[Chem. Pharm. Bull. 19(4) 718—721 (1971)] UDC 547.475.2.04 : 546.658.04 : 546.215.04

Free Radicals formed during the Oxidation of L-Ascorbic Acid or Hydroxytetronic Acid with Hydrogen Peroxide and Titanium (III) Ions

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(Received August 31, 1970)

The oxidation of L-ascorbic acid with Ti^{3+}-H_2O_2 has been investigated by a rapid-mixing flow technique coupled with electron spin resonance. Two kinds of spectra were distinguished, one of them being not reported hitherto. To assign these electron spin resonance spectra, the oxidation of hydroxytetronic acid, a simple analog of L-ascorbic acid, was further investigated. In the light of the obtained electron spin resonance parameters possible radical structures were discussed.

Both enzymic and nonenzymic oxidations of L-ascorbic acid in aqueous solutions have been investigated by a number of workers. In particular, the formation of intermediary free radicals has recently been demonstrated by using an electron spin resonance (ESR) spectroscopy.[2-4] Unfortunately, however, the spectral parameters were not well characterized, leaving some questions of interest on the structure of the free radicals formed.

We have reinvestigated the oxidation of L-ascorbic acid with Ti^{3+}-H_2O_2 in a continuously flowing aqueous solution passing through the cavity of an ESR spectrometer, and have found that at least two kinds of ESR spectra were distinguishable, and that one of them was a new spectrum. So, our problem is now to solve what type of radical could account for the observed ESR spectra.

In order to identify the observed two radical species, further studies were made on the oxidation of a simple analog of L-ascorbic acid, namely, hydroxytetronic acid. In the present paper are presented the ESR spectra of free radicals formed during the oxidation of L-ascorbic acid and of hydroxytetronic acid as well with Ti^{3+}-H_2O_2. Possible structures of these free radicals will be discussed.

Experimental

The detection of radicals formed upon mixing an aqueous solution of L-ascorbic acid or hydroxytetronic acid with a solution of hydrogen peroxide and titanium (III) salt was carried out by a rapid-mixing flow technique coupled with ESR.[5] A JEOL-P-10 type (X-band) spectrometer with 100 kcps field modulation and JEOL-mixers of different volumes were used for this purpose. Reaction times were calculated, as usual, from the observed flow rate and the known hold-up volume. The flow rates were controlled by means of a cylinder nitrogen at high pressures while four mixers of different volumes corresponding to 0.015, 0.070, 0.25 and 0.87 ml hold-up volumes respectively, were served to provide different reaction times. It was possible in this way to obtain reaction times of the order of 3—800 msec. The relative concentration of radicals formed was determined by a first moment calculation of the corresponding spectrum.

Materials——A purified sample of L-ascorbic acid, supplied by Takeda Chemical Industries, Inc., was used without further purification.

Hydroxytetronic acid was synthesized according to Micheel, *et al.*[6] and purified by recrystallizations. All the other reagents used were of highest grade.

1) Location: *Hongo 7-3-1, Bunkyo-ku, Tokyo.*
2) I. Yamazaki, H. S. Mason, and L. H. Piette, *J. Biol. Chem.*, **235**, 2444 (1960); I. Yamazaki and L. H. Piette, *Biochim. Biophys. Acta*, **50**, 62 (1961); T. Ohnishi, H. Yamazaki, I. Iyanagi, T. Nakamura, and I. Yamazaki, *ibid.*, **172**, 357 (1969).
3) C. Lagercrantz, *Acta Chem. Scand.*, **18**, 562 (1964).
4) G. v. Foerster, W. Weis, and H. Staudinger, *Ann. Chem.*, **690**, 166 (1965).
5) M. Setaka, Y. Kirino, T. Ozawa, and T. Kwan, *J. Catalysis*, **15**, 209 (1969).
6) F. Micheel and F. Jung, *Chem. Ber.*, **66**, 1291 (1933); *idem, ibid.*, **67**, 1660 (1934).

Result and Discussion

Oxidation of L-Ascorbic Aicd with Ti^{3+}-H_2O_2

Acidified (0.1 M H_2SO_4) aqueous solutions of Ti^{3+} (0.01 M $TiCl_3$) and of H_2O_2 (0.1 M), each containing L-ascorbic acid (0.02 M), were introduced in the JEOL mixing-cell and allowed to pass through the cavity of the ESR spectrometer at a constant rate. A typical ESR spectrum recorded at 10 msec after mixing is shown in Fig. 1. The spectrum of Fig. 1 was partly different from those reported earlier[2-4]; it was accompanied by a new spectrum in the higher magnetic field. The spectrum was certainly composed of two different radical species because these were entirely different in dynamical character. So, these will be denoted, for convenience, as l and m from the lower magnetic field, respectively. The spectruml was a doublet (splitting 1.7 G) of triplet (0.17 G) with g=2.0054 while m was a doublet (0.8 G) with g=2.0040. The spectrum l was identical with that of monodehydroascorbate radical reported hitherto,[2-4] while m was a newly found one detected in this system for the first time.

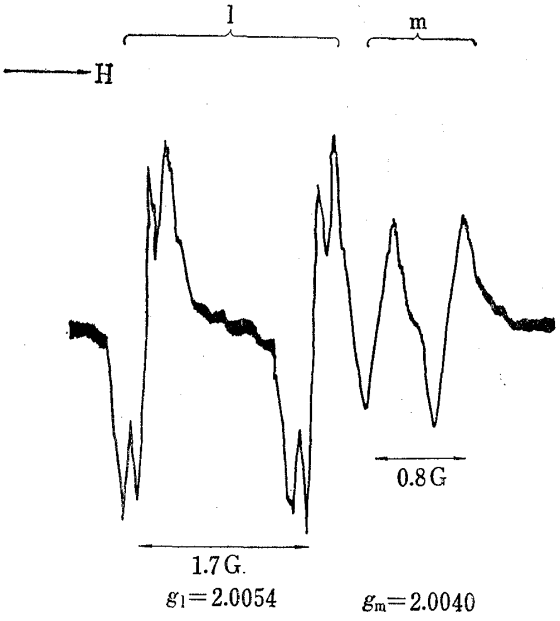


Fig. 1. ESR Spectrum for the Oxidation Intermediate of L-Ascorbic Acid with Ti^{3+} -H_2O_2

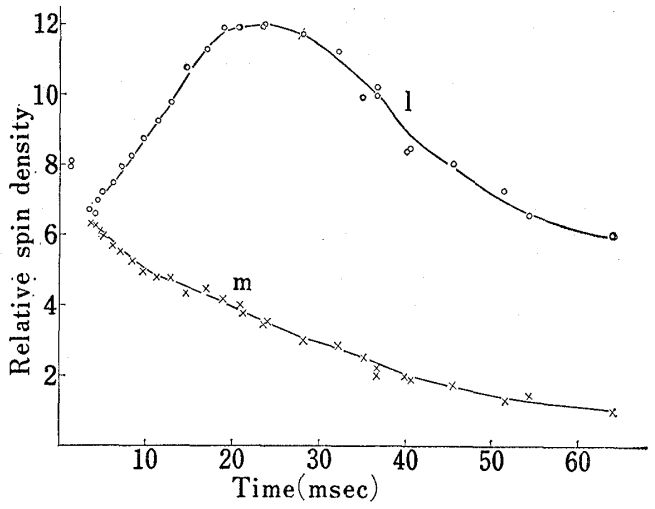
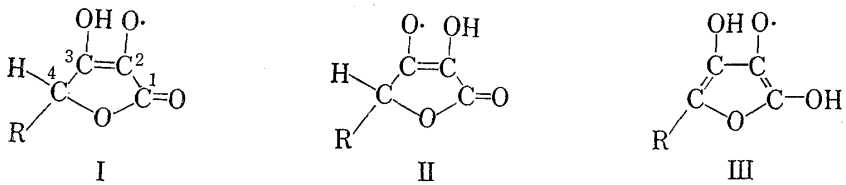


Fig. 2. Time-Dependence of the Intensities of l- and m-Signal

In Fig. 2 are shown relative concentrations of radicals as a function of time. As can be seen in Fig. 2, l seems to be generated more slowly than m at the initial stage and moreover it is rather stable compared with m. A glance of these curves in Fig. 2 would lead one to a supposition that l could be generated in the course of the consecutive reaction, m→l, but the view does not seem to be the case as will be discussed in a subsequent note.



I II III

Chart 1. Possible Structures for Monodehydroascorbic Acid Radical

R=-CH(OH)-CH_2-OH for L-ascorbic acid

R=H for hydroxytetronic acid

If L-ascorbic acid is assumed to yield, upon oxidation, monodehydroascorbic acid radicals, one may expect that the radical structure will be represented by I, II or III,[7] as shown in Chart 1.

The spectrum l presents an isotropic hyperfine interaction with a hydrogen nucleus and the other two equivalent hydrogen nuclei. Among the structure I, II and III postulated for the radicals formed III would be the most apt to explain the hyperfine structure, because the structure III involves a hydrogen nucleus and a methylene group in R (See Chart 1). It would, however, be almost unlikely that C_4-H is eliminated to yield the monodehyroascorbic acid radical of the structure III during the course of the oxidation of L-ascorbic acid to give dehyroascorbic acid. Indeed, the possibility of structure III was excluded, as will be described in the next section dealing with the oxidation of hydroxytetronic acid.

The spectrum m indicated that the unpaired electron of the corresponding radical inter acts with a hydrogen uncleus. It may then be attributable to either I or II, as the C_4-H still remains there.

Oxidation of Hydroxytetronic Acid by Ti^{3+}-H_2O_2

In Fig. 3 are shown the ESR spectra of the radicals formed during the oxidation of hydroxytetronic acid under the same condition as those for L-ascorbic acid. At 10 msec after mixing, a main triplet signal (denoted as l′ for convenience) was obtained with a minor signal (denoted as m′) overlapped with l 'in the region of higher magnetic field. At 36 msec after mixing, m′ almost disappeared while l′ remained as a distinct triplet signal with the ESR parameters of g=2.0050 and A=2.3 G. The minor signal, m′, is believed to be also a triplet, because the difference spectrum obtainable from those at 10 and 36 msec gave rise to a well-resolved triplet, as shown in Fig. 3c. Its ESR parameters were found to be g=2.003_7 and A=0.7 G.

ESR parametars of the l′- and m′-species will be compared with those of l and m as below.

	g	A	
l	2.0054	1.7G	0.17G
m	2.0040	0.8G	
l′	2.0050	2.3G	
m′	2.003_7	0.7G	

Inspection of the g-value and time-dependence of these signals may lead us to assume that l′ and m′ are quite similar in character to l and m obtained for the oxidation of L-ascorbic acid, respectively.

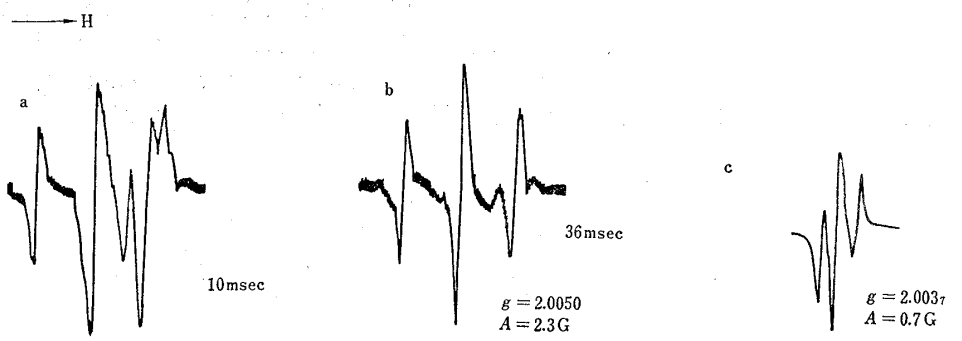


Fig. 3. ESR Spectra for the Oxidation Intermediate of Hydroxytetronic Acid with Ti^{3+}–H_2O_2

7) G. A. Russel, "Radical Ions," ed. by E. T. Kaiser and L. Kevan, Interscience Publishers, 1968, p. 88.

Possible structures I, II, and III, in analogy with the case of L-ascorbic acid, may be considered, substituting R for H, for the radicals to which l′ and m′ are to be assigned. Among these structures, however, III which has a furan ring with only one C_4–H, should be excluded, because both l′ and m′ are triplets with the intensity ratio of 1: 2: 1. On the other hand, both I and II have two equivalent hydrogen nuclei at C_4 consistent with the observed hyperfine structure. It seems almost certain therefore that either l′ or m′ should be attributable to the structure I or II. It should be noted however that the hyperfine constant of l′ was much greater than that of m′. The problem is still under investigations.

From the above results obtained for the oxidation of hydroxytetronic acid it can be considered that the radical which has a furan ring structure, namely III, would probably be absent during the oxidation of hydroxytetronic acid nor during the oxidation of L-ascorbic acid. On the other hand, the monodehydroascorbic acid radicals of the structure I or II may probably be present during the reaction, although the electronic structure of the radicals appropriate to the obtained ESR parameters should further be investigated.

Acknowledgement The authers wish to thank Takeda Chemical Industries, Inc. for generous supply of the pure sample of L-ascorbic acid.