# On the Radical Species produced by the Reaction of L-Ascorbic Acid and It's Analogs with Hydrazine and It's Derivatives

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L-Ascorbic acid and its analogs were found to react with hydrazine and substituted hydrazines in an aerobic, alkaline aqueous solution to give rise to some stable radical intermediates. The ESR parameters of the radical species were determined, and a possible radical structure common to all the reactions concerned was presented.

Electron spin resonance (ESR) studies on the radical species that appear during the autoxidation of the alkaline aqueous solution of L-ascorbic acid with hydrazine were first reported by Burlamacchi, et al.[2] The work was reinvestigated by us leading to suggest an assignment different from their's for the radical species.[3] The structure (A) which they proposed was based on the wrong structure for mono-dehydro L-ascorbic acid.[4]
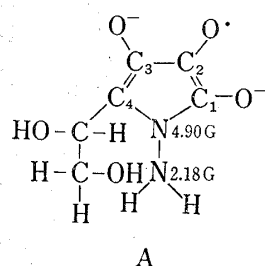
As to the ESR parameters of this radical, it has been noted that the ratio of the two nitrogen hyperfine splittings ($a_{N1}/a_{N2}=2.24$) or and the $g$-value (2.0044) are somewhat different when compared with that of the unstable hydrazyl radical[5] ($a_{N1}/a_{N2}=1.0$—1.5, $g=2.003$) of the stable hydrazoxyl radical[6] ($a_{N1}/a_{N2}=10$—20, $g=2.005$). Unfortunately, however, no convincing assignment has been put forward so far for the radical species.

A

Further studies were extended therefore on the reaction using some analogs of L-ascorbic acid (denoted as I) as well as derivatives of hydrazine (denoted as II) to identify the radical species. The purpose of the present work is to propose a reasonable radical structure in terms of the ESR parameters obtained under various experimental conditions, and also to suggest that some precursor must be present before the radical species are generated.

## Experimental

Materials——A purified sample of L-ascorbic acid (Ia), supplied by Takeda Chemical Industries, was used without further purification. 3-Hydroxy tetronic acid (Ib) was synthesized by the Claisen condensation of ethyl benzoylglycolate according to Micheel, et al.,[7] and purified by recrystallizations. 3-Hydroxy-5-methyl tetronic acid (Ic) was also prepared by the mixed Claisen condensation of ethyl benzoylglycolate and ethyl benzoylacetate, and purified by fractional crystallizations. Triose reductone (Id) was prepared from glucose by the method of von Euler and Hasselquist[8] and used without purification. All of these compounds were identified by melting point, elemental analyses and infrared spectra, and nuclear magnetic resonance (NMR) spectra. These four compounds have the same basic structure as

$$-C(OH)=C(OH)-C(=O)-$$
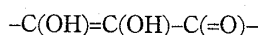
the former three being $\gamma$-lactone. Following hydrazine derivatives were subjected to reactions; hydrazine

1) Location: Hongo, Tokyo, 113, Japan.
2) L. Burlamacchi, P. Sarti-Fantoni and E. Tiezzi, Tetrahedron Letters, 1969, 5005.
3) H. Utsumi, Y. Kirino, and T. Kwan, Chem. Pharm. Bull. (Tokyo), 22, 1417 (1974).
4) Y. Kirino and T. Kwan, Chem. Pharm. Bull. (Tokyo), 19, 718 (1971).
5) V. Malatesta and K.U. Ingold, Tetrahedron Letters, 1973, 3307.
6) S. Terabe and R. Konaka, J. Am. Chem. Soc., 91, 5655 (1969).
7) F. Micheel and F. Jung, Chem. Ber., 66, 1291 (1933); idem, ibid., 67, 1660 (1934).
8) H. von Euler and H. Hasselquist, Arkiv, Kemi., 26B, Nr. 5 (1948).

hydrate (IIa), methylhydrazine (IIb), N,N-dimethylhydrazine (IIc), N,N'-dimethylhydrazine dihydrochloride (IId), phenylhydrazine hydrochloride.   Semicarbazide hydrochloride (IIe) was also employed as the reactant. Dimethylsulfoxide (DMSO), used as the solvent, was of the highest grade commercially available.

**Reactions and ESR Measurements**——Alkaline aqueous solutions of compounds (Ia—Id) with hydrazine and its derivatives (II) were prepared in a pyrex test tube.   The solutions were adjusted to pH 9 and then exposed to the air at room temperature.   After standing for some dozen hours (the time depended on the reactants), the ESR spectra were recorded at room temperature on the sample solution kept in a quartz tube with JEOL-PE-1 spectrometer with 100 KHz field modulation.   The DMSO solution of I and II was prepared by adding three times as much II as I by molarity and operated in the same manner as in the case of aqueous solutions.

**Thin–Layer Chromatography**——Thin–layer chromatography (TLC) was performed on "Silica Gel $GF_{254}$" with acetone–ethanol (3:1).

To detect the spots, the UV absorption at 254 nm and Tillman's Reagent were employed.   Then, each spot in the preparative TLC was scraped off, extracted with distilled water, and subjected to ESR measurements after adjusting to pH 9.

## Result and Discussion

In Fig. 1 was reproduced a typical ESR spectrum observed during the autooxidation of the alkaline aqueous solution of L-ascorbic acid with hydrazine.   This spectrum apparently consisted of nine resonance lines, and was identical with that of the Burlamacchi's, except that each line was somewhat broader.   The nine resonance lines were already interpreted
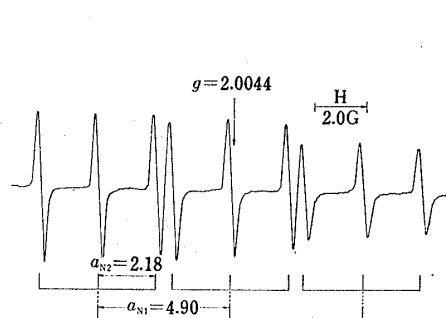


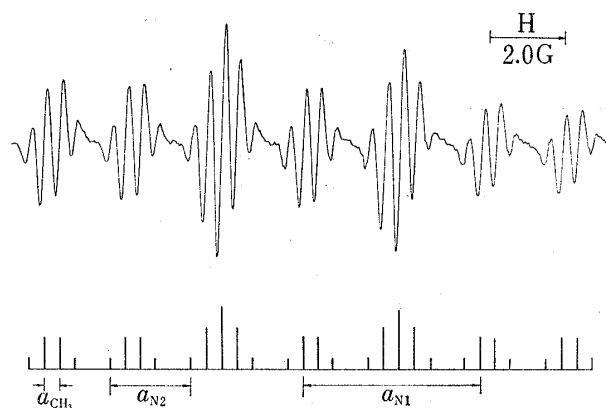Fig. 1.   The ESR Spectrum of Free Radical Formed during the Reaction of L-Ascorbic Acid with Hydrazine in $H_2O$



Fig. 2.   The ESR Spectrum of Free Radical Formed during the Reaction of L-Ascorbic Acid with Methyl Hydrazine in $H_2O$ ($a_{N1}=4.74G$, $a_{N2}=2.23G$, $a_{CH_3}=0.41G$)

as due to hyperfine interaction of odd electron with two non-equivalent nitrogen atoms. When DMSO was employed as the solvent instead of distilled water, each line appeared to split into doublet.   Similar splittings were found also in DMF.   The origin of this doublet has already been proved by us[3] to be due to the $C_4$–H of L-ascorbic acid moiety of the radical species.

A spectrum similar to that of Fig. 1 was also found from the reaction of triose reductone (Id) with hydrazine (IIa) under the same reaction condition as L-ascorbic acid, suggesting that triose reductone reacts with hydrazine at $C_1=O$ or $C_3$–OH of triose reductone, since only one proton except for two nitrogen nuclei was hyperfine-coupled with the odd electron. The analog of ascorbic acid such as Ib or Ic also gave rise to essentially the same spectrum with that of ascorbic acid when reacted with hydrazine so that the skeleton of the radical species formed seems to be of the common structure.

On the other hand, to get more information about the structure of hydrazine side of the radical species, various hydrazine derivatives (IIb, IIc, IId) were subjected to reactions with

L-ascorbic acid. In Fig. 2 is shown a typical ESR spectrum observed during the reaction of the alkaline aqueous solution of L-ascorbic acid with methylhydrazine (IIb). As can be seen in Fig. 2, the ESR spectrum consisted of nine quartets (relative intensities 1: 3: 3: 1). The quartet may probably be ascribable to three methyl protons of methylhydrazine. A similar spectrum was also observed when N,N-dimethylhydrazine (IIc) was employed in place of monomethylhydrazine (IIb). Thus, it seems likely that one of two methyl groups was eliminated from dimethylhydrazine during the reaction to form the radical species. The reaction of L-ascorbic acid with semicarbazide (IIe) also gave a similar sort of spectrum, indicating that the radicals were not of the azine structure. No ESR spectrum was observed, however, with phenylhydrazine.

The ESR parameters of the radical species obtained from the reaction of the analogs of L-ascorbic acid with hydrazine derivatives were summarized in Table I. Apparently, they

TABLE I. Hyperfine Coupling Constants of the Radical formed by the Reaction
of Ascorbic Acids with Hydrazines

| Solvent | Ia | | | | Ib | | | Ic | | | Id | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_1$ | $N_2$ | H | $CH_3$ | $N_1$ | $N_2$ | H | $N_1$ | $N_2$ | H | $N_1$ | $N_2$ | H | $CH_3$ |
| IIa $H_2O$ | 4.95 | 2.20 | a) | | 4.85 | 2.17 | 0.20(2) | 4.72 | 2.15 | a) | 5.21 | 2.33 | 0.55 | |
| DMSO | 5.04 | 2.22 | 0.35 | | 4.87 | 2.18 | 0.28(2) | 4.81 | 2.17 | a) | 5.15 | 2.34 | 0.58 | |
| $D_2O$ | | | | | 4.85 | 2.17 | 0.20(2) | | | | 5.20 | 2.30 | 0.5 | |
| IIb $H_2O$ | 4.74 | 2.23 | a) | 0.41(3) | | | | | | | | | | |
| DMSO | | | | | | | | | | | 4.97 | 2.35 | 0.86 | 0.53(3) |
| IIc $H_2O$ | 4.74 | 2.23 | a) | 0.41(3) | | | | | | | | | | |
| DMSO | | | | | | | | | | | 5.05 | 2.47 | 0.87 | 0.54(3) |
| IId $H_2O$ | 4.90 | 2.20 | a) | a) | | | | | | | | | | |
| IIe $H_2O$ | 4.90 | 2.20 | a) | a) | | | | | | | | | | |

Coupling constants are given in gauss. The number of hydrogen atoms displaying the hyperfine constant is given in parentheses if more than one. The g factor for the radical is 2.0045 ± 0.0001.

a) Hyperfine coupling are not observed.

have similar characteristics with respect to the hyperfine splittings due to two nitrogen nuclei ($a_{N1}$=4.7—5.2G, $a_{N2}$=2.2—2.5G) and g-values (g=2.0044—2.0046), so that the basic structure of these radical species may be considered to be the same.

Consequently, it is suggested that the basic structure of the radical species under investigation is of the type:
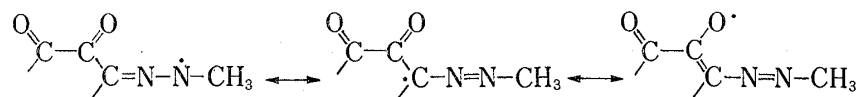


When compared with the reported ESR parameters of hydrazyl radicals[5] ($a_{N1}/a_{N2}$=1.0—1.5) and of hydrazoxyl radicals[6] ($a_{N1}/a_{N2}$=10—20), the assignment seems to be rather close to those of the former, although the present radicals are as stable as the latter. A highly rosonatated structure would have contributed to the stability of the present radical species.

Conditions under which the radical species are readily generated were investigated. When the molar ratio of hydrazine over L-ascorbic acid was changed from 1 to 4, the concentration of the radical species remained unchanged. On the other hand, when pH was allowed to increase from 5 to 11, the amount of the radical species increased to a remarkable extent especially around pH 9 as shown in Fig. 3. They were stable over days at room temperature. It is interesting to note that the radical species are formed only in the weak alkaline region, while monodehydro L-ascorbic acid radicals are readily detectable at pH 7.5 or above.

L-AsA(0.5M)
NH₂NH₂(1.5M) ╱ H₂O

Peak height

5.0

3.0

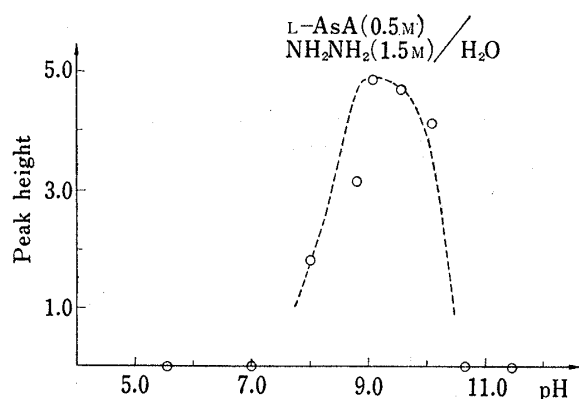1.0

5.0     7.0     9.0     11.0   pH

Fig. 3.   The pH Dependence of the Amount of
the Free Radical Formed

pH was adjusted by way of adding aq. HCl or aq. NaOH.

As described previously, the radical species were
never produced under nitrogen atmosphere in any pH
region, nor produced rapidly even in the presence of air;
some dozen hours' exposure to the air was necessary
for the ready formation of the radical species. The
radicals were not formed in the acidic and aerobic
solution, but immediately observed when sodium
hydroxide was added to pH 9.

Consequently, it can be considered that the reaction
of I with II takes place rather slowly to form some
diamagnetic intermediate *i.e.* a precursor of the radical
species. The formation of such intermediates would
probably be associated with some condensation of
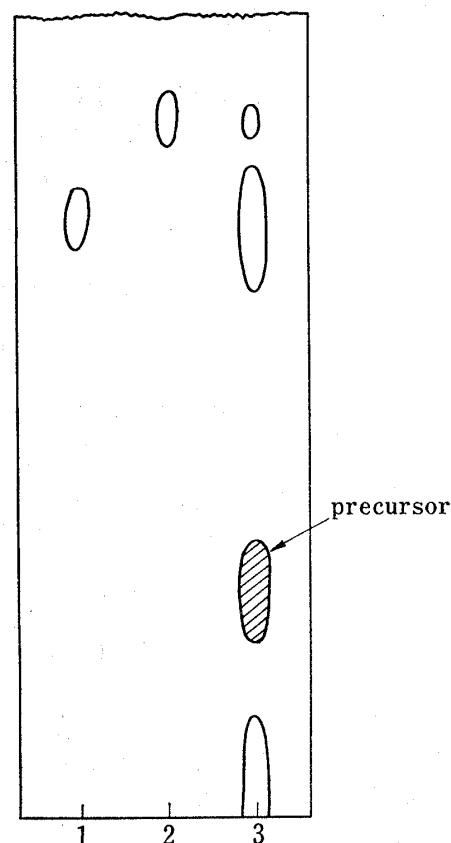
precursor

1     2     3

Fig. 4.   Thin–Layer Chromatography
of L-Ascorbic Acid (1), Hydrazine
Hydrate (2) and Reaction Mixture
Containing "Precursor" (3)

ascorbic acid (I) with hydrazine (II).   The intermediate is then quickly oxidized in the weak
alkaline region to yield the radical species. The oxidation took place with ceric salt or
potassium ferric cyanide likewise as air.   The reaction route of this kind was schematically
shown as:

$$I + II \quad \xrightarrow{\text{slow}} \quad \text{radical precursor} \quad \xrightarrow[\text{oxidant}]{\text{rapid}} \quad \text{radical species}$$
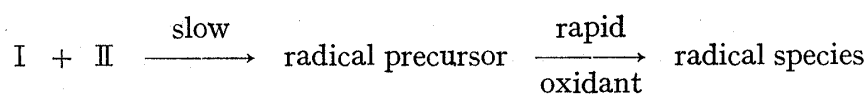
The existence of the precursor was actually confirmed by the thin–layer chromatography
(Fig. 4).   The precursor was colorless on the spot initially and inactive to ESR, but after
several hours' exposure to air it turned out to be purple and became active to ESR.   One of
reaction products, when oxidized with air, was identified to be oxalic dihydrazide, but accom-
panied by many other unknown products.   The isolation and identification of the radical
precursor, however, await further investigations.