CHEMISTRY LETTERS, pp.1193-1196, 1974. Published by the Chemical Society of Japan

ON THE STRUCTURES OF THE FREE RADICAL PRODUCTS FORMED BY THE REACTION OF DEHYDROASCORBIC ACID WITH AMINO ACIDS

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In addition to the radical products of a triplet esr signal (R-A) and a quintet-doublet (R-B), a new radical product of multiplet signal was isolated from tlc of the reaction mixture of dehydroascorbic acid with an amino acid. Structures were postulated for these radical products based on the esr spectral analysis and their properties in relation with the red pigment.

It has previously been reported[1] that the reaction of dehydroascorbic acid (DHA) with α-Ala in ethanol was found to give fairly stable radical products other than the red pigment which is a main product and has been isolated and identified as in Fig. 2.[2,3] The esr spectrum of this mixture was assumed to be composed of two kinds of spectra, a triplet one (Spectrum A) and a quintet-doublet one (Spectrum B), then the radical products corresponding to them are termed R-A and R-B respectively. R-A could be isolated on tlc as a blue fraction and its water extract exhibited a triplet signal as shown in Fig. 1-d. While, the same reaction done in water system gave the spectrum considered to be composed of Spectrum A and another multiplet one.

With our further investigations the latter multiplet product could be isolated and the present paper deals with its chemical and spectral properties as well as with the structural information of these radical products in relation with the red pigment.

Experimental conditions were essentially the same to the procedures described in the preceding paper.[1]

It was previously described that with the preparative tlc of the reaction mixture of DHA and α-Ala in ethanol the esr signal could be detected only on the blue band. However, in a detailed re-examination on tlc a weak but apparently different esr spectrum from that of R-A could be detected on the spot neighboring to the red pigment. The esr spectrum of water extract is shown in Fig. 1-a and this new radical product is termed R-C. The esr spectrum is resolved into the 9.18 G doublet, 8.27 G triplet and 0.92 G triplet responsible for a proton, a nitrogen and two equivalent protons respectively. This assignment is confirmed by the simulated pattern as shown in Fig. 1-b. With the examination of R-C in D_2O the 9.18 G proton splitting was replaced by a 9.18 G / 6.514 = 1.41 G deuterium splitting as shown in Fig. 1-c. Then it is concluded that the 9.18 G proton is attached to the 8.27 G nitrogen nucleus. The reaction of DHA with Gly in water gave the spectrum identical with

that seen in the DHA and α-Ala system, therefore, R-C was known to be absent of the amino acid residue. The reduction of the water extract of the red band with ascorbic acid (AsA) resulted in a remarkable increase in Spectrum C, which was hardly detected originally. Then R-C may be a reduced product of the red pigment and must have a proton on the nitrogen nucleus as the proposed structure shown in Fig. 2. This structure is similar to that of ascorbic acid free radical where the unpaired electron spread over a conjugated tricarbonyl system.

The water extract of R-C being allowed to stand at room temperature, the spectrum was found to change remarkably, namely that of R-C has faded out and contrary to this Spectrum A has appeared and hereafter increased. This indicates the possibility of the conversion of R-C to R-A. The spectrum of R-A resolved into the 8.45 G triplet and the 0.81 G triplet responsible for a nitrogen and two equivalent protons, respectively, as shown by the stick spectrum in Fig. 1-d. This assignment is also supported by the simulated pattern presented in Fig. 1-e. The fact that no significant change was detected in Spectrum A by the treatment with D_2O suggests that no proton is attached to the nitrogen nucleus in R-A. Similar reactions done in ethanol with such amino acids as Gly, Leu, Phe, Glu, Try, Lys, His, provided the blue spot of the same Rf value on tlc and from where the radical product R-A was extracted in each case. These results indicate that the amino acid residue was absent from the structure of R-A.

As previously reported, R-A is considered to be identical with the blue substance and it was easily oxidized in air to give the red pigment. There remained some doubt that new formation of the red pigment may be caused by the oxidation of contaminated scorbamic acid (SCA), the possible precursor of the red pigment. However, a comparison in ninhydrin test of authentic SCA with the ethanol reaction mixture developed on tlc neglected the contamination of SCA in the blue substance. Thus, the oxidative formation of the red pigment from the blue substance was confirmed. In view of these facts R-A should also be a reduced compound of the red pigment.

On the basis of these results, it seems reasonable to consider that R-A has the structure shown in Fig. 2, where the unpaired electron on nitrogen can conjugate in a long range with the carbonyl groups in DHA moieties, which may contribute to an abnormally high stability of R-A as a radical product. Furthermore, it should be pointed out that the triplet spectrum of R-A is accompanied always by a minor signal in their wings. The details of this spectrum are not yet made clear, but because of its abnormal intensity and splitting it might be due to some other product closely resemble to R-A.

It seems to be some contradiction that R-C which is produced by the reaction in water system was detected from the ethanol reaction mixture. However, when water was added gradually to the ethanol reaction mixture, the spectrum originally mixed with A and B types was changed to give a splitting of B and an enhancement of A along with an appearance of C. These facts suggest that R-B may easily alter to R-C with some changes as in the dissociation caused by the addition of water, then the reactions employed in water and in ethanol should not be essentially different each other.

Preliminary experiment done with DHA and SCA in water indicated that the red

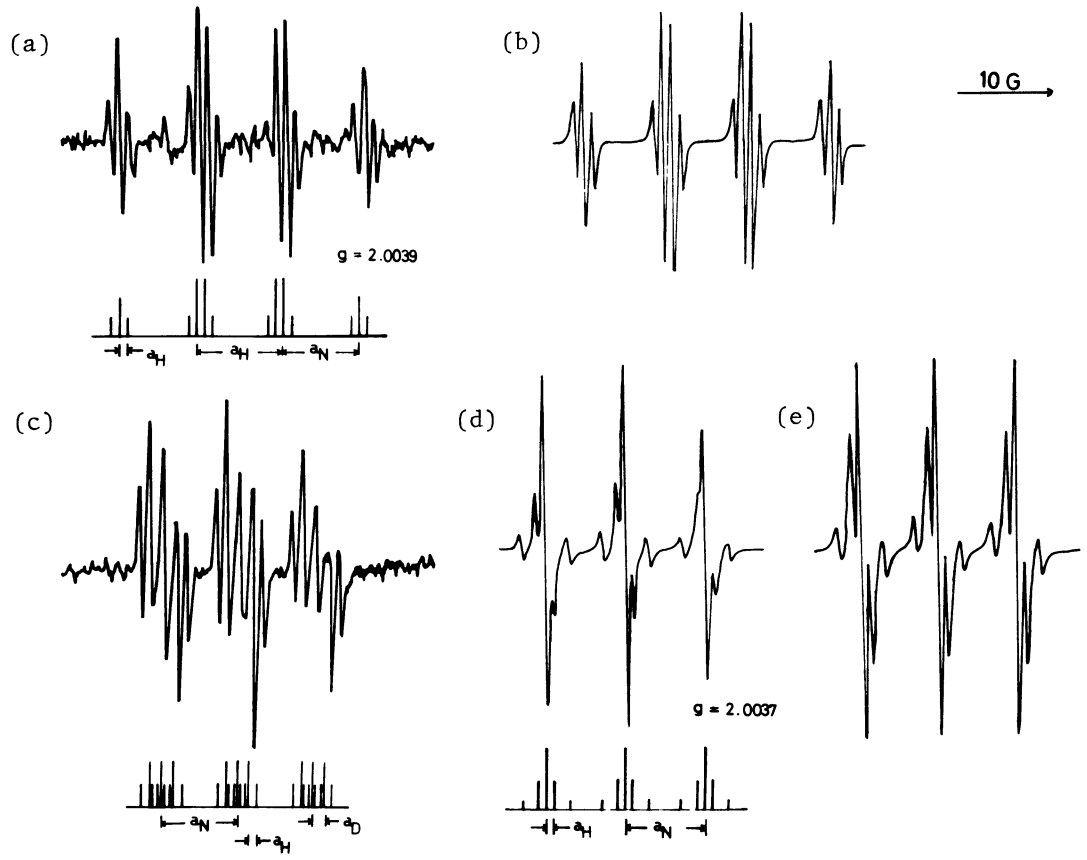

Fig. 1. ESR spectra of the extracts of the fractions on tlc of the reaction mixture of DHA and α-Ala in EtOH :(a) H_2O extract of the red spot :(b) Spectrum synthesized from the hyperfine pattern indicated by the stick spectrum in (a) : (c) D_2O extract of the red spot :(d) H_2O extract of the blue spot :(e) Spectrum synthesized from the hyperfine pattern indicated by the stick spectrum in (d), assuming the ratio of R-A and the unknown radical species accompanied in the wings of R-A is 5000:1500.

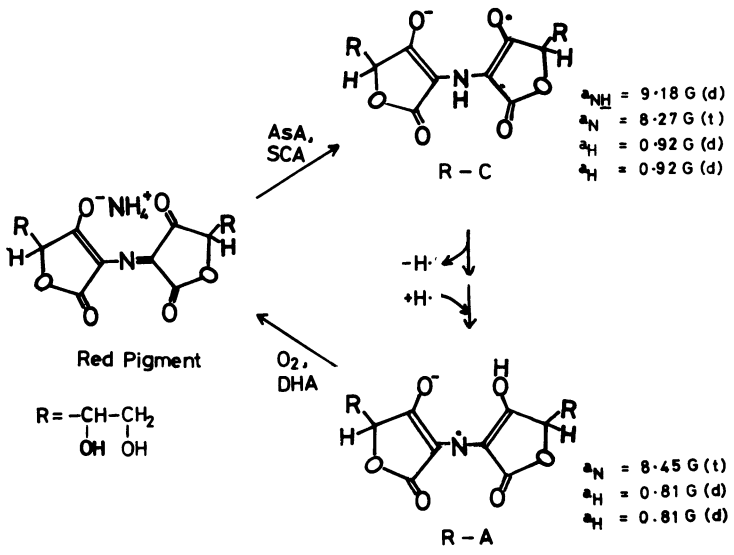

Fig. 2. Proposed structures of radical products and the relationship with the red pigment.

pigment formation occurred instantaneuosly and the esr signal appeared initially as the R-C type which followed by the development of the R-B type and finally changed to the R-A type. This fact is of interest in connection with understanding the stage of the formation of these radical products and the formation mechanism may tentatively be speculated as follows; the red pigment is produced by way of SCA as proposed by Kurata et al., then the radical species R-C is formed by the reduction of the red pigment with such as AsA and/or SCA[4] which are probably produced in the course of the reaction. The fact that Spectrum C changed to A especially in the presence of the red pigment suggests that R-A is formed from R-C and the reaction may not proceed directly but may be made through a set of proton transfer reaction in the presence of some redox reagent as the red pigment. Further investigations on the structure of R-B and on details of the reaction processes are being undertaken.

Acknowledgement

The authors wish to express their gratitude to Dr. Tadao Kurata of the University of Tokyo for suppling authentic scorbamic acid and to Dr. Keiichi Tsuji of the Institute of Physical and Chemical Research for valuable discussions.

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(Received July 15, 1974)