Oxidation of Triose Reductone by Oxidizing Inorganic Radicals Generated by Pulse Radiolysis

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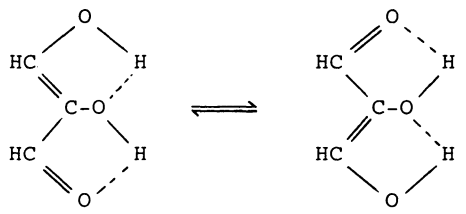
Triose reductone (2,3-dihydroxy-2-propenal) was oxidized by $Cl_2^{\cdot-}$, $Br_2^{\cdot-}$, $I_2^{\cdot-}$, $(SCN)_2^{\cdot-}$, and $N_3^{\cdot}$ to produce a triose reductone radical which had an absorption maximum at 398 nm, $\varepsilon=5500\ M^{-1}\,cm^{-1}$ (1 M=1 mol dm^{-3}) and decayed by second-order kinetics. The rate constants for the reactions of triose reductone with these oxidizing inorganic radicals were obtained. The pK_a of this radical was found to be 1.4.

Triose reductone (2,3-dihydroxy-2-propenal) was first synthesized by the decomposition of glucose in an alkaline aqueous solution and its structure was determined to be CH(OH)=C(OH)CHO by H. von Euler and C. Martius.[1] A very important characteristic of "reductone" is the strong reducing ability which is due to its enediol group, -C(OH)=C(OH)-.[2] Euler *et al.*[3] proposed that triose reductone has a double five-membered ring. Obata *et al.*[4] provided experimental proof for this by NMR and mass spectra of triose reductone and presumed the following two formulas in D_2O, $(CD_3)_2CO$, DMSO solution.



Well-known reductones, L-ascorbic acid,[5-7] adrenaline,[8] and dopamine[9] which play significant roles in biological systems, have been studied by pulse radiolysis. Thus, the oxidation of the reductones have been shown to proceed through the intermediate radicals. Furthermore, it is important and interesting to clarify the oxidation mechanisms of other enediol compounds. Triose reductone is yielded in biological systems[1,10-12] and in the process of browning reaction.[13] Also, it reacts with amino compounds to yield anil compounds[2] or causes a DNA-breaking action in the presence of Cu^{2+}.[14] The enzymatic oxidation of triose reductone has been reported to produce a free radical in the ESR study.[15] It is useful to study the oxidation mechanism of triose reductone, as it is one of the most simple reductones. We have studied the oxidations of triose reductone using oxygen and hydrogen peroxide,[16] a peroxodisulfate ion,[17] and a hexacyanoferrate(III) ion,[18] kinetically. It was found in our previous paper[19] that triose reductone is oxidized by $(SCN)_2^{\cdot-}$ and $Br_2^{\cdot-}$ to yield a one-electron oxidized radical. In this paper we studied oxidations of triose reductone by oxidizing species $Cl_2^{\cdot-}$, $I_2^{\cdot-}$, $N_3^{\cdot}$ including $(SCN)_2^{\cdot-}$, and $Br_2^{\cdot-}$ [19] kinetically and disclosed the reaction mechanisms.

Experimental

Pulse irradiation was accomplished using the 18 Mev linac (High Voltage Engineering Co.) of the Radiation Center of Osaka Prefecture at room temperature (about 20 °C). Pulses of 10-MeV electrons of 0.5—2 μs duration were diffused by an aluminum plate (0.5—3 mm thick) for regulating the pulse dose. The dose of a pulse was monitored by the current which was collected on a brass plate located directly behind the irradiation cell. The 1.5-cm long irradiation vessel was made of thermal quartz for light path windows. The sample solution was refreshed from a Pyrex stock bottle after each pulse-irradiation by a magnetic valve under gas (N_2O or Ar) pressure. All solutions were prepared from triply distilled water. Dinitrogen monoxide (Showa Denko Co., High Purity, upper limit of O_2 content: 10 ppm) was used after the deaeration of solutions by argon (Osaka Sanso Co., Instrument Argon). The solutions were bubbled before irradiation for ≈60 min with Ar and N_2O which converts the hydrated electron into the OH radical. Triose reductone was prepared and purified by the method of Euler and Martius.[1] All other chemicals used were of analytical reagent grade. The light of a 500 W Xe lamp (Ushio Denki Co.) was focused on the irradiation cell and was detected by a photomultiplier (1P28, Hamamatsu TV) after passing through a grating monochromator (Nikon, G-250). The signals were recorded by a personal computer (YHP Co., 9815A) through a transient recorder (Riken Denshi Co., TCH-1000) and analyzed.[20] Dosimetry was performed with N_2O-saturated 0.01 M KSCN solutions taking the value of G(OH) and $\varepsilon_{472\,nm}$ for $(SCN)_2^{\cdot-}$ as 6.13 and 7580 $M^{-1}cm^{-1}$.[21] The pH of the solution was generally adjusted by an addition of appropriate amounts of $HClO_4$ or NaOH.

Results and Discussion

Rate Constants for the Oxidations of Triose Reductone with Inorganic Radicals. The rate constants for the oxidation of triose reductone with $(SCN)_2^{\cdot-}$, $Cl_2^{\cdot-}$, $Br_2^{\cdot-}$, and $I_2^{\cdot-}$ were measured by following the exponential decay of each absorbance (472, 340, 340, and 380 nm) as a function of the substrate concentration. The rate constant, in the case of $N_3^{\cdot}$, was measured by following the first-order decay and growth of the absorbance due to triose reductone at 310 nm and the triose reductone radical at 400 nm, respectively. The rate constants were identical with each other regarding both decay and growth within experimental errors. The rate constants

Table 1. Rate constants in $M^{-1}s^{-1}$ for the reactions of triose reductone and ascorbic acid with inorganic oxidizing radicals

Oxidant	$k(TRH_2)$	$k(TRH^-)$	$k(AH_2)$[a]	$k(AH^-)$[a]
$Cl_2^-\cdot$	1.1×10^9		6.8×10^8	
$N_3\cdot$		4.0×10^9		
$Br_2^-\cdot$	2.2×10^8	1.8×10^9	1.1×10^8	1.1×10^9
$(SCN)_2^-\cdot$	2.7×10^7	9.0×10^8	1.0×10^7	6.0×10^8
$I_2^-\cdot$	$\leqq1\times10^6$	3.4×10^8	5×10^6 $\leqq6\times10^5$[b]	1.4×10^8

TRH_2: Triose reductone. TRH^-: Monoanion of triose reductone AH_2: Ascorbic acid. AH^-: Monoanion of ascorbic acid. a) Ref. 5. b) This study, at pH 2.

Table 2. One-electron reduction potentials of inorganic radicals

inorganic radical	E^0(V)
$Cl_2^-\cdot$	2.3
$N_3\cdot$	1.93
$Br_2^-\cdot$	1.7
$(SCN)_2^-\cdot$	1.25
$I_2^-\cdot$	1.0

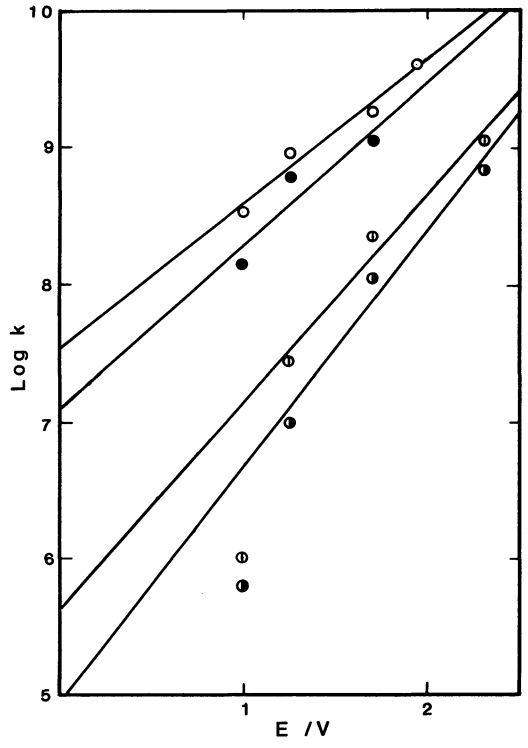


Fig. 1. Dependence of k (rate constant) on the reduction potential of inorganic radicals.
TRH_2 (Φ), TRH^- (○), AH_2 (◑), AH^- (●).

of both dissociated and undissociated forms are summarized in Table 1. For the sake of a comparison, the data of L-ascorbic acid[5] are also listed in Table 1. The reactivities of triose reductone with these oxidizing species are in the order of $Cl_2^-\cdot > N_3\cdot > Br_2^-\cdot > (SCN)_2^-\cdot > I_2^-\cdot$, similar to ascorbic acid. One-electron reduction potentials[22] of the oxidizing species (Table 2) explain the reactivity order well. The rate constants for oxidation are larger in triose reductone than in ascorbic acid, except that of $I_2^-\cdot$ with undissociated ascorbic acid quoted from a reference.[5] We found from the decay of $I_2^-\cdot$ at 410 nm that the reaction rate constant of $I_2^-\cdot$ with ascorbic acid was smaller than 6×10^5 M^{-1} s^{-1}. The latter value seems more reasonable from the relation mentioned above. The stronger reducing power of triose reductone in comparison with ascorbic acid was also shown in the reductions of peroxodisulfate[17,23] and hexacyanoferrate(III).[18,24] The dependences of log k (k: rate constants for oxidation by inorganic radicals) on the reduction potential (E (ev)) in both triose reductone and ascorbic acid are presented in Fig. 1. They are almost linear except for reactions of $I_2^-\cdot$ with undissociated triose reductone and ascorbic acid. This result shows that the electron-transfer reactions that occur in these systems are as predicted from the Marcus theory.[25] Triose reductone has two dissociation constants[16] ($pK_1=5.0$, $pK_2=13.0$). Therefore, the reaction scheme can be described as follows.

$$H_2O \rightsquigarrow e_{aq}^-, H, OH, H_2O_2, H_2 \quad (1)$$

$$N_2O + e_{aq}^- \xrightarrow{H_2O} OH + OH^- + N_2 \quad (2)$$

$$X^- + OH \longrightarrow X\cdot + OH^- \quad (3)$$
(X=SCN, Cl, Br, I)

$$X^- + X\cdot \longrightarrow X_2^-\cdot \quad (4)$$

$$N_3^- + OH \longrightarrow N_3\cdot + OH^- \quad (5)$$

$$TRH_2 \overset{K_1}{\rightleftharpoons} TRH^- + H^+ \overset{K_2}{\rightleftharpoons} TR^{2-} + 2H^+ \quad (6)$$

$$TRH_2 \text{ (or } TRH^-) + X_2^-\cdot \longrightarrow TRH\cdot \text{ (or } TR^{-}\cdot) + 2X^- \quad (7)$$

$$TRH_2 \text{ (or } TRH^-) + N_3\cdot \longrightarrow TRH\cdot \text{ (or } TR^{-}\cdot) + N_3^- \quad (8)$$

Absorption Spectra. Triose reductone is oxidized with $X_2^-\cdot$ (X=SCN, Cl, Br, I) or $N_3\cdot$ radicals to generate transient species, as mentioned above. The absorption spectra observed in these systems (Fig. 2) are identical with each other within experimental errors. The absorption spectrum (Fig. 2) almost completely disappears upon the addition of 0.2 M *t*-BuOH which scavenges OH radicals. The result supports the idea that transient absorption is produced by a reaction of triose reductone with the inorganic radicals. The absorption spectrum shows a quite symmetrical (Gaussian-type) band with a maximum at 398 nm and a width at half-maximum of 71 nm. The absorption coefficient at 398 nm is 5500 $M^{-1}cm^{-1}$. The solid line in Fig. 2 represents the simulated Gaussian-curve with the deviation of 32 nm, and fits well with a observed ab-

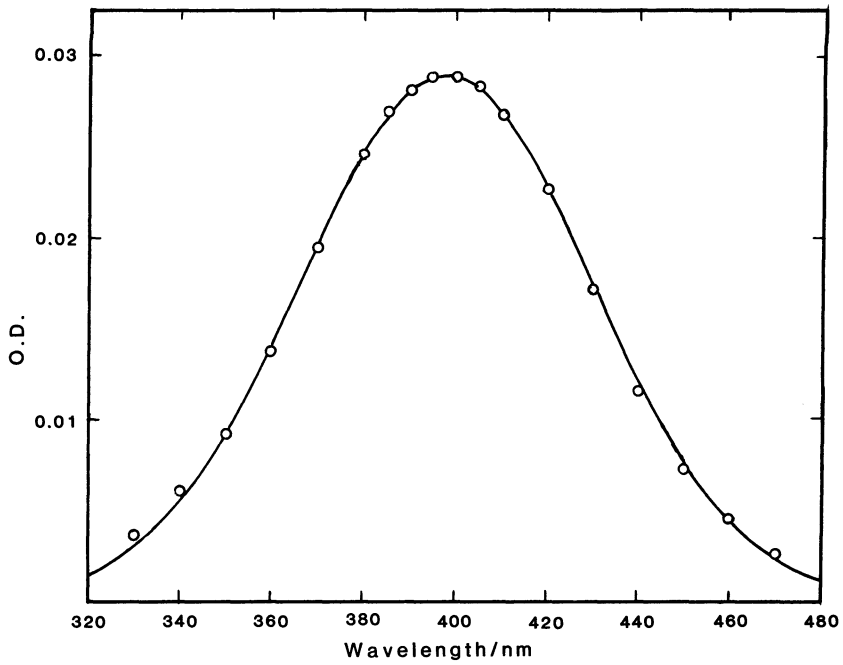


Fig. 2. Transient absorption spectrum produced by pulse irradiation of N_2O saturated 3×10^{-4} M triose reductone solution. Average dose, 536 rad; 20 mM KSCN, KBr, NaN_3; pH 9.6.

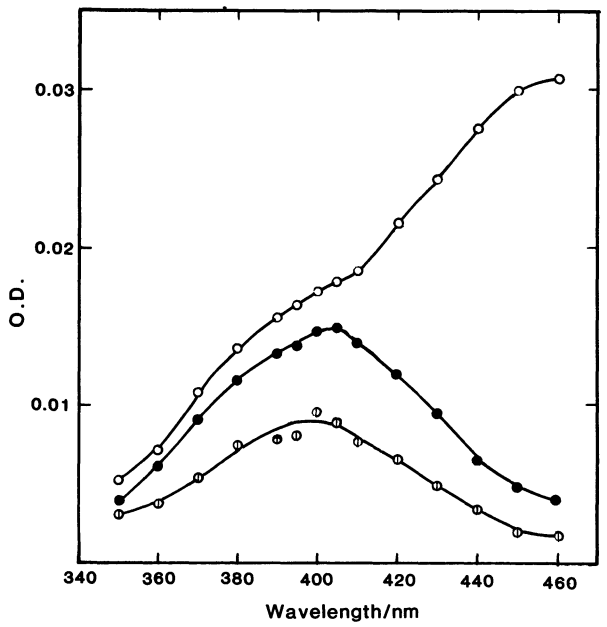


Fig. 3. Time change of transient spectrum produced by $(SCN)_2^-$ at pH 3.2. N_2O saturated 3×10^{-4}M triose reductone, 20 mM KSCN; Average dose, 536 rad; 0(○), 200(●), 900(Φ), μs after the pulse.

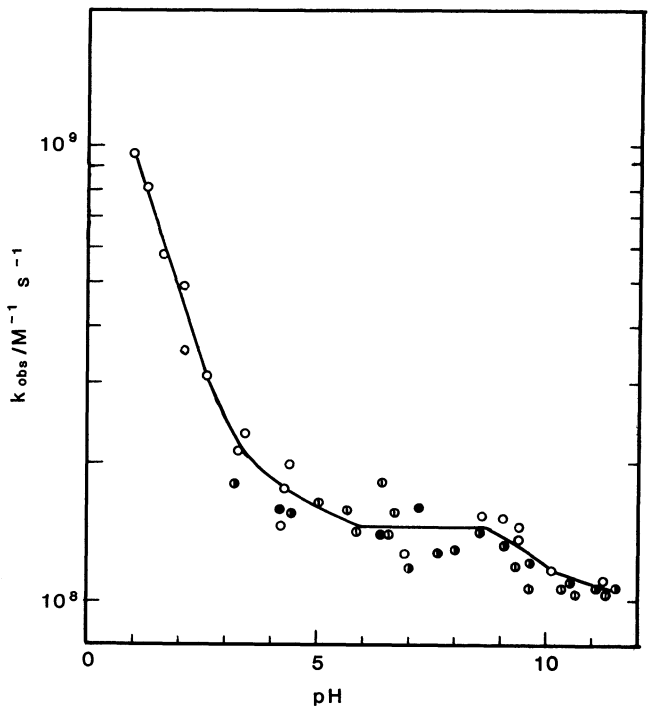


Fig. 4. Dependence of decay rate constants of the triose reductone radical corrected for ionic strength on pH. N_2O saturated solution of 2×10^{-4}, 3×10^{-4} M triose reductone; 10, 20 mM KBr (○); 5, 20 mM KSCN (◑); 5, 10, 20 mM NaN_3 (Φ); 3, 20 mM KI (●).

sorption data. The ascorbic acid radical also has a very symmetric-type band with a maximum at 360 nm and a half-maximum width of 50 nm.[26] Figure 3 shows the time-changes of the transient absorption spectrum produced by $(SCN)_2^-$ radicals at pH 3.2. It indicates that the $(SCN)_2^-$ radical (λ_{max}=472 nm) is gradually converted into the triose reductone radical (reaction 7), since the rate constant for the reaction $TRH_2+(SCN)_2^-$ is rather small (2.7×10^7 $M^{-1}s^{-1}$) in comparison with that for dissociated triose reductone (9.0×10^8 $M^{-1}s^{-1}$).

Rate of Radical Decay. Triose reductone radicals including both TRH· and TR^-· (*vide infra*) decay by

second-order kinetics over a wide pH range (pH 1—11.5). Figure 4 shows the relationship between the second-order rate constants for the decay of the radical (TRH· and/or $TR^{-\cdot}$) and the pHs of solutions, where the rate constants were corrected for ionic strength. The rate constant decreases slightly from pH 9 to 12.5 and is almost constant in the range of pH 6—9 and increases steeply from pH 4 to pH 1. The change of the rate constant around pH 10 is not at present understandable. The dependence of the decay rate of the radical on pH is probably due to H^+ as well as the ascorbate radical[5] and the decay rate may be affected by the dissociation of the radical below pH 2.5, whose pK_a is 1.4 as shown later. But, the hydrated electron reacts with H^+ competitively with N_2O to yield the H radical below pH ≈2.5. Also the transient species produced by the reaction of triose reductone with the H radical might affect the apparent rate constants. Hence, the decay mechanism of the radical is complicated below pH ≈2.5. The rate of a reaction between two ions is dependent on the ionic strength (μ), as given approximately[27] by

$$\log k/k_0 = Z_A Z_B(1.02\mu^{1/2})/(1+\mu^{1/2}) = Z_A Z_B m \qquad \text{(I)}$$

where k_0 is the value of k at zero ionic strength, and Z_A, Z_B are the charges of two ions, and μ is the total ionic strength. The effect of the ionic strength on the decay rate of the triose reductone radical was studied at pH 8.0, where the radical is presumed to be dissociated from the pK_a (1.4) as shown later, using sodium sulfate to vary μ. The results are shown in Fig. 5, where the observed values of log k are plotted as a function of m. The slope of the line in Fig. 5 is about 0.7. Hence, the oxidized intermediate can be assigned to the anion radical $TR^{-\cdot}$

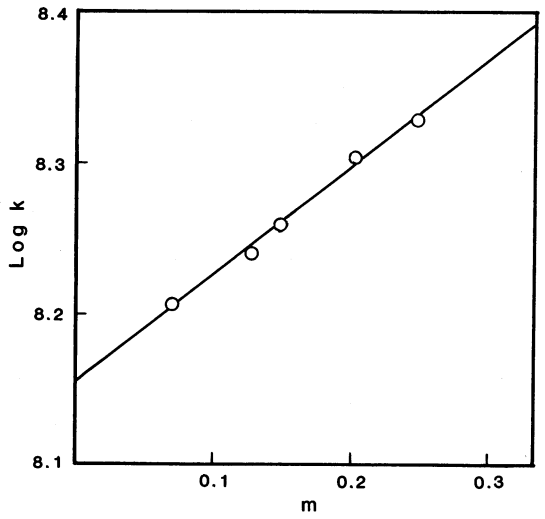


Fig. 5. Effect of ionic strength on decay rate of the triose reductone radical.
N_2O saturated solution of 3×10^{-4} M triose reductone; 5 mM KSCN; pH 8.0.

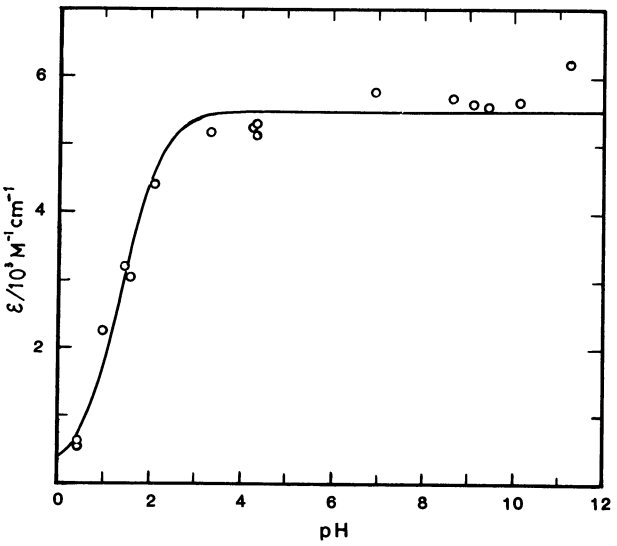


Fig. 6. Dependence of molar extinction coefficient (ε) of the triose reductone radical on pH. N_2O saturated solutions of 3×10^{-4} M triose reductone; 10, 20 mM KBr.

pK_a of the Triose Reductone Radical. The molar extinction coefficients (ε) of triose reductone radical determined at 400 nm as a function of pH are shown in Fig. 6. Below pH 3 the hydrated electron (e^-_{aq}) reacts with proton (H^+) to produce the H radical. But, the transient species which is produced by the reaction of triose reductone with the H radical has only a small extinction coefficient ($\varepsilon_{400\,nm}$=300). The extinction coefficient was obtained in the system of 1 mM TRH_2, 0.2 M *t*-BuOH, at pH 1. The numerical values of ε were corrected for the H^+ effect, as H^+ reacts with e^-_{aq} to yield the H radical. The smooth line in Fig. 6 was computed by assuming that the pH dependence of the extinction coefficient results from the acid dissociation process, using $\varepsilon^{TRH\cdot}_{400\,nm}$=200 $M^{-1}cm^{-1}$, $\varepsilon^{TR^{-\cdot}}_{400\,nm}$= 5500 $M^{-1}cm^{-1}$. The acid dissociation constant giving the best fit to the data is pK_a=1.4. In the γ-ray radiolysis of 5 mM KBr, 26 μM TRH_2, N_2O saturated solution at pH 8.5, G(-TRH_2) (the consumption G value of TRH_2) was obtained as about 3. As $G(Br_2^{-\cdot})$ is 6.1 in the system above, the triose reductone radical is presumed to decay by disproportionation mechanism in the same way as the ascorbic acid radical.[5] Consequently, the triose reductone radical decays according to the following scheme.

$$TRH\cdot \overset{pK_a=1.4}{\rightleftharpoons} TR^{-\cdot} + H^+ \qquad (9)$$

$$TR^{-\cdot} + TR^{-\cdot} \xrightarrow{H^+} TRH_2 \text{ (or } TRH^-) + TR \qquad (10)$$

However, it is not clear in this study whether the triose reductone radical is in equilibrium with a dimer similar to the ascorbic acid radical,[7] as follows.

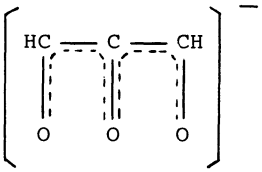
$$TR^{-\cdot} + TR^{-\cdot} \rightleftharpoons TR_2^{2-} \qquad (11)$$

TABLE 3. DISSOCIATION CONSTANTS FOR TRIOSE REDUCTONE, ASCORBIC ACID, AND THEIR RADICALS

	Triose reductone	Ascorbic acid
pK_1	5.0	4.3[a]
pK_2	13.0	11.6[a]
Radical		
pK_a	1.4	−0.45[b]

a) Ref. 31. b) Ref. 28.

Fessenden, Schuler, and co-workers[28,29] showed by an ESR study that the ascorbic acid radical has a structure with an unpaired electron spread over a highly conjugated tricarbonyl system with a pK_a of −0.45. Therefore, the triose reductone radical ($TR^{-\cdot}$) probably has the following conjugated structure, as it has a similar enediol group with a carbonyl group as well ascorbic acid. In the molecular orbital calculation



study[30] of triose reductone, the result also shows that the radical is in a resonated anion form, while the spin density is localized somewhat on the central oxygen atom.

The dissociation constants of ascorbic acid, triose reductone, and their radicals are summarized in Table 3. In each case, pK_a of triose reductone and its radical is larger than that of ascorbic acid and ascorbate radical. Namely, it shows that ascorbic acid and its radical are stronger acids than those of triose reductone, respectively. The dissociation constants of the reductone radicals are greater by a factor of over 10^3 than that of the respective reductones. This fact shows that these reductone radicals are far more acidic than the corresponding reductone.

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References

1) H. von Euler and C. Martius, *Liebigs. Ann. Chem.*, **505**, 73 (1933).
2) D. Nomura and H. Omura, "Chemistry of Reductone," Uchida Rokakuho Publishing Co., Ltd., Tokyo, (1969).
3) H. von. Euler and H. Hasselquist, *Arkiv. Kemi.*, **26A**, Nr. 25 (1948).
4) H. Obata and T. Tokuyama, *Nippon Kagaku Zasshi*, **91**, 1098 (1970).
5) M. Schöneshöfer, *Z. Naturforsh.*, **27b**, 649 (1972).
6) B. H. J. Bielski, D. A. Comstock, and R. A. Bowen, *J. Am. Chem. Soc.*, **93**, 5624 (1971).
7) B. H. J. Bielski, A. O. Allen, and H. A. Schwarz, *J. Am. Chem. Soc.*, **103**, 3516 (1981).
8) W. Bors, M. Saran, C. Michel, E. Lengfelder, C. Fuchs, and R. Spöttl, *Int. J. Radiat. Biol.*, **28**, 353 (1975).
9) H. W. Richter and W. H. Waddell, *J. Am. Chem. Soc.*, **105**, 5434 (1983).
10) G. Hesse and H. Schildknecht, *Angew. Chemie.*, **68**, 75 (1956).
11) S. Adachi, *Nippon Nôgeikagaku Kaishi*, **30**, 713 (1956).
12) H. von Euler and E. Klussmann, *Z. Physiol. Chem.*, **217**, 167 (1933).
13) J. E. Hodge, *J. Agr. Food Chem.*, **1**, 928 (1953).
14) K. Shinohara, M. So, M. Nonaka, K. Nishiyama, H. Murakami, and H. Omura, *J. Nutr. Sci. Vitaminol.*, **29**, 481 (1983).
15) I. Yamazaki and L. H. Piette, *Biochim. Biophys. Acta*, **77**, 47 (1963).
16) Y. Abe, H. Horii, S. Taniguchi, K. Kamai, and M. Takagi, *Bull. Chem. Soc. Jpn.*, **56**, 467 (1983).
17) Y. Abe, H. Horii, and S. Taniguchi, *Bull. Chem. Soc. Jpn.*, **57**, 222 (1984).
18) Y. Abe, T. Dohmaru, H. Horii, and S. Taniguchi, *Can. J. Chem.*, **63**, 1005 (1985).
19) H. Horii, Y. Abe, S. Taniguchi, S. Maeda, and T. Watanabe, *Chem. Lett.*, **1983**, 1313.
20) H. Horii, S. Fujita, T. Mori, and S. Taniguchi, *Radiat. Phys. Chem.*, **19**, 231 (1982).
21) R. H. Schuler, L. K. Patterson, E. Janata, *J. Phys. Chem.*, **84**, 2089 (1980).
22) J. Butler, E. J. Land and A. J. Swallow, *Radiat. Phys. Chem.*, **23**, 265 (1984).
23) M. Kimura, A. Kobayashi, and K. Boku, *Bull. Chem. Soc. Jpn.*, **55**, 2068 (1982).
24) U. S. Mehrotra, M. C. Agrawal, and S. P. Mushran, *J. Phys. Chem.*, **73**, 1996 (1969).
25) R. A. Marcus, *J. Chem. Phys.*, **43**, 3477 (1965).
26) R. H. Schuler, *Radiat. Res.*, **69**, 417 (1977).
27) R. E. Weston, H. A. Schwarz, "Chemical Kinetics," Prentice-Hall, Inc., Englewood Cliffs, N. J., 169 (1972).
28) G. P. Laroff, R. W. Fessenden, and R. H. Schuler, *J. Am. Chem. Soc.*, **94**, 9062 (1972).
29) R. W. Fessenden and N. C. Verma, *Biophys. J.*, **24**, 93 (1978).
30) Y. Abe, H. Horii, S. Taniguchi, S. Yamabe, and T. Minato, 50th National Meeting of the Chemical Society of Japan, Tokyo, April 1985, Abstr., No. 1U36.
31) E. Schauenstein, *Monatsh. Chem.*, **79**, 495 (1948).