

# Electron Paramagnetic Resonance Study of the Imino Ascorbyl Radicals Formed in the Photochemical Reaction in the Presence of Nitrite between Ascorbic Acid and the Amino Compounds DL-Alanine, Methylamine, Ethylamine, Glycine, Ammonia or Hydrazine

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## Dedicated to Professor Lennart Ebersson on the occasion of his 65th birthday

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Irradiation with UV light at room temperature of a mixture of the amino compounds DL-alanine, methylamine, ethylamine, glycine, ammonia or hydrazine, and sodium nitrite-ascorbic acid dissolved in alkaline water solution gave rise to radicals detectable by EPR spectroscopy. The observed radicals were considered to be mono imino or mono hydrazine ascorbyl radicals formed by a one-electron oxidation of the corresponding imino or hydrazyl derivatives formed in the nucleophile reaction between the amino compounds and an oxo group of ascorbic acid. The reactions are believed to be promoted by a photoexcited state of the nitrite ion.

In connection with EPR studies of the reaction of the nitrite ion, it was found that stable radicals were produced in the reaction with the amino group of aminoarenes such as 4-aminobenzoic acid, sulfanilic acid, 3,5-dimethoxyvaniline or aminobenzene.<sup>1</sup> The radicals were formed at room temperature in mixtures containing the parent aminoarenes, sodium nitrite and ascorbic acid when first dissolved in water followed by the addition of NaOH to an alkaline pH. The radicals appeared without any previous irradiation with UV light. The reaction involves the formation of the aryldiazonium ions  $\text{ArN}^+=\text{N}$  (acidic condition) in the reaction with nitrite followed by their reduction to aryl radicals  $\text{Ar}^\bullet$  in the reaction (alkaline condition) with ascorbic acid. The radicals  $\text{Ar}^\bullet$  combine with nitrite ions to give the observed aryl nitro anion radicals  $\text{ArNO}_2^{\bullet-}$ .

Similar studies with the amino compounds DL-alanine, methylamine, ethylamine, glycine, ammonia or hydrazine as parent substances gave rise to completely different

radical species. Reaction between an oxo group of ascorbic acid and the amino group of these substances gave rise to mono imino or hydrazone derivatives of ascorbic acid which were oxidized to the corresponding ascorbyl radicals.

## Experimental

Electron paramagnetic resonance (EPR) spectra were obtained using a Varian E-9 spectrometer at 20 °C with a microwave power of 1–5 mW and a 100 kHz modulation amplitude of 0.01–0.05 mT. The samples were contained in a flat aqueous solution cell. Hyperfine splittings were measured by comparison with the splittings of Fremy's radical ( $a_{\text{N}14} = 1.3$  mT).<sup>2</sup> The samples were irradiated with UV light (Osram HBO 200) *in situ* in the EPR cavity. Spectra simulations were performed by use of a computer program constructed by Oehler and Janzen.<sup>3</sup> The chemicals, including peroxyamine disulfonate, for producing Fremy's radical were from Sigma or Aldrich and were used as supplied.  $\text{Na}^{15}\text{NO}_2$  was from ICON Inc.

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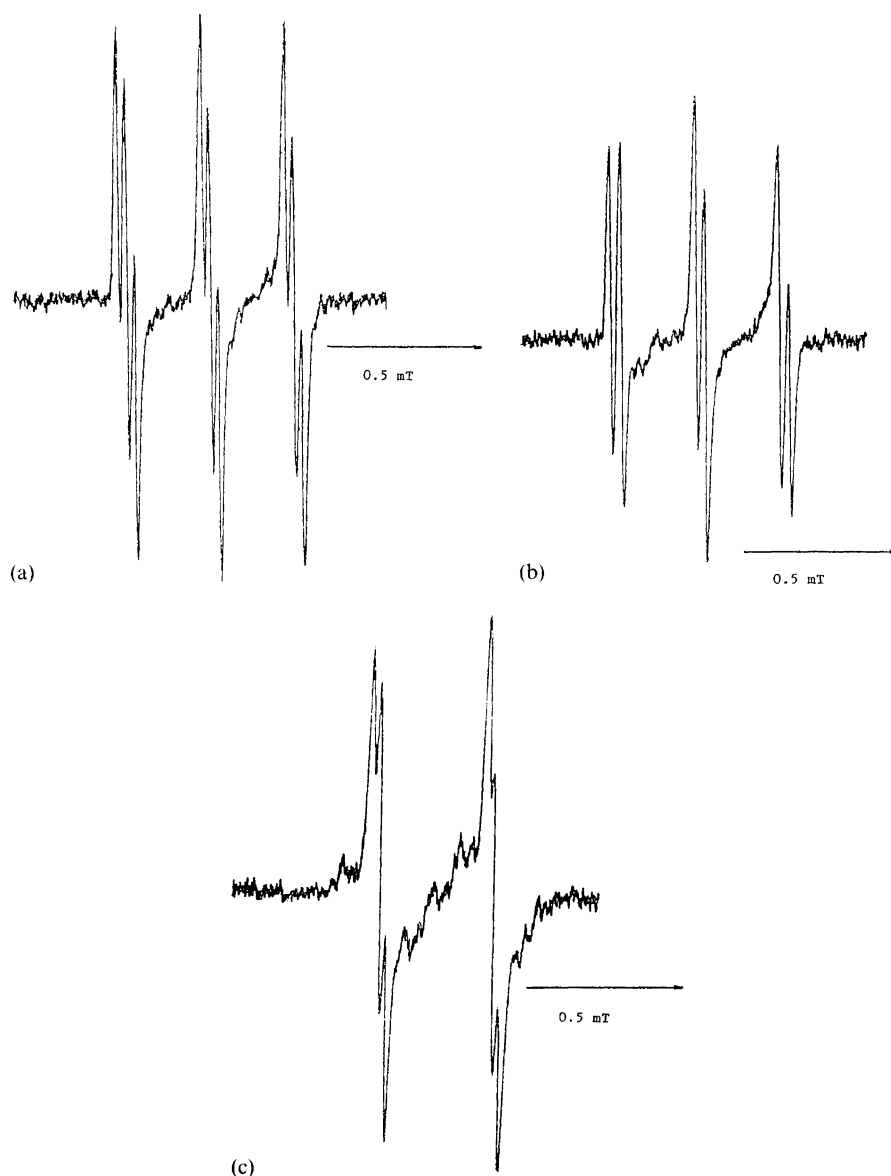


Fig. 1. The EPR spectra of the radicals formed in the photochemical reaction between DL-alanine, sodium nitrite and ascorbic acid: (a) with  $^{14}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$ ; (b) with 2,3,3,3- $\text{d}_4$ - $^{14}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$ ; (c) with  $^{15}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$ .

The radicals were prepared by simultaneously dissolving the parent substance (60–80 mg), sodium nitrite (50–70 mg) and ascorbic acid (30–50 mg) in 150  $\mu\text{l}$  of  $\text{H}_2\text{O}$  + 100  $\mu\text{l}$  of 1 M HCl followed by the addition of 250  $\mu\text{l}$  of 1 M NaOH after the mixture had been kept standing for ca. 30 s.

## Results

### Radicals obtained from DL-alanine.

1. *With  $^{14}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$ .* Figure 1a shows the EPR spectrum observed when the reaction mixture was irradiated with UV light *in situ* in EPR cavity. The radicals appeared immediately after the start of the irradiation. The spectrum shows a triplet (1:1:1) with a splitting constant of 0.26 mT. Each of the three lines was

further split into three narrow lines with a splitting constant of ca. 0.03 mT. The radicals were stable and were observed for ca. 10 min of irradiation. The radicals decayed almost immediately when the light was turned off.

2. *With 2,3,3,3- $\text{d}_4$ - $^{14}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$ .* Figure 1b shows the spectrum obtained with this system. The triplet splitting of 0.26 mT is preserved but the narrow secondary triplet is replaced by a narrow doublet with a splitting of ca. 0.03 mT.

3. *With  $^{14}\text{N}$ -alanine and  $\text{Na}^{15}\text{NO}_2$ .* The spectrum was identical with that obtained with  $^{14}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$ , i.e. with that of Fig. 1a.

4. *With  $^{15}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$ .* The spectrum (Fig. 1c) shows a doublet splitting of  $1.4 \times 0.26 = 0.36$  mT, where the factor 1.4 is the ratio between the

coupling constants of  $^{15}\text{N}$  and  $^{14}\text{N}$  nuclei.<sup>2</sup> Each of the two lines is incompletely split into a narrow triplet.

5. With  $^{14}\text{N}$ -alanine and  $\text{Na}^{14}\text{NO}_2$  in  $\text{D}_2\text{O}$  and 1 M NaOH in  $\text{D}_2\text{O}$ . The spectrum was identical with that observed in  $\text{H}_2\text{O}$ .

6. No radicals were observed in reaction mixtures without added ascorbic acid or sodium nitrite.

The experiments indicate that the triplet (0.26 mT) and the doublet (0.36 mT) originate from an interaction of the unpaired electron with the nitrogen nucleus derived from the amino group of the parent alanine. The narrow triplet splitting (Figs. 1a and 1c) is considered to originate from an interaction with two accidentally equivalent hydrogen nuclei. The narrow doublet splitting of Fig. 1b originates from one of these nuclei only. One of these two hydrogens constitutes the  $\alpha$ -hydrogen of alanine. Identification of the other hydrogen will be discussed below.

*Radicals from methylamine.* Figure 2 shows the EPR spectrum obtained with methylamine hydrochloride in the reaction with sodium nitrite and ascorbic acid. The radicals were prepared as described for the radicals from alanine. The spectrum exhibits  $3 \times 4 (1:3:3:1) = 12$  lines, each incompletely resolved into doublets:  $a_{\text{N}14} = 0.25$  mT (1 N);  $a_{\text{H}}^1 = 0.31$  mT (3 H);  $a_{\text{H}}^2 \approx 0.03$  mT (1 H). This corresponds to an interaction with one nitrogen, three equivalent methyl hydrogens and a further hydrogen.

*Radicals from ethylamine.* The spectrum observed with ethylamine hydrochloride in the photochemical reaction with nitrite-ascorbic acid is a superposition from two species. An acceptable fit to the experimental spectrum of the main species was obtained with the simulation parameters:  $a_{\text{N}14} = 0.228$  mT (1 N) and two non-equivalent hydrogens:  $a_{\text{H}}^1 = 0.336$  mT (1 H);  $a_{\text{H}}^2 = 0.280$  mT (1 H) and  $a_{\text{H}}^3 = 0.027$  mT (1 H), linewidth 0.025 mT. A minor amount of a further radical species was present also.

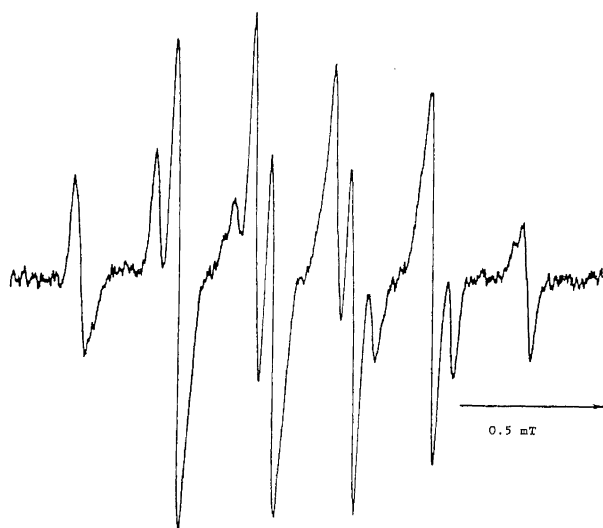


Fig. 2. EPR spectrum observed with methylamine hydrochloride.

*Radicals from glycine.* The radicals from glycine were prepared as described for alanine. The main spectrum exhibited a  $3 \times 2 \times 2 = 12$  line system which indicated an interaction with one nitrogen, and two hydrogen nuclei which were non-equivalent:  $a_{\text{N}14} = 0.25$  mT (1 N),  $a_{\text{H}}^1 = 0.34$  mT (1 H) and  $a_{\text{H}}^2 \approx 0.03$  mT (1 H).

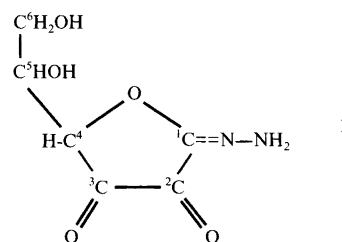
*Radicals obtained with ammonia.* The spectrum obtained with ammonium hydrochloride exhibited six lines. The central lines were incompletely resolved into doublets:  $a_{\text{N}14} = 0.22$  mT (1 N);  $a_{\text{H}} = 0.18$  mT (1 H). The spectrum obtained with  $\text{D}_4\text{NCl}$  in  $\text{H}_2\text{O}$  and 1 M NaOH in  $\text{H}_2\text{O}$  exhibited a triplet splitting by interaction with the nitrogen nucleus:  $a_{\text{N}14} = 0.22$  mT (1 N). The expected deuterium triplet could not be recorded depending on exchange of deuterium for hydrogen. However, the deuterium triplet was resolved when the reaction was made in  $\text{D}_2\text{O}$  and 1 M NaOH in  $\text{D}_2\text{O}$ :  $a_{\text{D}} = 0.028$  mT (1 D). This value is in conformity with the splitting observed with  $\text{H}_4\text{NCl}$  in  $\text{H}_2\text{O}$ , i.e.  $0.18/6.5 = 0.028$  mT where the factor 6.5 is the ratio between the coupling constants of H and D.<sup>2</sup>

*Radicals from hydrazine.* The radicals were prepared by dissolving hydrazine dihydrochloride (40 mg) and sodium nitrite-ascorbic acid (30 mg each) in 200  $\mu\text{l}$  of  $\text{H}_2\text{O}$  followed by the addition of an equal volume of 1 M NaOH. The EPR spectrum is shown in Fig. 3. There are nine lines of an equal amplitude which indicate an interaction with two non-equivalent nitrogens:  $a_{\text{N}14}^1 = 0.22$  mT (1 N) and  $a_{\text{N}14}^2 = 0.48$  mT (1 N). No splittings from hydrogen nuclei could be resolved.

The splitting constants of the radicals of this series are collected in Table 1.

## Discussion

*Structure of the radicals.* The radicals are considered to be derivatives of monoimines or monohydrazone of ascorbic acid formed in the nucleophilic reaction between an oxo or hydroxyl group of ascorbic acid and the amino group of the compounds of this series. Nitrogen derivatives of ascorbic and dehydroascorbic acid are well known, such as the monohydrazone derivative formed with hydrazine (**1**).<sup>4,5</sup>



The reactions which lead to the observed radicals **6** can be formulated as follows, where **3** is ascorbic acid and **4** the parent amino compound of this series. First

Table 1. Coupling constants of the monoimino radicals in mT.

Parent compound	$a_{N14}$	$a_H^1$	$a_H^2$	$\frac{a_H^3}{a_N}$
DL-Alanine $\text{CH}_3\text{C}(\text{H})(\text{COOH})-\text{N}=\text{A}^{\cdot-}$	0.26	0.03 (1 H)	—	0.03
Methylamine $\text{CH}_3\text{N}=\text{A}^{\cdot-}$	0.25	0.31 (3 H)	—	0.03
Ethylamine $\text{CH}_3-\text{CH}_2-\text{N}=\text{A}^{\cdot-}$	0.23	0.336 (1 H)	0.28 (1 H)	0.03
Glycine $(\text{HOOC})\text{CH}_2-\text{N}=\text{A}^{\cdot-}$	0.25	0.34 (1 H)	—	0.03
Ammonia $\text{H}-\text{N}=\text{A}^{\cdot-}$	0.22	0.18 (1 H)	—	—
Hydrazine $\text{H}_2\text{N}-\text{N}=\text{A}^{\cdot-}$	0.22	—	—	0.48 (1 N)

A, ascorbic acid.

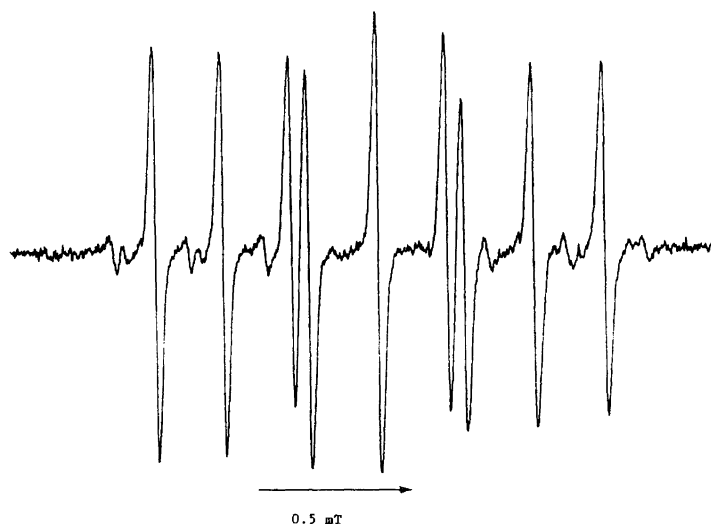
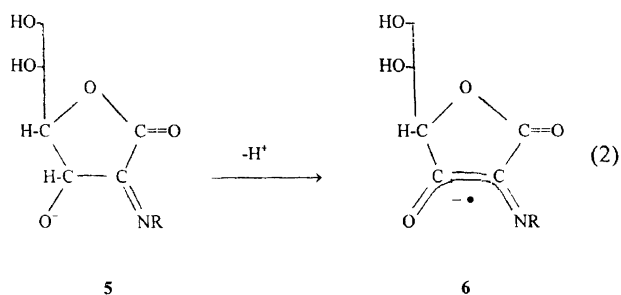
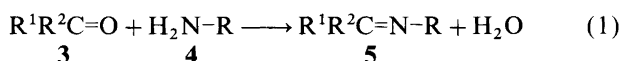


Fig. 3. EPR spectrum obtained with hydrazine dihydrochloride.

the corresponding monoimino of hydrazine derivative of ascorbic acid **5** is formed [eqn. (1)]. The imino or hydrazyl derivative **5** is then oxidized to the observed radicals, **6** [eqn. (2)].



It is suggested that the reaction which gives rise to the radicals is promoted by a photoexcited state of the nitrite ion,<sup>6</sup> i.e.  $[\text{NO}_2^-]^*$ . The EPR spectra of radicals **6** exhibit interactions with nitrogen and hydrogen nuclei of the group R of the parent amino compounds **4**. Of the two narrow doublet splittings, ca. 0.03 mT observed in the spectra obtained with alanine, one originates from interaction with the hydrogen in the  $\alpha$ -position of the parent substance. The other is suggested to originate from an

interaction with the hydrogen attached to carbon atom 4 of ascorbic acid (cf. structure **1**). This interpretation is supported by the statement that the doublet splitting of the ascorbyl radical, i.e. 0.14 mT originates from an interaction with this hydrogen.<sup>5</sup> It seems possible that the narrow and uncompletely resolved doublet splittings observed in the spectra from methylamine, ethylamine and glycine originate from a similar interaction also. Evidently the spin density of the imino ascorbyl radicals is displaced towards the substituent groups, so that the interaction with the hydrogen in position 4 (cf. **1**) becomes very small. The doublet splitting, which could be exchanged to a deuterium triplet in the spectra with ammonia, originated from an interaction with the hydrogen in the group R, i.e.  $=\text{NH}$ . In the present reactions, the substitution was formulated to take place on carbon atom 2 of ascorbic acid, cf. eqn. (2). The substitution could be on carbon 1 or 3 just as well. No means seems to be at hand to decide at which of the three carbon atoms the substitution takes place.

Alternative structures of the observed radicals might involve the formation of aminyl radicals of the type  $\text{R}-\text{N}^\bullet\text{H}$  ( $\text{R}=\text{H}, \text{NH}_2, \text{alkyl}$ ). Such radicals are expected to be very short-lived and not able to be observed directly at room temperature, and to exhibit much larger nitrogen coupling constants. Thus the aminium radical  $\text{N}^\bullet + \text{H}_3$

observed in a matrix at low temperature had a nitrogen coupling constant of 1.95 mT and  $a_{\text{H}}=2.58$  mT (3 H), which indicated a planar structure.<sup>7</sup> In view of these statements the formation of aminyl radicals seems to be ruled out. Finally it should be emphasized that a number of oxo compounds gives rise to *gem*-nitronitroso dianion radicals in their reaction with sodium nitrite–ascorbic acid.<sup>8</sup> However, these radical species have much larger nitrogen coupling constants, i.e.  $a_{\text{N}14}^1=1.00\text{--}1.20$  mT (1 N) and  $a_{\text{N}14}^2=0.50\text{--}0.60$  mT (1 N). Further these radicals decay almost immediately when irradiated with UV light.

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