Electron Spin Resonance Spectra of Radicals Related to the Intermediates in the Oxidation of Ascorbic Acid. The Radical Produced from γ-Methyl-α-hydroxytetronic Acid[1]

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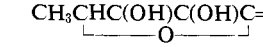
Abstract: The radical intermediate produced in the oxidation of aqueous solutions of γ-methyl-α-hydroxytetronic acid by OH radicals has been examined by the *in situ* radiolysis-esr method. The *g* factor (2.00519) and proton hyperfine constants ($a^{H}(H_4)$ = 1.84 G and $a^{H}(CH_3)$ = 0.05 G) for the radical produced in neutral and basic solutions are very similar to those for the radical produced in the chemical and radiation chemical oxidation of ascorbic acid. Spectra of four of the five ^{13}C-containing radicals have been observed at the natural abundance level (a^{C} = 5.69, 3.54, 2.76, and 0.91 G). It is clear that above pH 1 the radical exists as an anion with the unpaired electron delocalized in a highly conjugated tricarbonyl system. From the dependences of the *g* factor and the proton hyperfine constant on pH, the p*K* for the protonation of the radical anion has been determined to be −0.40.

The principal radical produced in the oxidation of ascorbic acid by radiation generated OH radicals has been studied in considerable detail by esr methods,[2] and it has been shown that this radical is identical with that present in enzymatic[3] and chemical[4-6] oxidation experiments. In the radiation chemical experiments the radicals can be produced in sufficient concentration that those containing ^{13}C at the natural abundance level are observable. The spectra resulting from the ^{13}C-containing radicals are sufficiently complex and the hyperfine constants sufficiently small that analysis of the patterns is difficult. In particular, assignment of a pattern to the radical containing ^{13}C at the C_5 position is ambiguous.[2] It is useful, therefore, to extend the previous study to the model compound γ-methyl-α-hydroxytetronic acid where the side chain of ascorbic acid has been replaced by a methyl group. Determination of the hyperfine constant of the methyl group protons should also assist in the assignment of the smaller proton hyperfine constants observed in the ascorbic acid system. We have carried out a study of the radical intermediates present during the oxidation of γ-methyl-α-hydroxytetronic acid by the *in situ* radiolysis-esr method[7] and report the results here.

Experimental Section

γ-Methyl-α-hydroxytetronic acid (the enol of the γ lactone of 2,4-



dihydroxyacetoacetic acid, also known as 3,4-dihydroxy-5-methyltetrone) was prepared by the method of Micheel and Haarhoff.[8] The product (2 g) was purified by recrystallization from ether–petroleum ether. These samples, however, contained a small amount of by-product α-hydroxytetronic acid (estimated to be ~5% from the esr measurements). The lines of the radical produced from this latter substance (see ref 2) unfortunately mask certain regions of interest in the ^{13}C studies. The sample was purified further by preparative thin layer chromatography on silica gel and the resultant material shown (from the esr studies) to contain less than 0.5% α-hydroxytetronic acid. The final sample (14 mg) was dissolved in 500 ml of solution to give a 0.2 m*M* solution which was satisfactory for observing the ^{13}C spectra at the natural abundance level.

The *in situ* radiolysis-esr method[7] was used as in the previous study.[2] Solutions were saturated with N_2O to convert hydrated electrons to OH radicals. Except where noted, studies were carried out at pH ~10.5.

The CH_3 proton hyperfine constant of the radical produced from γ-methyl-α-hydroxytetronic acid was determined by comparing the detailed shape of the observed lines with the shape of synthesized compound lines. It was assumed that the observed lines represent unresolved 1:3:3:1 quartets with a component width governed by the lines for the radical from α-hydroxytetronic acid. The calculation–plotting routine developed by Laroff (Hewlett-Packard Co. Program No. 09100-73208; see ref 2) was used. A line shape with 50% Lorentzian–50% Gaussian character was chosen as most closely resembling the lines of α-hydroxytetronic acid under the experimental conditions. This shape exhibits slightly lower resolution than lines of 100% Lorentzian character and places a less stringent upper limit on the magnitude of the CH_3 hyperfine constant.

Results and Discussion

The dominant esr spectrum observed during the oxidation of γ-methyl-α-hydroxytetronic acid by radiation produced OH radicals consists of two lines corresponding to a radical with a *g* factor of 2.00519 and a single proton having a hyperfine constant of 1.84 G. The *g* factor is essentially identical with those of the radicals produced from ascorbic (L-xyloascorbic), araboascorbic (D-araboascorbic), and α-hydroxytetronic and reductic acids, and the proton hyperfine constant is very similar to that of the radicals from ascorbic and araboascorbic acids. It was not possible to resolve the individual lines resulting from the protons on the methyl group. One can, however, show that the hyperfine constants of these protons are 0.05 ± 0.01 G by comparing the width and shape of the spectral lines obtained in the γ-methyl-α-hydroxytetronic acid system with that of the lines for the radical from α-hydroxy-

(1) Supported in part by the U. S. Atomic Energy Commission.
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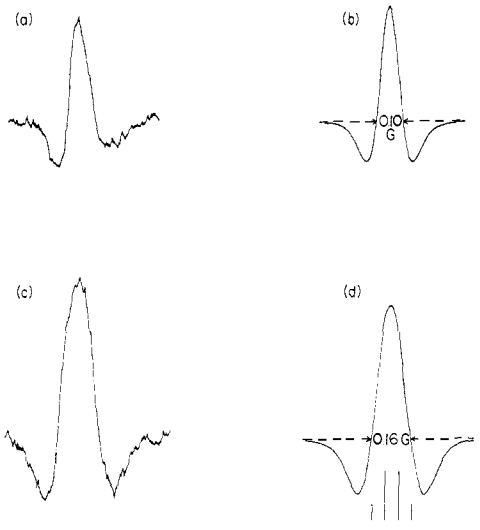


Figure 1. Second derivative esr lines of the radicals derived from α-hydroxytetronic acid and γ-methyl-α-hydroxytetronic acids and the corresponding synthetic lines. (a) The lowest field line of the proton hyperfine structure experimentally obtained from α-hydroxytetronic acid with an observed line width of 0.10 G. (b) Spectral line synthesized assuming 50% Lorentzian–50% Gaussian shape with a width of 0.10 G. (c) The low field line obtained from γ-methyl-α-hydroxytetronic acid under the same experimental conditions as in a. In this case the apparent width is 0.16 G. (d) Spectral line synthesized by superposition of lines of 50% Lorentzian–50% Gaussian shape with an individual width of 0.10 G and intensity ratio of 1:3:3:1 separated by 0.05 G as indicated by the stick diagram.

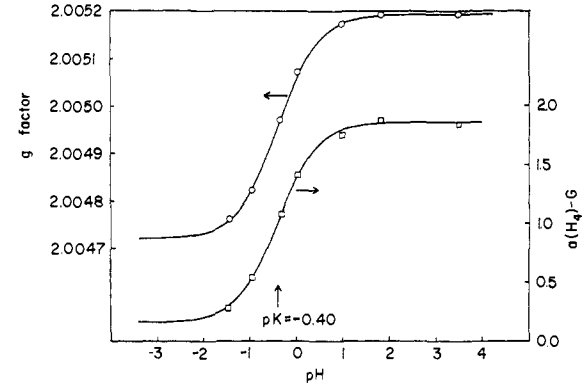


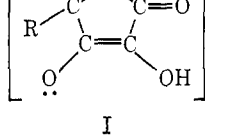
Figure 2. The pH dependences of the g factor (○) and H_4 proton hyperfine constant (□). The points for 2, 4, and 6 M perchloric acid solutions have been plotted at corrected pH values taking into account the very high activity coefficients of the H^+ ion in these solutions. The solid curves have been 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 $pK = -0.40$.

tetronic acid. This comparison, which was made under identical spectrometer conditions, is illustrated in Figure 1. The apparent width of the observed line in Figure 1c (0.16 G) is unquestionably greater than that of the component width as indicated in the α-hydroxytetronic acid system (Figure 1a; 0.10 G). Synthesis of compound lines (as illustrated in Figure 1d) shows that the hyperfine constant cannot be less than 0.04 G since this would give rise to a narrower than observed compound line. A CH_3 proton hyperfine constant larger than 0.06 G would give rise to a resolvable pattern.

A CH_3 proton hyperfine constant of the magnitude of 0.05 G substantiates the previous assignment[2] of the 0.07 G proton hyperfine constant observed in the ascorbic acid system to the C_5 proton and leaves the remaining two constants of 0.19 G to be attributed to the C_6 protons as in the original assignment of Lagercrantz.[4] Since the two C_6 protons need not necessarily be equivalent (see, for example, the previous discussion of the araboascorbic acid spectrum),[2] there had been a slight ambiguity on this point.

The spectrum obtained with a 2 mM solution of γ-methyl-α-hydroxytetronic acid containing ~5% α-hydroxytetronic acid impurity exhibited the lines of the principal radical with a S:N of ~2000:1. In this spectrum the lines of radicals containing ^{13}C with hyperfine constants of 5.69, 3.54, and 0.91 G are readily observable with S:N of ~10:1. The radicals produced from the α-hydroxytetronic acid impurity, however, mask the spectral region for radicals having ^{13}C hyperfine constants in the range 2.5–3.2 G. The spectrum obtained with the chromatographically purified material is somewhat less intense (~5:1) but clearly shows one additional ^{13}C-containing radical with $a(^{13}C)$ = 2.76 G. No contribution from a fifth ^{13}C-containing radical was observable. In the spectrum obtained with the purified γ-methyl-α-hydroxytetronic acid, there is no line at the position corresponding to $a(^{13}C)$ = 2.3 G so that the previous tentative assignments of ~2.3 G to ^{13}C at the C_5 position of ascorbic and araboascorbic acids would seem to be incorrect. Laroff and Fessenden[9] have shown that in certain aliphatic radicals the hyperfine constants of γ carbon atoms are ~40% of those of β protons where the two nuclei have comparable orientations relative to the unpaired electron orbital. If this relationship is transferable to the present example, one expects a hyperfine constant of the carbon atom of the methyl group of only ~0.7 G, and the lines of a radical having such a small value would be masked by the tails of the lines of the principal radical and not be observable at the natural abundance level. The lines of the radical with a^C = 0.91 G are distorted somewhat by these tails but careful examination of these lines indicates that they represent only a single ^{13}C-containing radical. We conclude that the fifth ^{13}C-containing radical must have a hyperfine constant <0.8 G.

The esr spectral parameters obtained in this study are summarized in Table I and compared with those of the related radicals from ascorbic, araboascorbic, and α-hydroxytetronic acids. Above pH 5 the parent compounds exist predominantly in the anionic form having the structure



I

where R is H for α-hydroxytetronic acid, CH_3 for γ-methyl-α-hydroxytetronic acid, and $HO(CH_2)CH(OH)$ for the diastereomers ascorbic and araboascorbic acids. The similarity of the hyperfine constants between the various radicals is obvious and it is clear that the radical

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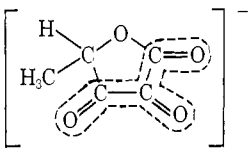
Table I. Esr Spectral Parameters

	Ascorbic acid[a]	Arabo-ascorbic acid[a]	α-Hydroxy-tetronic acid[a]	γ-Methyl-α-hydroxy-tetronic acid
g factor[b]	2.00518	2.00519	2.00519	2.00519
$a(H_4)$[c]	1.76	1.84	2.32	1.84
$a(H_5)$	0.07	<0.04		0.05
$a(C_1)$	5.74	5.70	5.72	5.69
$a(C_3)$	3.62	3.72	3.65	3.54
$a(C_2)$	0.96	0.92	1.03	0.91
$a(C_4)$	2.78	2.62	2.85	2.76
$a(C_5)$	*d*	*d*		<0.8

[a] From ref 2. [b] The *g* factors are known with an absolute accuracy of 0.00003 and relative to each other with an accuracy of 0.00001. [c] The hyperfine constants are accurate to 0.02 G. [d] The previous assignment of hyperfine constants of ~2.3 G to the C_5 carbon atoms (ref 2) now appears to be incorrect by virtue of the fact that no such constant is observed in the present case.

present in the γ-methyl-α-hydroxytetronic acid system has the structure



II

with the unpaired electron being delocalized in the tricarbonyl system.

Radical II can be produced from the parent anion by OH attack either directly by electron transfer to the OH radical or indirectly by addition of the OH to the double bond at either the C_2 or C_3 position followed by loss of water, *i.e.*

$$\cdot OH + I \longrightarrow II + H_2O \quad (1)$$

Pulse radiolysis experiments[10] show that for ascorbic acid reaction 1 is complete within a millisecond.

The protonation of radical I was examined by determining the pH dependence of the *g* factor and proton hyperfine constant and the results are illustrated in Figure 2. These parameters are independent of pH above pH 2. Studies were carried out in more acidic solutions (to solutions 6 *M* in $HClO_4$). The detailed interpretation of these results is somewhat more simple than those for the ascorbic acid system, since the additional structure resulting from the protons on the C_6 position is absent. The *g* factor of the protonated form of the radical is estimated as 2.00472, and the hyperfine constant of the C_4 proton as 0.14 G (compared with the estimates[2] of 2.00482 and 0.16 G in the ascorbic acid case). The p*K* for the protonation of radical I is −0.40.

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Electronic Structure and Spectroscopy of Parabanic Acids[1]

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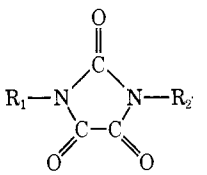
Abstract: The four lowest energy excited electronic states of parabanic acid and three of its alkyl derivatives have been characterized by means of empirical generalizations, CNDO/s–CI computational results, and a composite-molecule analysis of the acid in terms of its oxamide and carbonyl residues. The lowest energy $S_i \leftarrow S_0$ transitions are assigned, in order of increasing energy as $^1B_2 \leftarrow {}^1A_1$ ($^1\Gamma_{n_+\pi_+^*} \leftarrow {}^1\Gamma_1$), $^1B_1 \leftarrow {}^1A_1$ ($^1\Gamma_{\pi_\ominus\pi_+^*} \leftarrow {}^1\Gamma_1$), and $^1A_1 \leftarrow {}^1A_1$ ($^1\Gamma_{\pi_\ominus\pi_+^*} \leftarrow {}^1\Gamma_1$). The luminescence of parabanic acid and methylparabanic acid is assigned as $^3B_2 \rightarrow {}^1A_1$ ($^3\Gamma_{n_-\pi_+^*} \rightarrow {}^1\Gamma_1$). The luminescence of the dialkyl derivatives of parabanic acid involves decay of an excited state of mixed $^3\Gamma_{n\pi^*}/{}^3\Gamma_{\pi\pi^*}$ character. The drastic change of emissive characteristics caused by dialkylation is attributed to a decrease of the $^3\Gamma_{n\pi^*}/{}^3\Gamma_{\pi\pi^*}$ energy gap. Finally, a composite-molecule analysis indicates that the T_1 state of all parabanic acids is highly localized on the dicarbonyl residue, a prediction substantiated by vibronic analyses of the phosphorescence spectra.

A large group of molecules is related, at least in a formal sense, by the presence of —C(=O)N< units bonded directly to identical units or to such functional units as >C=O, —N<, >C=C, etc. Included in this group are urea, oxamide, parabanic acid, alloxan, barbituric acid, and uric acid. It has been shown[2] that a useful interpretation of the absorption and emission characteristics of oxamides, >N–CO–CO–N<, in terms of amide properties may be obtained using a composite-molecule analysis of CNDO/s–CI computational results. The formal similarity of parabanic acid (imidazolidinetrione) and *cis*-oxamide suggests that a composite-molecule analysis of the more complex molecule can also lead to useful results.



The following derivatives were studied (R_1, R_2, acronym): parabanic acid (H, H, PA); methylparabanic acid (CH_3, H, MPA); dimethylparabanic acid (CH_3, CH_3, DMPA); di-*n*-propylparabanic acid (C_3H_7, C_3H_7, DPPA).

(1) This work was supported by contract between the United States Atomic Energy Commission-Biology Branch and The Louisiana State University.

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