experimentally observed spectrum and (b) spectrum synthesized assuming lines of Lorentzian shape with a width of 0.11 G located at the line positions indicated in (c). The spectral center is indicated by the position of the arrow and the entire spectrum is symmetrical with respect to this center.

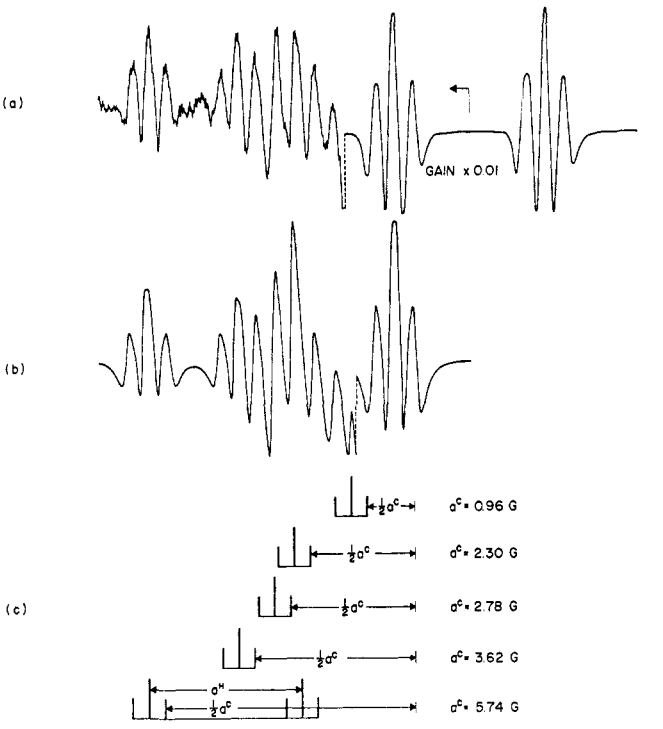



Figure 4. Low field portion of the esr spectrum of the $^{13}C$-containing radicals present during the irradiation of ascorbic acid: (a) experimentally observed spectrum and (b) spectrum synthesized assuming pairs of 0.11 G wide lines separated by 0.07 G with the centers of the pairs located at the positions indicated in (c). The entire spectrum is symmetrical with respect to the position of the arrow.

factors of the two radicals are identical within less than 0.00001. The observed spectrum can be simulated from the data of Table I assuming equal abundances of the two radicals.

The spectrum of Figure 2a was observed during the irradiation of ascorbic acid deuterated at the 4 position. The spectrometer conditions here were identical with those used in obtaining Figure 1b. As can be seen by comparing the observed spectrum with the position of the lines of the protonated radical (indicated as Figure 2d) the entire spectrum of the deuterated radical exists in the window between the positions for the two sets of "triplets" of the latter. It is quite obvious from this fact that the proton at the 4 position is responsible for the 1.76 G doublet of the ascorbic acid radical.[16] The spectrum synthesized by assuming that the 1.76 G proton splitting is replaced by a 1.76/6.514 = 0.27 G deuterium splitting (with the other coupling constants as in the protonated radical and a line width of 0.082 G) is illustrated in Figure 2b (stick spectrum in Figure 2c). Under higher modulation conditions very weak lines of the protonated radical can be seen outside the spectrum of the deuterated radical. It is estimated that these lines arise from about a 2% impurity of the protonated substance in the sample (*i.e.*, about the normal level of isotopic impurity expected in this type of preparation). No exchange appears to occur between the $C_4$ proton of the radical and the water within the ~50 msec observation time applicable to these experiments.

Examination of ascorbic acid in $D_2O$ gave a spectrum (Figure 1b) identical with that seen in $H_2O$. Since the protons of the hydroxyl groups of the side chains will have been exchanged by deuterium, all four remaining protons are accounted for in terms of the observed coupling constants of 1.76, 0.19, 0.19, and 0.07 G. It is reasonable that, as assigned by Lagercrantz,[4] the two equal splittings in the ascorbic acid radical are associated with the protons on the $C_6$ carbon, leaving the 0.07 G splitting to be assigned to the $C_5$ position. The observation of a slight difference between the two splittings at the $C_6$ position in the araboascorbic acid radical, however, shows that these two splittings are not necessarily identical in ascorbic acid. The lack of symmetry of the radical, of course, allows such a difference to exist. The proton at the $C_5$ position is expected to be most affected by the stereochemical difference between ascorbic and araboascorbic acids and it is not surprising that a significant difference between its coupling constant in the two radicals exists.

**Ascorbic and Araboascorbic Acids. $^{13}C$ Hyperfine Structure.** Spectra which illustrate the $^{13}C$ patterns outside the $^{12}C$ structure are shown in Figures 3 and 4 for ascorbic and araboascorbic acids. In each case the observed spectrum should represent the superposition of the individual spectra of the six different $^{13}C$-containing radicals on the 200-fold more intense spectrum of the unlabeled species. These spectra were obtained at high beam currents and under modulation conditions

(16) Piette, *et al.* (ref 3), assigned the 1.8 G doublet to the proton on the $C_4$ atom. This interpretation of the original assignment has been confirmed by L. H. Piette (private communication). Lagercrantz (ref 4), however, misinterpreted the notation used by Piette, *et al.*, to mean that the proton was on the $C_5$ atom. The confusion apparently arose because the terminology used by most esr spectroscopists differs from that of organic chemists. Esr spectroscopists commonly refer to the position on which the electron resides as the $\alpha$ position.

such that the spectra of the $^{12}C$ radicals have the appearance of two simple triplets. The entire spread of the spectrum is less than 8 G in each case. The spectra have good mirror symmetry so that, in spite of the high gain necessary, there is no evidence that any impurity radical contributes to these spectra.

The spectra of the two sets of radicals are clearly different. The spectrum from araboascorbic acid appears to be much more highly resolved than that from ascorbic acid and has a more discernible structure: 12 "lines" are clearly observable outside of the main spectrum of the radical from araboascorbic acid but only ten outside that from ascorbic acid. In both cases one $^{13}C$-containing radical with a carbon coupling constant of $\sim$5.7 G and a second with a constant $\sim$3.7 G are apparent. The outermost two sets of triplets have an intensity as expected from the natural abundance level of $^{13}C$. The spectral center for each of these radicals is identical with that of the $^{12}C$ radical. The $^{13}C$ coupling constants for each of these carbon-13-containing radicals are quite accurately known (to $\pm$0.02 G) and are given as the uppermost entries for $a^C$ in Table I.

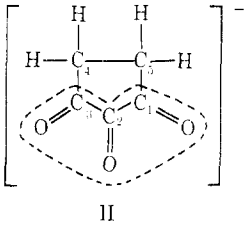
Disentangling the remaining structure is much more problematical. Because of its greater simplicity the spectrum from araboascorbic acid will be discussed first. It must be recognized that in this case the species with $a^C$ = 5.70 G will contribute a second triplet at a field 1.84 G above the first. This pattern is indicated by the lowest stick spectrum of Figure 3c. Two unaccounted for lines appear at a field just below this triplet so that a third $^{13}C$-containing radical with a coupling constant $\sim$2.6 G must contribute in this region. There is no obvious excess intensity within the region of the six outermost lines of the spectrum so that the seventh line is assigned as the outermost line of the triplet structure of this third radical (with a coupling constant of 2.62 G). One further line, unrelated to any of these three $^{13}C$-containing radicals, is seen as the twelfth from the left and one additional line, which was observed only with difficulty and is not displayed in the figure, was shown to be present 0.2 G higher on the tail of the $^{12}C$ spectrum. These latter lines require the existence of a fourth $^{13}C$ radical with a coupling constant of $\sim$1.0 G. A tentative assignment of the twelfth line in Figure 3a is made as the outermost line of a triplet pattern and gives a $^{13}C$ coupling constant of 0.92 G. The spectrum synthesized from the five triplet patterns indicated above clearly has insufficient intensity at the position of the ninth maximum and makes it apparent that a fifth $^{13}C$-containing radical must also be present. Adding in an additional triplet pattern at the position corresponding to a $^{13}C$ coupling constant of 2.34 G gives the synthetic spectrum illustrated in Figure 3b which has apparent line centers at the experimentally observed positions. The appearance of this spectrum is extremely sensitive to differences of the detail in which the individual lines overlap so that one has very little freedom in the adjustment of the hyperfine parameters in fitting the observed spectrum.

The $^{13}C$ coupling constants of the araboascorbic acid radical are from the above treatment 5.70, 3.72, 2.62, 2.34, and 0.92 G. Presumably these represent the five radicals labeled at positions 1–5. The $^{13}C$ hyperfine constant of the radical labeled at position 6 is expected to be sufficiently small that, at the natural abundance level, it will be masked by the spectrum of the unlabeled species. As mentioned above the 5.70 and 3.72 G constants are quite accurately known. The 2.62 coupling constant would appear also to be known accurately. The 0.92 G value is subject to an uncertainty of assignment which makes an alternative value of 1.30 possible though, from synthesized spectra, this seems unlikely. The fourth value (2.34 G) is subject to the greatest uncertainty since it comes from detailed fitting of the total spectrum. There is, however, reasonable certainty that a radical with approximately this coupling constant is present.

The spectrum from ascorbic acid was treated in a like manner except that the basic triplet pattern was replaced by two triplets of line width 0.11 G separated by the unresolved splitting of 0.07 G. The patterns resulting from the two largest $^{13}C$ coupling constants (5.74 and 3.62 G) are readily seen in Figure 4a. As in the case of araboascorbic acid, the triplet pattern on the left requires an image shifted inward by the proton hyperfine constant of 1.76 G. The remaining structure requires the presence of at least two additional $^{13}C$-containing radicals with coupling constants $\sim$2.7 and 1.0 G. Examination of synthesized patterns indicates that a fifth radical must also be present. The synthetic spectrum obtained, assuming $^{13}C$ coupling constants of 5.74, 3.62, 2.78, 2.30, and 0.96 G, is given in Figure 4b. Both the 2.78 and 2.30 G coupling constants are somewhat uncertain because of the distorted appearance of the sixth maximum. The 0.96 G constant is subject to an ambiguity similar to that in the case of araboascorbic acid and an alternative value of 1.34 G is possible. In this case in particular it would be desirable to have $^{13}C$ enriched material for better measurements of these latter three coupling constants.

It is seen in Table I that in spite of the difference in the appearance of the spectra obtained from ascorbic and araboascorbic acids the $^{13}C$ hyperfine constants of the two radicals are very similar. Because the two sets of hyperfine constants essentially represent independent determinations, the general agreement lends weight to the analysis given above. The hyperfine constants are, however, not identical in the two radicals. The small variations lead to pronounced differences in the appearance of the two spectra because of the high degree of overlap of the lines.

**The Radical from Reductic Acid.** Reductic acid can be considered as a model compound for the ring system of ascorbic acid. Oxidation of this compound was shown by Yamazaki, *et al.*,[2,3] to lead to a radical with a 1:4:6:4:1 quintet esr spectrum so that the radical must have four equivalent protons. This radical is, on the average, symmetrical with equal spin densities on the $C_1$ and $C_3$ carbons and is presumably planar with the structure



II

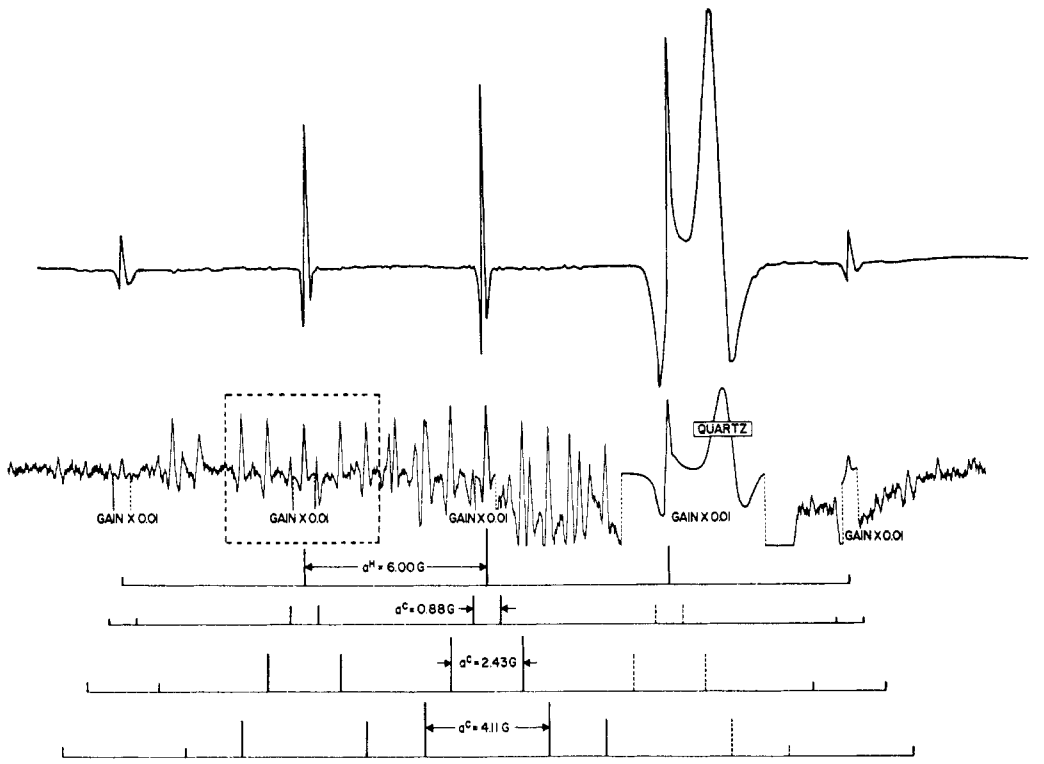



Figure 5. The esr spectrum of the radical present during the irradiation of a $10^{-3}$ *M* solution of reductic acid. A very intense 6.00 G quintet of radical II is present. At a 100-fold higher gain a considerable number of additional lines are apparent. The only lines present that can be appropriately assigned to $^{13}C$-containing radicals are at the positions indicated by the three lowermost stick spectra. The three pairs of lines of relative intensities 0.01, 0.01, and 0.005 are readily seen in the enclosed area around the second line of the main spectrum.

We have observed a very similar spectrum during the irradiation of a $10^{-3}$ *M* solution of reductic acid at pH 11.3. The spectrum obtained is illustrated in Figure 5a and appears to be identical with that reported by Yamazaki, *et al.*, except that the proton hyperfine constant of 6.00 G determined in the present study is 0.3 G smaller than the value previously reported. The *g* factor of this radical is 2.00519.

The spectrum in Figure 5b was obtained at a 100-fold higher gain and somewhat lower modulation. A fairly large number of lines appear with an intensity of approximately 1% of those of the main radical. The only lines of this intensity that are symmetrically disposed around the lines of the $^{12}C$ species are sets of six lines separated from each of the main lines by ±2.055, ±1.215, and ±0.44 G. This pattern can be seen particularly well within the area indicated around the second main line on the left side of the spectrum. The lines with the two larger splittings are ~1% as intense as the main lines. The lines with the smallest splitting, which are barely observable in the figure, are readily resolvable under lower modulation conditions and slower scan rates and have an intensity half that of the other lines. The separations of the remaining lines from those of the $^{12}C$ spectrum do not repeat so that these other lines can be dismissed as resulting from impurity species. No attempt was made to purify the small (0.2 G) amount of sample available since there was no interference with the interpretation of the main features of the spectrum. The lines of 0.5% intensity split by a $^{13}C$ hyperfine constant of 0.88 G are ascribable to the radicals labeled at the unique position $C_2$. The equivalence of the carbon atoms at positions $C_1$ and $C_3$ and also at $C_4$ and $C_5$, as required by the proton hyperfine pattern, is also demonstrated by the doubled intensity of the lines with the larger splittings.

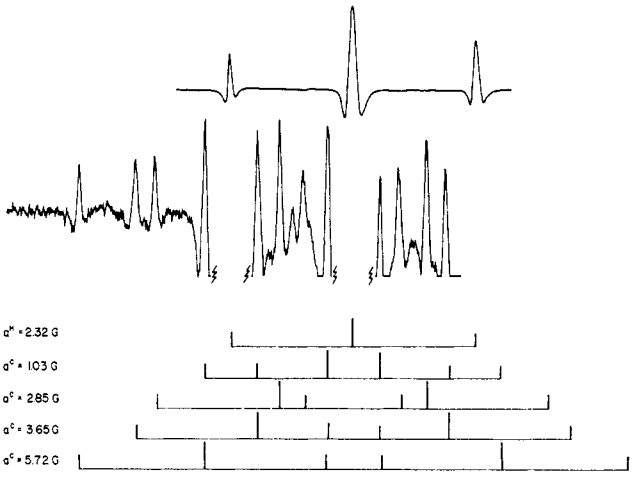
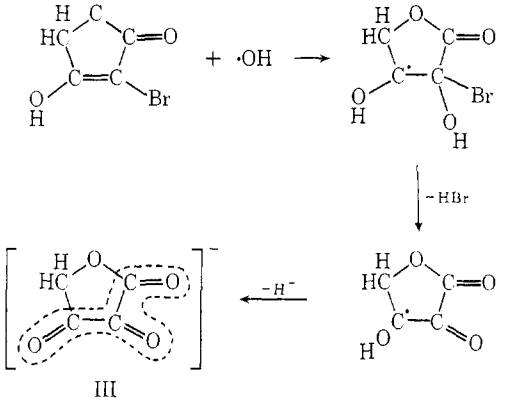



Figure 6. Esr spectra observed during the irradiation of a $1.7 \times 10^{-3}$ *M* solution of α-hydroxytetronic acid ($N_2O$ saturated, pH 9.5). The upper and lower traces were obtained at relative gains of 1 and 200.

**Radical from α-Hydroxytetronic Acid.** The second and more appropriate model is α-hydroxytetronic acid. In this compound the side chain of ascorbic acid has been replaced by a hydrogen atom. Kirino and Kwan have examined this compound in their flow studies[6] at pH 1 and report a radical which has a triplet esr pattern with $a^H$ = 2.3 G and $g$ = 2.0050. It was obvious from their work that this radical has a structure very similar to that of the radical produced from ascorbic

acid. We have irradiated a sample of α-hydroxytetronic acid at pH 9.5 and have observed a similar triplet (see Figure 6) with the parameters $a_H$ = 2.32 G and $g$ = 2.00519 (line width = 0.1 G) which we assign to radical III illustrated below. There is no evidence of nonequivalence of the two protons so that these protons are presumably symmetrically disposed with respect to a plane containing the remaining atoms of the radical.

A radical identical with the above was also prepared by irradiating α-bromotetronic acid in a solution saturated with $N_2O$. In this latter case it is quite evident that hydroxyl radicals add to the double bond and produce radical III by dehydrohalogenation of the adduct (see below). The intensity of the spectrum of



III

radical III obtained from α-bromotetronic acid although high ($S/N \approx 50:1$) was, however, insufficient to observe the $^{13}C$-containing radicals. Subsequent pulse radiolysis experiments showed that in this system radical III disappears by second-order reactions with other radicals produced from the α-bromotetronic acid.

The sample of α-hydroxytetronic acid kindly supplied by Professors Kirino and Kwan allowed determination of the $^{13}C$ hyperfine constants of radical III. Irradiation of an $N_2O$ saturated $1.7 \times 10^{-3}$ $M$ solution at pH 9.5 resulted in the spectrum of Figure 6. Lines of the $^{13}C$-containing radicals are readily observable at a $S/N$ of 5:1 (see the lower spectrum of Figure 6). The three outermost lines on the low-field side clearly define the three largest $^{13}C$ hyperfine constants (5.72, 3.65, and 2.85 G) and the remaining lines of their spectra are observable at the appropriate positions. The fourth and fifth lines from the end of the spectrum are principally satellites of the central line (relative intensity 2) and mask any lines of the fourth $^{13}C$-containing radical in this region. This fourth radical cannot have a $^{13}C$ hyperfine constant larger than 1.1 G. The two innermost lines surrounding the central line of the $^{12}C$ species appear to be too intense to be ascribable to satellites of the end lines of the spectrum. A 1.03 G hyperfine constant was determined on the assumption that the excess intensity results from the fourth $^{13}C$-containing radical.

**The Electronic Structure of the Radicals.** As indicated in the introduction, a major matter of concern is the state of ionization of the ascorbic acid radical. While it seems obvious from the esr data that the radical exists as an anion, an argument can be advanced from the high value of the second p$K$ of ascorbic acid (11.79; the first p$K$ is 4.10) that in neutral solutions oxidation of the parent anion will lead to a neutral radical. The fact that there is no change in the spectrum up to a pH of 13 suggests at that no ionizable proton exists in the radical. The equivalence of the four protons in the related radical from reductic acid clearly shows this radical to be an anion. It should also be noted that the radical produced from biacetyl is known to exist in the anionic form ($CH_3\dot{C}OCOCH_3^-$) at all pH values above 6.[17] Bielski, *et al.*, have demonstrated from the effect of ionic strength on the radical recombination rate constant that at pH 9.8 the radical from ascorbic acid is charged.[8]

The high values of the $g$ factors for the four radicals given in Table I imply that in each case there is considerable spin density on the oxygen atoms. This conclusion is corroborated by the low values of the $^{13}C$ coupling constants which show that the spin density must be highly delocalized. Only anionic radicals with the conjugation indicated by structures I–III would seem to be completely consistent with these facts. The simplest of these radicals is that from reductic acid where the spin density on carbon atoms 1 and 3 can be estimated as 0.16 from the observed 6.0 G β-proton hyperfine constant. This estimate uses an approximate value of 25 G for $Q_\beta$ and takes into account the orientation of the CH bond with respect to the π orbital. The low spin density on these carbon atoms can also be seen from a comparison of the 6.0 G constant with that of 23 G found in cyclopentenyl radical[18] where the analogous spin density is close to 0.6. This comparison also gives a carbon spin density of 0.16 and is subject only to the effect of charge on $Q_\beta$. The proton hyperfine constant can also be compared with the values of 5.6 and 7.1 G observed for $a^H_{CH_3}$ in the trans and cis forms of biacetyl radical anion.[17,19] In the latter case Russell, *et al.*,[19] have estimated that the spin density on the carbon atoms of the carbonyl groups is ~0.28 which in turn leads to an estimate of 0.16–0.20 for the spin density on the $C_3$ atom of the radical from reductic acid.

Assignment of the larger two $^{13}C$ coupling constants of radical II to specific carbon atoms is extremely difficult because of the canceling effects in conjugated radicals produced by spin density on the atom in question and that on the adjacent atoms of the π system. (This cancellation is very obvious for biacetyl radical anion where the value of $a^C$ for the carbon of the carbonyl groups is considerably smaller than that of the adjacent $CH_3$ carbon atoms.[19]) Two assignments are possible and arguments can be raised for and against both. From the work on the cyclic semidione radical anions[19] it seems likely that the carbon atoms at the 4 and 5 positions will have a larger coupling constant than those at the 1 and 3 positions. However the work on the semidiones also indicates that the ratio of the coupling constant of a β carbon atom to that of a β proton should be only 0.4 if each is taken as a measure of the spin density on the adjacent carbon

(17) H. Zeldes and R. Livingston, *J. Chem. Phys.*, **47**, 1465 (1967). The p$K$ for dissociation of the radical $CH_3COC(OH)CH_3$ has been measured to be 4.4: J. Lilie, G. Beck, and A. Henglein, *Ber. Bunsenges. Phys. Chem.*, **72**, 529 (1968).

(18) R. H. Schuler and R. W. Fessenden, "Radiation Research," G. Silini, Ed., North-Holland Publishing Co., Amsterdam, 1967, p 99.

(19) G. A. Russell, D. F. Lawson, H. L. Malkus, and P. R. Whittle, *J. Chem. Phys.*, **54**, 2164 (1971).

atom. This parallel leads to the assignment of the observed 2.43 G coupling constant to the carbon atoms at the 4 and 5 positions and, as a consequence, the larger value (4.11 G) to those at the 1 and 3 positions. In any event the smallest coupling constant represents a unique carbon atom and must be assigned to the 2 position. The smallness of this latter value to a large extent reflects the canceling effect of the spin density on the three adjacent atoms.

The similarity of the $g$ factors and of the magnitude of the carbon coupling constants indicates that the electronic structures of all of the radicals are very closely related. The proton at the $C_4$ positions of the radicals from ascorbic, araboascorbic, and hydroxytetronic acids, however, have coupling constants only *ca.* one-third as great as that for the radical from reductic acid. Since these radicals are unsymmetrical such a difference can arise as the result of a shift in spin density away from the $C_3$ atom. A shift sufficiently large to cause the effect observed seems unlikely; however, an alternative explanation involves the existence of spin density on the ring oxygen and an electronic configuration such that the wave function has opposite signs at the ring oxygen and at $C_3$. It has already been pointed out that it is possible for a small spin density on this type of oxygen atom to have a disproportionately large effect on $\beta$ hydrogen splittings.[20] A spin density of only 0.03 on the ring oxygen atom would account for the change observed here.

The $^{13}C$ hyperfine constants observed for the radicals from $\alpha$-hydroxytetronic acid correlate extremely well with four of those found in the ascorbic and araboascorbic acid systems and allow the fifth (2.3 G) hyperfine constant observed in the latter cases to be assigned to the $C_5$ carbon atom. Correlation of the smallest observed coupling constants with that of the unique carbon atom in reductic acid allows assignment of these values to the $C_2$ atoms. The hyperfine constants of 2.6–2.9 are similar to the 2.4 G value assigned above to the $C_4$ atom in reductic acid and are so assigned here. Note that the existence of only one hyperfine constant in this range in the case of hydroxytetronic acid confirms the assignment of the 2.43 G value to the $C_4$ atom in reductic acid. The two remaining (and largest) $^{13}C$ hyperfine constants are assigned to the $C_1$ and $C_3$ carbon atoms. The difference between these values shows that considerable asymmetry in the electron distribution is induced by the oxygen atom. The abnormally small proton hyperfine constant at the $C_4$ position suggests that the smaller of these $^{13}C$ hyperfine constants be assigned to the adjacent carbon atom ($C_3$). Ambiguity in any of the assignments does not, in any event, affect the principal conclusion derived from the small values observed from the $^{13}C$ hyperfine constants, *i.e.*, that there is no substantial localization of spin density on any one carbon atom.

### Spectra Observed in Acidic Solutions of Ascorbic Acid

Spectra were taken in acidic solutions of ascorbic acid with the expectation, from the optical studies of Bielski, *et al.*,[8] that radical I would become protonated in the region of pH 4 and that as a result the esr spectrum would be altered. It was found, however, that no significant change in the spectrum of radical I occurred even down to a pH of 1. Rather, esr spectra of two radicals were observed at all pH values less than 6. The most intense lines are similar to those which also appear in basic solution and which have been assigned to radical anion I. In the following discussion the radical responsible for this spectrum will be called the principal radical. The second radical produces a doublet esr spectrum which at pH 1 is described by the parameters $a^H = 0.73$ and $g = 2.00394$. At this pH these lines are rather broad (0.3 G width). The presence of two radicals in the oxidation of ascorbic acid has already been noted by Kirino and Kwan[6] in their studies with the $Ti^{3+}$–$H_2O_2$ system. (Their parameters for the second radical at pH 1 are $a^H = 0.8$ G and $g = 2.0040$.) They were, however, unable to generate the second radical by direct oxidation with agents such as $Ce^{4+}$.[21] From the agreement of the esr parameters there is little doubt that both the $Ti^{3+}$–$H_2O_2$ reagent and radiolysis produce the same two radicals in ascorbic acid solution and that OH is the precursor of both radicals. This conclusion is confirmed by the fact that the spectrum of the second radical is observed at pH >3 with $N_2O$ present. Demonstration of this fact is very important because H atoms are known to react rapidly with ascorbic acid ($k = 10^8\ M^{-1}\ sec^{-1}$)[22] and it might otherwise be supposed that the second radical is the result of such reaction as no steps were taken to scavenge the H atoms. Bielski, *et al.*,[8] used a mixture of dehydroascorbic acid and ascorbic acid to scavenge both H and OH in acid. It must be emphasized that the conclusions reached below regarding the protonation behavior of the two radicals in acid does not depend on how the radicals are formed.

**The Principal Radical.** It is obvious from the qualitative fact that the spectrum does not change with pH that the principal radical is extremely acidic in character and that the p$K$ of 4.25 assigned by Bielski, *et al.*,[8] to the protonation equilibrium of this radical, in fact, reflects a change in the radical formation reactions resulting from a change in the state of protonation of ascorbic acid itself (p$K$ = 4.1). The intensity of the spectrum of radical I is of the same magnitude over the pH range of 5–10, as is indicated in Figure 7. In more acidic solutions the intensity drops by more than an order of magnitude and more or less plateaus in the region of pH 1. It seems likely that this drop is caused both by a decrease in the production rate and also by an increase in the disappearance rate which results from the presence of other radicals in solution (see below). Bielski, *et al.*,[8] report an increase in the second-order disappearance rate constant by better than 3 orders of magnitude in going from pH >7 to pH <2.

In very acidic solutions (*i.e.*, $[H^+] > 1\ M$) a change in the spectrum is observed as is indicated in Figure 8. At pH 0.7 the spectrum of this radical is still essentially identical with that found at higher pH. In 2 $M$ perchloric acid the low-field triplet has moved toward higher field and in 4 $M$ perchloric acid it has almost merged with the high field triplet. As far as the high

(20) R. W. Fessenden and R. H. Schuler, *Advan. Radiat. Chem.*, **2**, 103 (1970).

(21) Y. Kirino and T. Kwan, *Chem. Pharm. Bull.*, **19**, 831 (1971).

(22) P. Neta and R. H. Schuler, *Radiat. Res.*, **47**, 612 (1971).

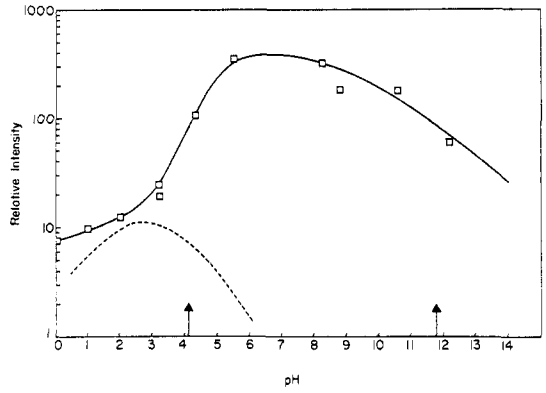



Figure 7. Dependence of the relative intensity of the spectra of the principal (solid curve) and second (dashed curve) radicals on pH. The p$K$ values for the two ionization equilibria of ascorbic acid (4.0 and 11.8) are indicated by the arrows.

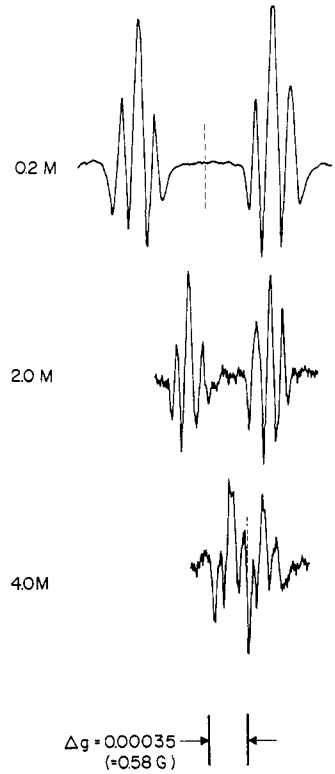



Figure 8. The esr spectra during the irradiation of ascorbic acid in the presence of high concentrations of perchloric acid. The spectrum observed in the presence of 0.2 $M$ $HClO_4$ is essentially identical with that observed at all higher pH values (up to pH 12). At higher acidity the doublet spacing collapses and the center of the spectrum shifts toward higher field by 0.58 G as indicated.

field lines are concerned the decrease in the $g$ factor approximately compensates for the decreased coupling constant so that the positions of these lines move only slightly toward lower field, as is indicated in the figure.

The dependences of the coupling constants and $g$ factor on pH are summarized in Figure 9. It is seen that the changes occur in a parallel fashion and it is evident that a simple protonation equilibrium is involved. The fact that one changing spectrum is observed rather than two superimposed spectra shows that rapid exchange between two forms of the radical is occurring. As a result, the esr parameters observed should be the weighted average of those obtained

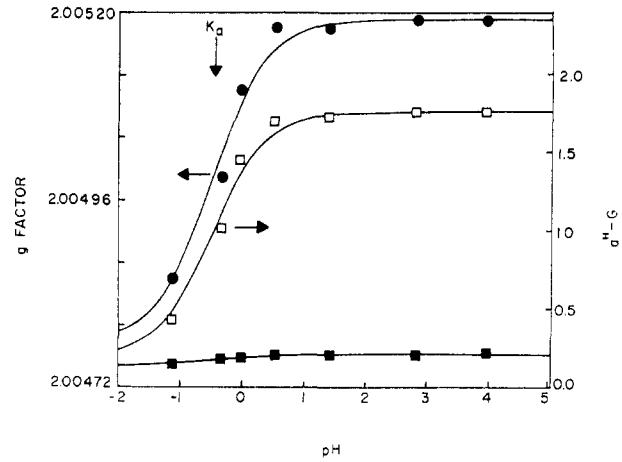
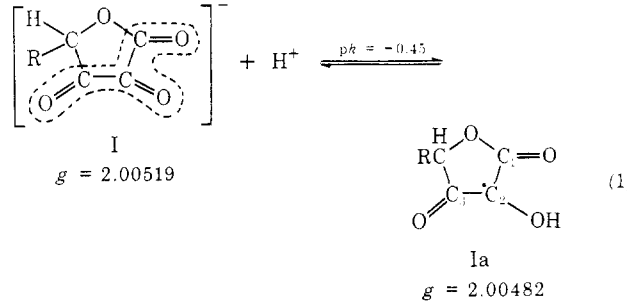



Figure 9. The dependences of the esr parameters of the principal radical produced from ascorbic acid on pH: ●, $g$ factor; □, $C_4$ proton coupling constant; and ■, $C_6$ proton coupling constant. The points for 4 and 2 $M$ perchloric acid have been plotted at corrected pH values taking into account the very high activity coefficients of $H^+$ ion in these solutions. The solid curves have been calculated assuming that the observed parameters represent the weighted average of those of the two forms of the radical present with concentrations governed by a simple acid–base equilibrium with the p$K$ of −0.45 (indicated by the arrow).

from the two limiting spectra. Because the proton coupling constant approaches a very small value in the highly acidic solutions (<0.2 G) it is possible to determine the equilibrium constant quite accurately (p$K$ = −0.45 ± 0.10). The limiting $g$ factor in very acidic solutions should be only slightly less than the value measured in 4 $M$ $HClO_4$. A value of 2.00482 is estimated. The predicted pH dependences of the $g$ factor and $C_4$ coupling constant are then completely determined by the p$K$ and are given by the upper two curves in Figure 9. In the very acidic solutions the small triplet spacing does decrease slightly in the region of pH 0 although, as indicated in the figure, it does not go to zero. The low pH limit here is estimated as 0.14 G but considerable error is, of course, possible because of the relatively poor quality of the spectrum in the concentrated acid.

The changes detailed above require that the protonated radical have very little spin density on $C_3$ in order to explain the resulting low value of the $C_4$ proton coupling constant. We tentatively suggest that protonation occurs at the $C_2$ position.




Structures alternative to Ia with spin density on the oxygen atoms at positions 1 and 3 can be written. Inclusion of oxygen orbitals because of conjugation among these three structures can result in the relatively high $g$ factor observed for the protonated rad-

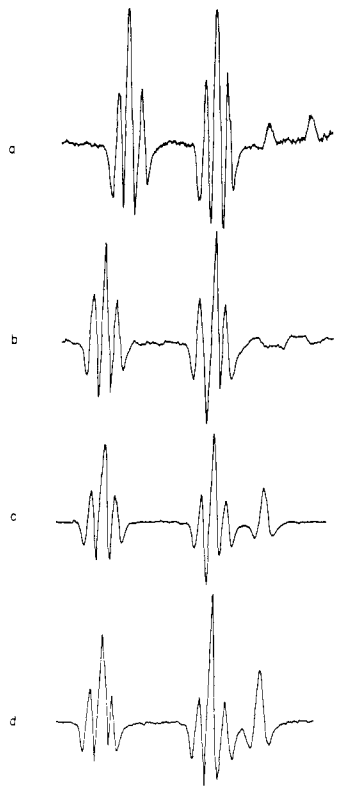


Figure 10. The esr spectra observed during the irradiation of ascorbic acid solutions at pH (a) 0.0, (b) 1.4, (c) 2.8, and (d) 4.0. A doublet spectrum of a second radical is readily apparent in (a). With increase in pH the doublet shifts toward lower field until it distorts the appearance of the high field triplet of the principal radical. At still higher pH values these additional lines decrease in intensity and are not observable for all pH values >6. Note also the change in line width manifest in (b).

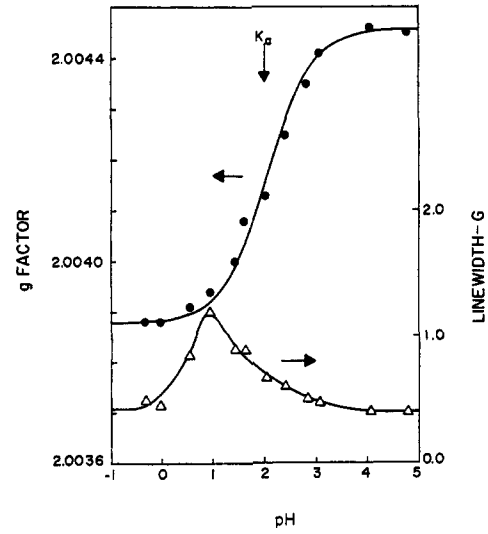



Figure 11. The pH dependence of (●) $g$ factor and (△) line width of the second radical produced from ascorbic acid. The upper curve is calculated assuming a simple acid–base equilibrium with a p$K$ of 2.0 as indicated by the arrow.

ical. With protonation as indicated in Ia no electronic structure with appreciable spin density on either the $C_1$ or $C_3$ carbon atoms can be written. The negligible coupling constant observed for the $C_4$ proton would seem to require this to be the case. Assignment of the protonated form to Ia makes the radical some 4 orders of magnitude more acidic than ascorbic acid itself where the first ionization corresponds to a p$K_a$ of 4.1.

**The Second Radical.** The doublet spectrum observed for the second radical at pH 1 is described above. In their studies on the oxidation of $\alpha$-hydroxytetronic acid by $Ti^{3+}$–$H_2O_2$ reagent Kirino and Kwan[6] found a second radical which exhibited a triplet spectrum with $a^H$ = 0.7 G and $g$ = 2.0037. It is clear from the fact that two equivalent protons are observed in this latter radical that the proton responsible for the doublet in the case of ascorbic acid is at the $C_4$ position. Studies at other values of pH show that the positions of the lines depend significantly on pH. At a pH of 4 the lowermost line overlaps the high field triplet of the principal radical, as is readily seen in examining Figure 10d. As one lowers the pH the lines broaden and shift toward higher field. The shift is sufficiently great that below a pH of 2 both lines are observable outside the spectrum of the main radical. The lines continue to broaden down to a pH ~1 and then narrow again in very acidic solutions. The observed dependence on pH of both the $g$ factor and line width is illustrated in Figure 11. While these other changes are occurring the proton coupling constant, interestingly, does not change significantly. Measurements of the positions of the low field line are difficult above a pH ~2 because this line is masked by the triplet of the principal radical. Up to a pH ~5 the position of this line can be determined reasonably well by comparing the details of synthesized spectra with those observed. A value of 0.75 G is found at a pH ~4. At higher pH values the relative intensity is insufficient for such a procedure and for pH values >6 the high field line is not observed at all. Where the low field line is not directly measurable, the $g$ factors given in Figure 11 have been determined from the position of the high field line on the assumption that the splitting is 0.75 G. Approximate relative concentrations of this second radical in these steady-state experiments, as estimated from the integrated signal intensities, are given in Figure 7 for various pH values.

The pH dependence of the $g$ factor follows that expected for a simple acid–base equilibrium involving two forms of a single radical. The limiting values are measurable on both the acidic ($g_{acidic}$ = 2.00388) and basic ($g_{basic}$ = 2.00446) sides of the equilibrium, and as is indicated by the solid curve the experimental data are very well defined in the intermediate region by these two limits and a p$K$ of 2.0. The change in line width should be associated with the dynamics of this equilibrium but the fact that the maximum width occurs at a pH below the p$K$ is puzzling. A direct relation between this line broadening and the acid–base equilibrium would require the maximum width to occur on the high pH side of the equilibrium. Examination of the shape of the high field line in Figure 10b suggests that this "line" is actually a poorly resolved doublet. The line broadening can be explained by the develpment of a small splitting ascribable to an OH proton as the equilibrium shifts toward the acidic form of the radical. Subsequent narrowing at even lower pH would then result from exchange of this proton in strong acid.

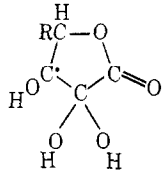
The fact that the spectrum of the second radical coexists with that of I in the region of pH 1 indicates

that these two radicals are not related by a simple protonation equilibrium. Such an explanation would ignore the separate equilibria observed for the two radicals and would require the forward rate constant to

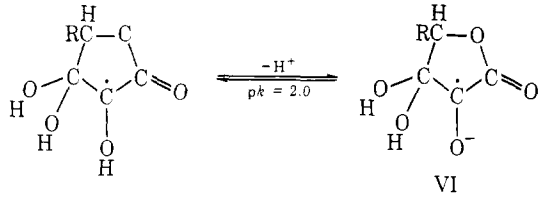
$$A^- + H^+ \rightleftarrows AH$$

be $<10^7\ M^{-1}\ sec^{-1}$ which is several orders of magnitude less than known rate constants for this type of reaction. The rapidity of the equilibria observed individually for the two radicals, as shown by the continuous change in the spectral parameters (see Figures 9 and 11), strongly substantiates this conclusion. Furthermore with increase in acidity below pH 2 the signal intensity of radical I does not decrease as rapidly as that of the second radical, as is noted in Figure 7. It is quite clear from these various arguments that the second radical is not simply a protonated form of the first.

From the fact that the second radical is not observed for pH values higher than 6 it appears that the neutral form of ascorbic acid must be present for its formation. Kirino and Kwan have shown that it is produced by reaction of OH radicals but not by other oxidizing agents.[21] The fact that a similar radical is observed in the case of $\alpha$-hydroxytetronic acid[6] shows that attack must be on the ring system. The simplest conclusion is that this radical is produced by OH addition to the $C_2{=}C_3$ double bond. Two possible initial radicals are IV and V. Radical IV must have a large proton



IV




V VI

hyperfine constant (>15 G) and can be ruled out while radical V is quite consistent with the observed spectrum. Tentatively we suggest that radical V, in equilibrium with radical VI, is responsible for the second spectrum. The $g$ factors observed are similar to those of radicals derived from a number of $\alpha$-hydroxy acids and the $g$ factor difference on protonation is also very similar (*e.g.*, the $g$ factor for the radical from lactic acid increases by 0.00037 upon ionization of the OH proton).[23] The main difficulty with this assignment is the very low value observed for the p$K$, a value which is considerably lower than, for example, those of radicals from hydroxy acids.[24] The explanation given here is also at variance with the work of Bielski, *et al.*,[8] where it was found that there is no effect of ionic strength on the rate of disappearance of radicals at pH 3.3. This result implies that the radicals present at this pH are neutral. From the esr data we, of course, know only that an acid–base equilibrium is involved but it is difficult to conceive of a positively charged radical which can give the doublet spectrum observed.

If radical V is formed, then it does not seem likely that addition will be completely selective and radical IV should also be produced. We propose that radical IV rapidly loses both water and a proton to form radical I. We have carried out preliminary pulse esr experiments which show that a significant yield of radical I is present 5 $\mu$sec after the pulse. Presumably radicals V and VI will also dehydrate to form I but, if the above explanation is correct, they must do so on a longer time scale. Radical I can also be produced directly by electron transfer from ascorbic acid. Where ascorbic acid exists in the anionic form, electron transfer appears to be dominant since there is no evidence in the esr spectra of the addition product.

### Comments on the Kinetics of Radical Disappearance

Bielski, *et al.*,[8] have shown that the second-order rate constant for radical disappearance in the ascorbic acid system increases markedly with decreased pH below 7. Two changes in rate constant are observed: the first in the pH region of 7–5 and the second in the region of 2.5–0.5. Parallel changes are, however, not found in the intensities of the esr spectra obtained in the steady-state experiments. It should be pointed out that these esr experiments focus attention on the radicals remaining on the millisecond time scale so that a direct comparison with the observations at much shorter times in the pulse radiolysis experiments is not necessarily possible. Bielski, *et al.*,[8] have ascribed the observed increase in rate constant to reactions of the neutral form of the radical. The esr experiments, however, show that the principal radical exists predominantly as the anion down to a pH of 0 so that the recombination rate constant for this species is not expected to be strongly dependent on pH. The observed increase in the rate constant must result from reaction of the ascorbic acid radical with other radicals produced in this system.

### Conclusions

These esr experiments show quite definitively that the principal radical formed from the oxidation of ascorbic acid by OH radicals is highly conjugated and is present as the anion over the pH range of 1–13. In very acidic solutions this radical protonates rapidly and reversibly with the p$K$ of the equilibrium being $-0.45$. Over the pH range of 0–6 oxidation by OH also produces a second radical which exists in two forms also related by a protonation equilibrium (with a p$K$ of 2.0). These facts must be taken into account in the analysis of results from pulse radiolysis experiments. The most obvious way of correlating observed radical decay rates with the esr data is to assign the drop by a factor of 5 which occurs over the range 0.5–2.5[8] with the protonation equilibria involving the second radical. The pronounced decrease in rate observed for pH values >5 can be ascribed to a change in the reaction mechanism which occurs as the result of ionization of ascorbic acid. It is obvious that time resolved esr experiments, which are yet to be carried out, can provide the answer to many questions regarding the mechanism

(23) A. Samuni, D. Behar, and R. W. Fessenden, *J. Phys. Chem.*, submitted for publication.
(24) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, 73, 4214 (1969).

and kinetics of the radical reactions which occur in this system. It would also appear in order to reinvestigate certain aspects of the pulse radiolytic work in acidic solutions keeping in mind the fact that more than one radical is now known to be present.

**Acknowledgment.** We wish to thank Dr. B. H. J. Bielski of Brookhaven National Laboratory for drawing our attention to questions about the structure of the ascorbic acid radical and for many valuable discussions on this subject. We are indebted to Dr. B. M. Tolbert of the University of Colorado for furnishing the sample of ascorbic-4-$d_1$ acid and for his various suggestions. We also wish to thank Dr. W. L. Mock of Carnegie-Mellon University for many helpful discussions on the synthesis of $\alpha$-hydroxytetronic acid and to thank Miss M. A. Schuler for her assistance in the preparation of this compound and Professors Y. Kirino and T. Kwan of the University of Tokyo for furnishing the final sample which allowed observation of the $^{13}C$ spectra of radical III.

# Three-Electron Oxidations. II. The Chromium(VI) Oxidation of Oxalic Acid[1]

**Fariza Hasan and Jan Roček***

*Contribution from the Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois 60680.*
*Received April 14, 1972*

**Abstract:** The chromic acid oxidation of oxalic acid ($H_2C_2O_4$) has been reinvestigated over a wide range of conditions. Oxalic acid is oxidized by chromium(VI) by two different mechanisms, each involving a different oxalic acid–chromic acid complex. The complete mechanism (Scheme III) leads to the rate law $v = [Cr_T][OxH_2]\{k_1h_0 + (kK_I \cdot K_{II}K_a^{OxH_2}[OxH_2])/(1 + h_0/K_a^{H_2CrO_4} + K_I[OxH_2]h_0 + K_IK_{II}'K_a^{OxH_2}[OxH_2]^2/h_0)\}$, where $[OxH_2]$ is the concentration of undissociated oxalic acid and $[Cr_T]$ the sum of the concentrations of all chromium(VI) species present. Kinetic evidence for the formation of a chromium(VI)–oxalic acid 1:1 and 1:2 complexes has been obtained and the equilibrium constants for their formation have been determined. The 1:1 complex exists as a neutral species and is most probably a cyclic anhydride. The 1:2 complex is stable as a dianion and exists most likely in an open chain form in which the usual coordination number of four for chromium(VI) is retained. The reactive intermediate in the second-order reaction is the monoanion of the 1:2 complex, $HO_2CCO_2CrO_3COCO_2^-$. It is proposed that this intermediate decomposes directly into $[Cr(H_2O)_6]^{3+}$, three molecules of $CO_2$ and a free radical $\cdot CO_2H$, in a one-step three-electron oxidation reaction. The formation of free-radical intermediates has been demonstrated. In the presence of an excess of acrylamide, the yield of carbon dioxide is reduced to a limiting value which is in agreement with the proposed mechanism. An unexpected exchange between $[Cr(H_2O)_6]^{3+}$ and oxalic acid under the reaction conditions has been observed.

In the first paper of this series[1] we reported what we believe to be the first documented case of a three-electron oxidation in organic chemistry.[2,3]

As organic substrates generally undergo at most a two-electron oxidation in a single step, a one-step three-electron reduction of an oxidant must involve more than one molecule of an organic substrate in the activated complex and the rate law will therefore be higher than first order in the total of organic compounds involved. In looking for further examples of three-electron oxidation reactions, we were therefore interested in those reactions for which a higher than first-order kinetic dependence in the substrate was reported. As chromium(VI) oxidation of oxalic acid is the prime example of such a reaction, we decided to investigate it in more detail.

The chromic acid oxidation of oxalic acid has been the subject of numerous studies.[4-13] According to Chakravarty and Ghosh,[8] the reaction is first order in hexavalent chromium, is second and third order in oxalic acid, and exhibits a too complex dependence on acidity to allow simple description in form of a rate law. Rao and Ayyar[9] reported similar results and proposed a mechanism involving chromium(VI) species with coordination numbers of six and eight. Chandra, Shukla, and Chatterji[10] reported a second-order dependence in oxalic acid and independence on the hydrogen ion concentration, but close examination of their data reveals a slightly higher order in oxalic acid and a noticeable acidity dependence. Bakore and Jain[11] were the first to notice that a much simpler rate dependence can be obtained if instead of the total oxalic acid concentration the concentrations of undissociated

(1) Part I: F. Hasan and J. Roček, *J. Amer. Chem. Soc.*, **94**, 3181 (1972).
(2) Haight earlier suggested a three-electron oxidation in an organic system involving chromium(VI) and hydrogen sulfite.[3]
(3) G. P. Haight, E. Perchoncek, P. Emmenegger, and G. Gordon, *J. Amer. Chem. Soc.*, **87**, 3835 (1965).
(4) K. Jablezynsky, *Z. Anorg. Chem.*, **68**, 38 (1908).
(5) N. R. Dhar, *J. Chem. Soc.*, **111**, 707 (1917).
(6) C. Wagner, *Z. Anorg. Allg. Chem.*, **168**, 279 (1928).
(7) H. C. S. Snethlage, *Recl. Trav. Chim. Pays-Bas*, **59**, 111 (1940).
(8) D. N. Chakravarty and S. Ghosh, *J. Indian Chem. Soc.*, **34**, 841 (1957).
(9) G. G. Rao and R. V. V. Ayyar, *Z. Phys. Chem. (Leipzig)*, **236**, 17 (1967).
(10) S. Chandra, S. N. Shukla, and A. C. Chatterji, *J. Inorg. Nucl. Chem.*, **31**, 805 (1969).
(11) G. V. Bakore and C. L. Jain, *ibid.*, **31**, 805 (1969).
(12) C. A. Durham, *ibid.*, **31**, 3549 (1969).
(13) G. P. Haight, Jr., Tracy J. Huang, and B. Z. Shakhashiri, *ibid.*, **33**, 2169 (1971).