

## Free Radicals in the Auto-Oxidation of Ascorbic Acid

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Piette *et al.*<sup>1-3</sup> have shown that free radicals are formed from ascorbic acid in the reaction between this substance and  $H_2O_2$  in the presence of peroxidase, and in the reaction with molecular oxygen in the presence of ascorbic acid oxidase. The kinetic mechanism of the oxidation was studied with the technique of electron spin resonance (ESR), especially at the pH optimum of the enzymes (4.8). The ESR-spectrum of the radicals exhibited a doublet structure with a splitting constant of 1.7 gauss which was suggested to be due to an interaction of the unpaired electron with the proton situated on the carbon atom in  $\beta$ -position to the double bond (position 5 in Fig. 1).

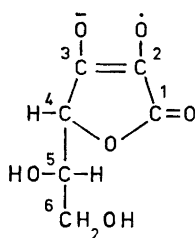


Fig. 1.

It has now been found that the spectrum of these radicals can be further resolved. The radicals were prepared by oxidation of ascorbic acid with dissolved molecular oxygen. A 0.1 M solution of ascorbic acid was prepared by dissolving 176 mg in 10 ml 0.1 N NaOH (non-degassed). The pH value of this solution was about 5.7. By further addition of small volumes of 0.1 N NaOH the pH was adjusted to higher values. Free radicals were found to be present in the pH interval between 6.6 and 9.6. Kept in stoppered sample tubes the ESR-spectrum of the radicals could be observed for several hours.

The ESR-spectrum exhibited a triplet splitting of each of the two previously described lines with a splitting constant of about 0.17 gauss (Fig. 2). The intensity

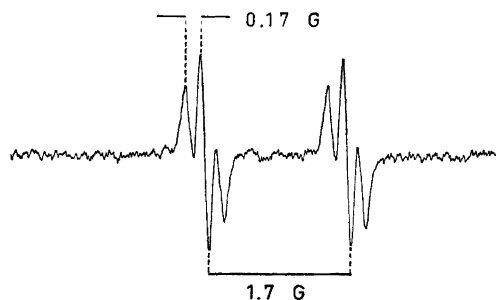


Fig. 2. ESR-spectrum of free radicals derived from ascorbic acid. pH = 8.2

ratio was close to 1:2:1. In order to resolve these structures, the microwave power applied to the sample had to be attenuated at least 20 db. The origin of the triplet splitting is due to an interaction with two equivalent protons, almost certainly those attached to the carbon atom in position 6 (Fig. 1). This interpretation is consistent with the assignment made by Piette *et al.*,<sup>1</sup> the doublet splitting being due to the proton on carbon atom 5. No splittings due to the proton situated on carbon atom 4 have been observed. Evidently the coupling to this proton is very small probably for steric reasons.

The spectra were obtained by a Varian 100 kc spectrometer.

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