

ESR Studies of Ascorbic Acid Free Radicals

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One-electron oxidations of L-ascorbic acid and its analogs or derivatives were investigated by electron spin resonance (ESR). ESR spectra were obtained from compounds not substituted at 3-hydroxyl group. Two types of spectra were observed when Ti^{3+} - H_2O_2 system was used as the oxidizing agent. On the basis of the comparison of the ESR spectra obtained from the compounds examined with one another and their pH-profile, the radical structures and hyperfine couplings were determined. One type of spectrum corresponds to an anion radical and the other corresponds to a neutral species. Variation in line width and the finding that only an anion radical was detected when oxidizing reagents other than Ti^{3+} - H_2O_2 system was used, were interpreted in terms of the complex formation of radicals with metal ions coexisting in the reaction system.

It has generally been accepted that the redox reaction between L-ascorbic acid (AsA) and dehydroascorbic acid (DAsA) proceeds in two one-electron-transfer steps with the formation of a free radical intermediate, monodehydroascorbic acid (MDAsA). Among a number of kinetic studies dealing with the metal ion-catalyzed oxidation of AsA with molecular oxygen, mention may be made to the recent reports^{2,3)} which have shown that the step at which a complex composed of metal ion, AsA and O_2 decomposes to form MDAsA is the rate-determining in the reaction in an acidic non-buffered aqueous solution.

As to the biological role of AsA, there is a possibility that it is a component of the electron-transport chain. It has been shown by Staudinger, *et al.*⁴⁾ and also by Frunder, *et al.*⁵⁾ that the redox system of AsA composes a cycle in the liver microsomal oxidation of NADH and that the electron acceptor is not DAsA but MDAsA in the cycle.⁶⁾

Even from only a few examples cited above, there needs little comment on the importance of detecting MDAsA directly and elucidating its structure and properties.

ESR studies on MDAsA have already been performed by several investigators.⁷⁻⁹⁾ Unfortunately, however, the spectral parameters were not well characterized, leaving some question of interest on the structure of MDAsA. Yamazaki, *et al.*⁷⁾ who observed for the first time a doublet spectrum proposed the radical structure A shown in Chart 1 and assigned the hyperfine (h.f.) splitting of 1.7 G to the proton bonded to the carbon atom of 4 position according to the nomenclature for ascorbic acids ($\text{C}_4\text{-H}$). Lagercrantz⁸⁾ observed a well-resolved spectrum composed of a doublet (1.7 G) of triplets (0.17 G) and assigned its h.f. coupling constants of 1.7 G to $\text{C}_5\text{-H}$ and of 0.17 G to two $\text{C}_6\text{-H}$'s in the radical A. Foerster,

1) Location: Bunkyo-ku, Hongo 7-3-1, Tokyo.

2) M.M.T. Khan and A.E. Martell, *J. Am. Chem. Soc.*, **89**, 4176 (1967).3) Y. Ogata, Y. Kosugi, and T. Morimoto, *Tetrahedron*, **24**, 4057 (1968).4) K. Krisch and H. Staudinger, *Biochem. Z.*, **331**, 195 (1959).5) H. Frunder, E. Blume, and H. Kluge, *Biochim. Biophys. Acta*, **65**, 146 (1962).6) H. Staudinger, R. Abraham, and W. Schneider, *Biochim. Biophys. Acta*, **74**, 547 (1963).7) I. Yamazaki, H.S. Mason, and L.H. Piette, *Biochem. Biophys. Research Commun.*, **1**, 336 (1959); *idem*, *J. Biol. Chem.*, **235**, 2444 (1960); L.H. Piette, I. Yamazaki, and H.S. Mason, "Free Radicals in Biological System," ed. by M.S. Blois, Jr., H.W. Brown, R.M. Lemmon, R.O. Lindblom, and M. Weissbluth, Academic Press, 1961, p. 195; I. Yamazaki and L.H. Piette, *Biochim. Biophys. Acta*, **50**, 62 (1961).8) C. Lagercrantz, *Acta Chem. Scand.*, **18**, 562 (1964).9) G.v. Foerster, W. Weis, and H. Staudinger, *Ann. Chem.*, **690**, 166 (1965).

*et al.*⁹⁾ also obtained a similar spectrum ascribing it to the radical B; the h.f. splittings of 1.7 G and 0.17 G were attributed to C₄-H and two of C₅- and C₆-protons, respectively.

On the other hand, the structure C quite different from those mentioned above was proposed by Russell.¹⁰⁾ According to this proposal one could apparently explain the ESR spectrum assuming that the splittings of 1.7 G and 0.17 G were due to C₅-H and two equivalent C₆-H's, respectively. However, the structure C lacking C₄-H in the furan ring does not sound enough if both AsA and DAsA should have C₄-hydrogen atom.

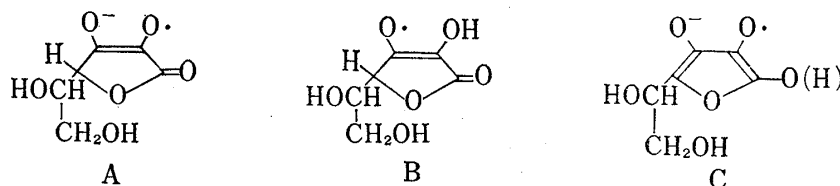


Chart 1

We have also investigated¹¹⁾ ESR of MDAsA where two types of ESR spectra were recognized, one of which being a new spectrum.

The purpose of this report which is a continuation and extension of the earlier work is to determine the radical structures and clarify their properties by using a series of analogs and derivatives of AsA as well.

Experimental

Materials—A purified sample of L-ascorbic acid (I), supplied by Takeda Chemical Industries, Ltd., was used without further purification. D-Araboascorbic acid (II) used was of reagent grade. 3,4-Dihydroxy-tetrone (III) was synthesized by the Claisen condensation of ethyl benzoylglycolate according to Micheel, *et al.*¹²⁾ and purified by recrystallizations. 3,4-Dihydroxy-5-methyltetrone (IV) was also prepared by the mixed Claisen condensation of ethyl benzoylglycolate and ethyl benzoyllactate and purified by fractional crystallizations. D-Glucoascorbic acid (V) was prepared from D-glucosone according to Reichstein, *et al.*¹³⁾ and Haworth, *et al.*¹⁴⁾ and the monohydrate was obtained by recrystallizations from water. 5,6-Isopropylidene-L-ascorbic acid (VI) was prepared according to Vargha.¹⁵⁾ 3-Methyl-L-ascorbic acid (VII) and 2,3-dimethyl-L-ascorbic acid (VIII) were prepared according to Haworth, *et al.*¹⁶⁾ by the methylation with equimolar and excessive diazomethane, respectively. Dehydroascorbic acid (anhydrous) was prepared according to Kenyon and Munro¹⁷⁾ and purified by the method of Staudinger and Weiss.¹⁸⁾ All of these compounds were identified by melting points, elemental analyses and infrared spectra, and nuclear magnetic resonance (NMR) spectra were also measured and analyzed except for dehydroascorbic acid. Compounds I—VI which have the same structures except their side chains were shown¹⁹⁾ to have the same chemical properties with respect to redox reactions.

Deionized water was used for preparation of solutions after it was confirmed to make no difference on ESR spectra from distilled water. All the other chemicals used were of highest grade.

Apparatus—The ESR spectrometer and the flow apparatus employed were just the same as described in the preceding paper.¹¹⁾

Oxidations and procedure—In most of oxidations in aqueous solutions, titanous ions and hydrogen peroxide (Ti³⁺-H₂O₂ system), which generate hydroxyl radicals on mixing each other, were used as an oxidizing reagent. When EDTA equimolar with Ti³⁺ was added, it was available in the wide range of pH below 9 without precipitation. Also was used Fenton's reagent (Fe²⁺-H₂O₂ system), with or without EDTA.

- 10) G.A. Russell, *Rec. Chem. Progress*, **27**, 26 (1966); *idem*, "Radical Ions," ed. by E.T. Kaiser and L. Kevan, Interscience Publishers, 1968, p. 88.
- 11) Y. Kirino and T. Kwan, *Chem. Pharm. Bull. (Tokyo)*, **19**, 718 (1971); *idem, ibid.*, **19**, 831 (1971).
- 12) F. Micheel and F. Jung, *Chem. Ber.*, **66**, 1291 (1933); *idem, ibid.*, **67**, 1660 (1934).
- 13) T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta*, **17**, 510 (1934).
- 14) W.N. Haworth, D.L. Hirst, J.K.N. Jones, and F. Smith, *J. Chem. Soc.*, **1934**, 1192.
- 15) L. von Vargha, *Nature*, **130**, 847 (1932).
- 16) W.N. Haworth, E.L. Hirst, and F. Smith, *J. Chem. Soc.*, **1934**, 1556.
- 17) J. Kenyon and N. Munro, *J. Chem. Soc.*, **1948**, 158.
- 18) H. Staudinger and W. Weiss, *Z. Physiol. Chem.*, **337**, 284 (1964).
- 19) F. Smith, *Carbohydrate Chem.*, **2**, 79 (1946).

In addition, potassium ferricyanide was employed in strongly alkaline solution and ceric ions, Ti^{3+} - NH_2OH system known to generate amino radicals, potassium bichromate and permanganate were used in strongly acidic solutions. In all the cases mentioned above, ESR measurements were achieved by the flow technique.

For autoxidation in basic DMSO solution including potassium *tert*-butoxide and for comproportionation of AsA and DAsA in neutral aqueous solution, ESR spectra were measured by a static method.

All the ESR measurements were carried out at room temperature and concentrations of radicals formed in aqueous solutions were determined by taking the first moments of the corresponding spectra and comparing them to that of one of the eight lines for an aqueous solution of vanadyl sulfate.

Attempts to trap MDAsA by using nitrosobenzene or 3-methyl-3-nitrosobutanone-2 as a spin trap were unsuccessful.

Result and Discussion

Oxidations in Aqueous Solutions at $\text{pH} > 5$

In Fig. 1 is shown a typical ESR spectrum observed during an oxidation of L-ascorbic acid (I) with Ti^{3+} (EDTA)- H_2O_2 system in an aqueous solution where pH was adjusted at 5—9 with ammonium hydroxide. This doublet-triplet spectrum, denoted as l_1 for convenience, is the same as those reported by earlier workers.

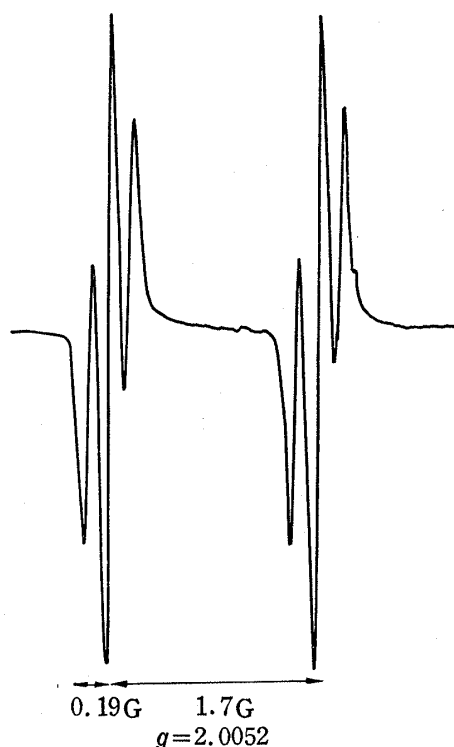


Fig. 1. ESR Spectrum (l_1) Observed 10 msec after Mixing Solution A and B at pH 8.5

Peak height ratio is (1:2.45:1.12):(1.01:2.39:1.02).

Soln. A (Ti^{3+} (EDTA): 0.005M)

AsA : 0.01M

Soln. B (H_2O_2 : 0.1M)

AsA : 0.01M

To judge whether hydroxyl protons contribute to the hyperfine structure (h.f.s.) or not, ESR spectra were investigated for AsA and DAsA admixed in neutral (pH 6—7) water or heavy water. As the result, both solutions gave the same spectra as l_1 , showing that none of the hydroxyl protons were hyperfine-coupled with the odd electron.

The same spectrum was obtained also from II, which is a C_5 -epimer of I, under a similar reaction condition to that shown at the bottom of Fig. 1.

On the other hand, any spectrum was observed from neither VII (methylated at the C_3 -hydroxyl group) nor VIII (methylated at both of the C_2 - and C_3 -hydroxyl). This suggests that the radical corresponding to the l_1 spectrum is an oxygen-centered radical produced by the attack of oxidizing agent to the C_3 -hydroxyl group. The value of g -factor, 2.0052, that is reasonable for an oxygen-centered radical, may support this suggestion.

The l_1 radical is considered to be an anion species, for the same spectra were observed during the oxidation of I or II with ferricyanide under a strongly alkaline condition (pH 12.6) where I and II are present as their dianion forms. This consideration is to be substantiated by the pH-

profile of the radical concentration later. Our problem is now to solve what protons are h.f. coupled among four protons bonded to C_4 , C_5 , and C_6 in the oxygen-centered anion radical. So, similar experiments were performed for III, IV, and V each of which is an analog of I having a side chain of different length. Figs. 2, 3, and 4 present the spectra l_{III} , l_{IV} , and l_{V} derived from III, IV, and V, respectively.

The spectrum l_{III} is a sharp triplet with an intensity ratio of 1:2:1, indicating the presence of two C_4 -H's in the radical formed. Therefore, such type of radical structure as C having a furan ring should be excluded in the case of l_{III} .

The spectrum l_{IV} , which is a somewhat broader doublet ($\Delta H_{msl}=0.18$ G), indicates again the presence of C_4 -H ($A(C_4-H)=1.8$ G) in the radical formed from IV and, therefore, the radical does not have a furan ring but a tetrone ring. Evidently, the broadening of each line is caused by the latent splittings due to the methyl protons. According to Voevodskii, *et al.*²⁰ who simulated ESR spectra using a ratio of line width and h.f.

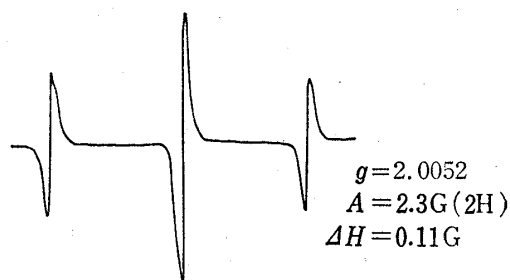


Fig. 2. ESR Spectrum (l_{III}) Observed during the Oxidation of 3,4-Dihydroxy-tetrone (III) with $Ti^{3+}(EDTA)-H_2O_2$ System at pH 8.5

microwave power 1.7 mW
modulation width 0.010 G

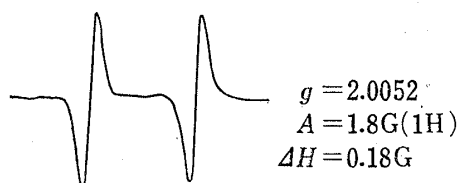


Fig. 3. ESR Spectrum (l_{IV}) Observed during the Oxidation of 3,4-Dihydroxy-5-methyltetrone (IV) with $Ti^{3+}(EDTA)-H_2O_2$ System at pH 8.5

microwave power 3.5 mW
modulation width 0.028 G

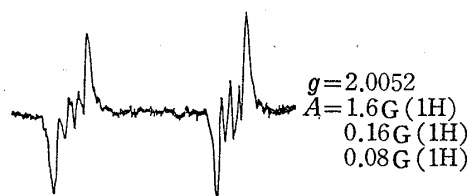


Fig. 4. ESR Spectrum (l_V) Observed during the Oxidation of D-Gluco-ascorbic acid (V) with $Ti^{3+}(EDTA)-H_2O_2$ at pH 8.5

microwave power 1.7 mW
modulation width 0.10 G

coupling constant as a parameter, the h.f. coupling constant of the methyl proton (C_5 -H) is estimated to be smaller than a half of line width, 0.09 G.

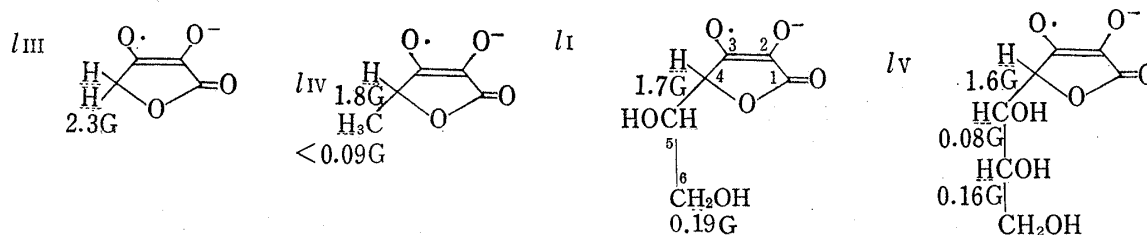
Consideration of the results for l_{III} and l_{IV} may lead us to assume that the l_I radical, too, has a tetrone ring where the h.f. splittings should be assigned as follows: $A(C_4-H)=1.7$ G, $A(C_6-H)=0.19$ G, because $A(C_5-H)$ could be too small to be resolved as found for l_{IV} and moreover there is no particular reason for the nonequivalence of the two C_6 -H's. From the above reasons, it is natural to presume that the compound II should also give the same spectrum as l_I , as it was actually the case.

In the spectrum l_V (Fig. 4), each of doublet lines is analyzed, according to the simulation curves by Voevodskii, *et al.*²⁰ to be composed of four lines of equal separation (0.08 G) and equal intensity one another. Therefore, the l_V radical has three h.f. coupled protons whose coupling constants are 1.6, 0.16, and 0.08 G, respectively. If they are assigned as $A(C_4-H)=1.6$ G, $A(C_5-H)=0.08$ G, and $A(C_6-H)=0.16$ G, there may be no contradiction with the interpretation of the spectra l_I-l_{IV} .

A surprisingly large value of h.f. coupling constant of C_6 -H in the radical l_I or l_V would lead one to suppose that C_6 -H might be located near the radical center by, for example, an intramolecular hydrogen bonding or a hemi-ketal ring formation of the side chain. This, however, is not the case, for the compound VI, 5,6-isopropylidene derivative of I, also gave the same spectrum as l_I during the oxidation in an alkaline solution. In the radical l_I , l_{III} , l_V , or l_{VI} the side chain would probably be free against the five-membered ring.

The structure of l -type radicals and the assignment of their proton hyperfine coupling constants were summarized in Chart 2.

20) Ya. S. Lebedev, D.M. Chernikova, N.M. Tikhomirova, and V.V. Voevodskii, "Atlas of Electron Spin Resonance Spectra, 1," Consultants Bureau, 1963.

Chart 2. Structure of *l*-type Radicals and Assignment of Hyperfine Coupling Constants

We shall now discuss the anomalously large coupling constant of C_6 -H. Electron spin density propagating through σ -electron system attenuates with increasing the distance from a radical center. In *l*-type radicals, C_6 -H is at the δ -position to C_3 atom in the π -system where the unpaired electron is present. So far as we know, there have been only a few reports on the observation of h.f. coupling of δ -H in a cyclic radical by means of ESR, such as equatorial protons of piperidine iminoxyl²¹⁾ or cycloheptane semidione.²²⁾ We do not know such observation in the past as that in this work where h.f. coupling of δ -H on the normal alkyl chain was observed.

The long-range coupling constant of δ -H (C_6 -H) larger than that of γ -H (C_5 -H) may be qualitatively explained in terms of two mechanisms by which electron spin density propagates through σ -system: spin delocalization mechanism and spin polarization mechanism. Experiments on isotropic paramagnetic shifts of NMR spectra and molecular orbital calculations have recently shown²³⁾ that the spin delocalization mechanism gives positive electron spin density to the nuclei of atoms contributing to the molecular orbital that has an unpaired electron while spin polarization effect gives rise to the spin density alternating in sign along the bonds from the radical center to the nucleus in question. In radicals with which we are concerned, consequently, the spin polarization mechanism brings about the negative spin density to the nucleus of C_5 -H and the positive one to the nucleus of C_6 -H while the spin delocalization mechanism gives positive spin densities to both the nuclei of C_5 -H and C_6 -H. So, the absolute value of spin density on the nucleus of C_6 -H could be larger than that of C_5 -H.

Oxidations in Aqueous Solutions at pH < 5

One-electron oxidation of I with $Ti^{3+}(EDTA)-H_2O_2$ system at pH=2 (adjusted with H_2SO_4) gave the ESR spectra presented in Fig. 5. Besides the l_I spectrum, another one appeared in the higher magnetic field. This doublet spectrum with h.f. splitting of 0.80 G and $g=2.0040$ was denoted as m_I .

ESR spectra in Fig. 6 were obtained from III under the same reaction condition as above. Again an *m*-type spectrum (m_{III}) appeared partly overlapped with l_{III} in the region of higher field. The m_{III} spectrum is a triplet with an intensity ratio of 1:2:1 and h.f. splitting of 0.7 G. Comparison of two observations above leads to the assignment of h.f. coupling constant (0.80 G for m_I and 0.7 G for m_{III}) to C_4 -H.

No spectrum was obtained from methyl ethers (VII and VIII) in acidic and in alkaline solutions.

The dependence of concentrations of l_I and m_I radicals on time after mixing and pH is shown in Fig. 7 and 8. The decay of these radicals is neither first- nor second-order from the apparent time-dependence curves. This may be owing to the difficulty of kinetic studies by

21) J.J. Windle, J.A. Kuhnle, and B.H. Beck, *J. Chem. Phys.*, **50**, 2630 (1969).

22) G.A. Russell, Post ISNA Symposium, Aug. 1970, Tokyo.

23) T. Yonezawa, I. Morishima, and Y. Ohmori, *J. Am. Chem. Soc.*, **92**, 1267 (1970); I. Morishima, T. Yonezawa, and K. Goto, *ibid.*, **92**, 6651 (1970); I. Morishima, K. Okada, T. Yonezawa, and K. Goto, *ibid.*, **93**, 3922 (1971); I. Morishima and T. Yonezawa, *J. Chem. Phys.*, **54**, 3238 (1971); W. DeW. Horrocks, Jr. and D.L. Johnson, *Inorg. Chem.*, **10**, 1835 (1971).

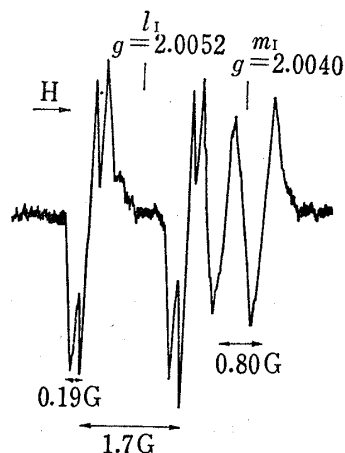


Fig. 5. ESR Spectra Observed during the Oxidation of L-Accorbic Acid (I) with $Ti^{3+}(EDTA)-H_2O_2$ at pH 2.0

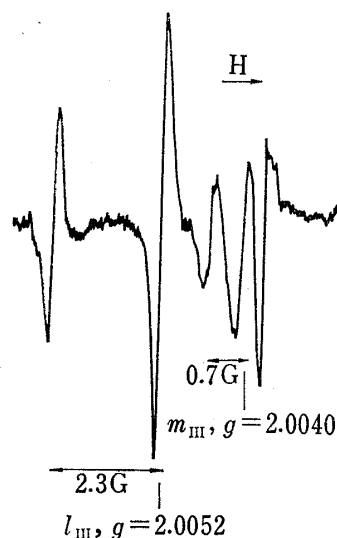


Fig. 6. ESR Spectra Observed during the Oxidation of 3,4-Dihydroxytetrone (III) with $Ti^{3+}(EDTA)-H_2O_2$ System at pH 2.0

continuous flow techniques as revealed by Czapski,²⁴ that is, the fact that the radical formation reaction is not complete immediately after mixing but proceeds also within the ESR cavity.

The concentration of l_I radicals increases with the increase of pH as shown in Fig. 7, corresponding to the anion character of l_I radical. Although the pH-dependence of the

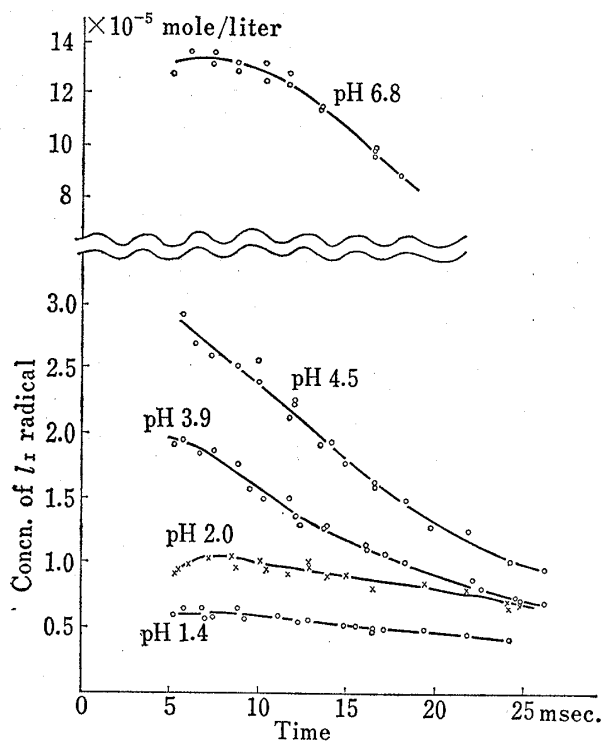


Fig. 7. pH Effect on the Concentration of l_I Radical as a Function of Time

$(Ti^{3+}(EDTA): 0.005M)$ $(H_2O_2: 0.1M)$
 $(AsA: 0.01M)$ $(AsA: 0.01M)$

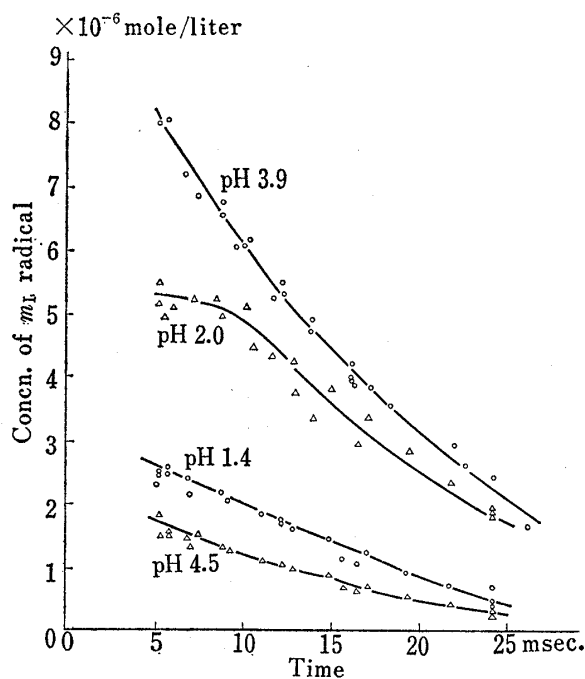


Fig. 8. pH Effect on the Concentration of m_I Radical as a Function of Time

$(Ti^{3+}(EDTA): 0.005M)$ $(H_2O_2: 0.1M)$
 $(AsA: 0.01M)$ $(AsA: 0.01M)$

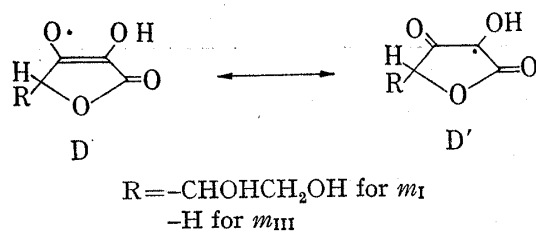


Chart 3

concentration of m_I radicals shown in Fig. 8 is not monotonous, an important finding is that m_I radicals do not appear above pH 5, which suggests that m -type radicals are neutral as D in Chart 3. It may be the reflection of the contribution of a canonical structure, D', that the h.f. coupling constant of C₄-H in m -type radicals (0.8 G for m_I and 0.7 G for m_{III}) is smaller

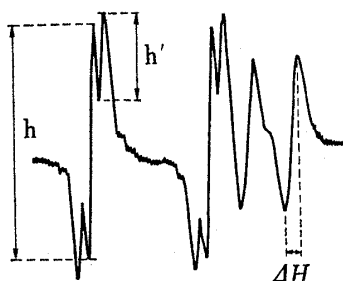
than that in l -type radicals (1.7 G for l_I and 2.3 G for l_{III}).

Another pH effect was that both l_I and m_I spectra became broader with increase of acidity as shown in Table I where the breadth of the spectra is represented in terms of the line width for m_I and the peak height ratio of the end line and the central one of the triplet lines for l_I .

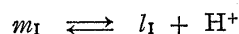
TABLE I. pH Effect on the Line Width

pH	ΔH_{msl} of m_I	h'/h in l_I
1.4	0.25 G	0.30
2.0	0.24	0.32
3.0	0.15	0.38
3.9	0.13	0.42
4.5	0.12	0.42
6.0	—	0.45
8.0	—	0.46

$(\text{Ti}^{3+}(\text{EDTA}): 0.005\text{M})$ $(\text{H}_2\text{O}_2: 0.1\text{M})$
 $(\text{AsA}: 0.01\text{M})$ $(\text{AsA}: 0.01\text{M})$



If the broadening with decrease of pH should arise from the rapid exchange between m_I and l_I , there would exist an acid dissociation equilibrium between them:



If this assumption is correct, the concentration ratio of m_I and l_I would be independent on time at a fixed pH and would depend on pH according to the following equation,

$$\log \frac{(m_I)}{(l_I)} = \text{p}K_a - \text{pH}$$

where K_a is the dissociation constant of m_I . However, results shown in Table II indicate that this is not the case, and therefore, the phenomenon described above is not due to the exchange broadening.

This will be further discussed on the basis of an interaction between metal ions and radicals in the following section.

Recently, Bielski, *et al.*²⁵⁾ have observed the ultraviolet absorption spectra of MDAsA generated in aqueous solutions by pulse radiolysis and estimated from the variation in extinc-

25) B.H.J. Bielski, D.A. Comstock, and R.A. Bowen, *J. Am. Chem. Soc.*, **93**, 5624 (1971).

TABLE II. pH- and Time-Dependence of the Concentration Ratio of m_I and l_I Radical

pH	Time after mixing (msec)				
	7.4	9.1	10.1	13.3	14.4
1.4	0.44	0.34	0.32	0.27	0.24
2.0	0.50	0.45	0.44	0.39	0.30
3.9	0.46	0.41	0.40	0.37	0.30
4.5	0.058	0.051	0.050	0.045	0.045
5.0	0	0	0	0	0

$(\text{Ti}^{3+}(\text{EDTA}): 0.005\text{M})$ $(\text{H}_2\text{O}_2: 0.1\text{M})$
 $(\text{AsA}: 0.01\text{M})$ $(\text{AsA}: 0.01\text{M})$

tion coefficient with pH the existence of dissociation equilibria among protonated, neutral and anion species. Such situation may be realized in their system which does not contain metal ions.

Interaction between Monodehydroascorbic Acid Radicals and Metal Ions

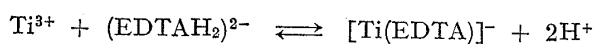
As shown in Table III, l_I and m_I spectra become broader with increase of the concentration of titanous ion in the system during the oxidation of I at pH=2 with Ti^{3+} — H_2O_2 system containing no EDTA. On the basis of this observation, the result in Table I is considered

TABLE III. Effect of Titanium Ion Concentration on the Line Width

Concn. of Ti^{3+}	ΔH_{msl} of m_I	h'/h in l_I
$1.4 \times 10^{-3}\text{M}$	0.26 G	0.45
2.1	0.27	0.33
2.8	0.28	0.31
8.8	0.28	0
13.9	0.30	0

$(\text{Ti}^{3+}: \text{variable})$ $(\text{H}_2\text{O}_2: 0.1\text{M})$ pH=2
 $(\text{AsA}: 0.01\text{M})$ $(\text{AsA}: 0.01\text{M})$

not to be a direct pH effect but to arise indirectly from the increase of the concentration of free titanium ions according to the following reaction: the equilibrium shifts to the left affording free titanium ions with increase of acidity.



Broadening of the spectra is assumed to be due to the complex formation of radicals with metal ions. To clarify this point of view, we further carried out microwave power saturation measurements. The result is shown in Fig. 9 where ordinate is relative peak height of the derivative absorption curve and abscissa is square root of microwave power which is proportional to the amplitude of oscillating magnetic field.

An extremely sharp l_I spectrum observable during the air oxidation of AsA in DMSO containing potassium *tert*-butoxide is shown in Fig. 9-1 to be easily saturated. A sharp l_I spectrum observed during the oxidation with $\text{Ti}^{3+}(\text{EDTA})$ — H_2O_2 system at pH=8.5 was also easily saturated (Fig. 9-2). On the other hand, somewhat broad spectra, l_I and m_I , obtained during the oxidation with Ti^{3+} — H_2O_2 system at pH=2 showed quite different saturation curves (Fig. 9-3) indicating their shorter relaxation times. Oxidations of AsA with Fe^{2+} — H_2O_2 system with or without EDTA at any pH examined, gave only broad l_I spectrum which was not easily saturated and the intensity of which was almost proportional to the amplitude of oscillating magnetic field employed as exemplified in Fig. 9-4.

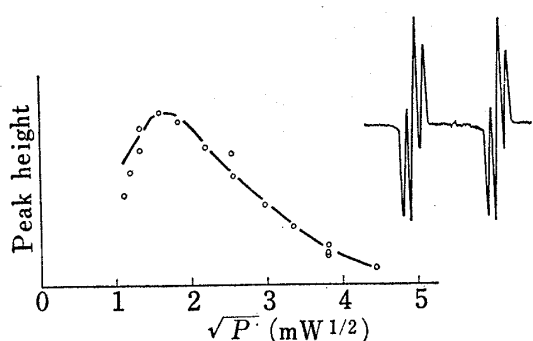


Fig. 9-1. Air Oxidn. in Basic DMSO

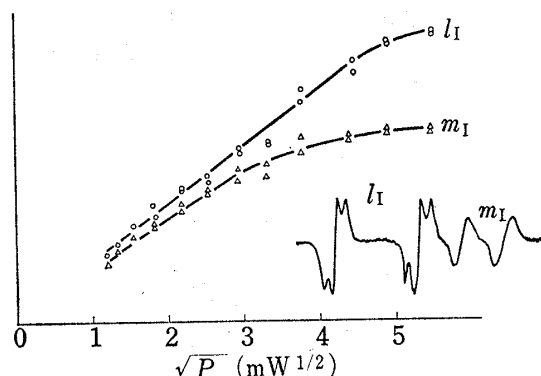
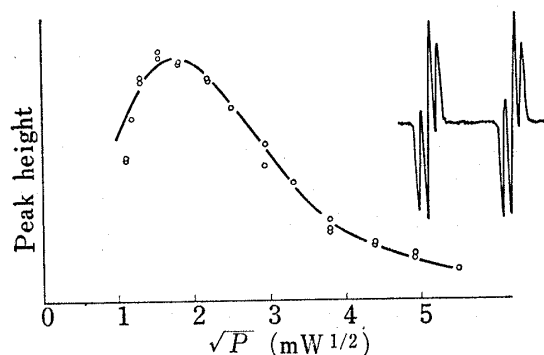
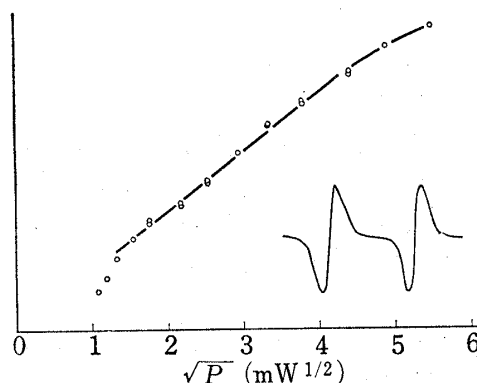
Fig. 9-3. Oxidn. with Ti^{3+} - H_2O_2 at pH 2.0Fig. 9-2. Oxidn. with $\text{Ti}^{3+}(\text{EDTA})$ - H_2O_2 at pH 8.5Fig. 9-4. Oxidn. with Fe^{2+} - H_2O_2 at pH 8.5

Fig. 9. Microwave Power Saturation Curves on ESR Spectra Observed during the Oxidation of L-Ascorbic Acid

Above results may suggest that the line broadening is due to the shortening of the relaxation time by the complex formation of radicals with metal ions.

Although attempts were unsuccessful to observe h.f.s. due to the nucleus of metal atom by the addition of such metal ions as cobalt, yttrium or lanthanum to the reaction system, microwave power saturation experiments described above showed that the broadened spectrum corresponds to the complex of radicals with metal ions. If so, the signal intensity of the radical concentration may depend on the stability of the complex.

On the basis of the above consideration, the result in Table II is interpreted as follows: the complex involving the m_I radical as a ligand is more unstable than the one involving the l_I radical.

In Table IV are summarized the type and relative intensity of ESR spectra, l_I and m_I , observed during the oxidation of AsA at pH=2 with various oxidizing agents. The m -type spectrum was observable only when Ti^{3+} - H_2O_2 system (independent of the addition of EDTA) was used as the oxidizing agent. One-electron oxidations with other reagents gave solely the l -type spectrum as a broad doublet as exemplified in Fig. 9-4.

This difference is interpreted to result from the difference in the stability of the complex of radicals with metal ions rather than from the difference in the character of active species for the oxidation since the hydroxyl radical is an active species both in Fenton's reagent and Ti^{3+} - H_2O_2 system as revealed by Norman, *et al.*²⁶⁾ They attributed the difference in ESR spectra between Fenton's reagent and Ti^{3+} - H_2O_2 system to the smaller oxidation potential of titanous ions than that of ferric ions formed in the reaction mixture. However, the small

TABLE IV. Type and Intensity of ESR Spectra, l_1 and m_1 , Observable during the Oxidation of L-Ascorbic Acid at pH 2

Oxidizing agent	Type and relative intensity of ESR spectra	
	l_1	m_1
$\text{Ti}^{3+}\text{--H}_2\text{O}_2$	++	++
$\text{Ti}^{3+}(\text{EDTA})\text{--H}_2\text{O}_2$	++	++
$\text{Ti}^{3+}\text{--NH}_2\text{OH}$	+	—
$\text{Ti}^{3+}(\text{EDTA})\text{--NH}_2\text{OH}$	+	—
$\text{Fe}^{2+}\text{--H}_2\text{O}_2$	+	—
$\text{Fe}^{2+}(\text{EDTA})\text{--H}_2\text{O}_2$	++	—
$\text{VO}^{3+}\text{--H}_2\text{O}_2$	+	—
Ce^{4+}	++	—
$\text{K}_2\text{Cr}_2\text{O}_7$	+	—
KMnO_4	+	—

oxidation potential of titanous ions is not the only reason in our case, for $\text{Ti}^{3+}\text{--NH}_2\text{OH}$ system did not bring about m -type radicals. That the m -type radical has a lifetime long enough to be detected by means of ESR coupled with the flow technique only when $\text{Ti}^{3+}\text{--H}_2\text{O}_2$ system is used as the oxidizing agent, may also be related to the fact that titanous ions and hydrogen peroxide form a stable peroxo complex.

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