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ESR SPECTRA OF ASCORBIC ACID RADICALS IN APROTIC SOLVENTS[1]

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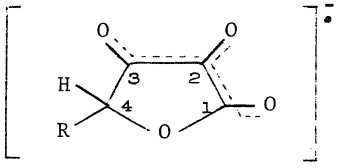
ESR spectral parameters of the radicals from L-ascorbic acid and its analogs in aprotic solvents are reported and compared with those for the same radicals in aqueous solutions. Marked differences in g factor as well as shifts (up to 16%) in proton and ^{13}C hyperfine coupling constants have been observed. Conformational changes in the side chain result in the C_6 protons becoming magnetically nonequivalent in the radical from L-ascorbic acid while equivalent in the radical from D-araboascorbic acid.

Extensive ESR studies have been carried out on intermediate radicals in the oxidation of ascorbic acids in aqueous solutions using enzymatic,[3] chemical,[4-6] radiation chemical,[7] and photochemical,[8,9] oxidation methods. Very recently Steenken and Olbrich[9] have reported ESR spectra of the radical from L-ascorbic acid in non-aqueous solutions where the two C_6 protons were found to be magnetically nonequivalent. In the present paper ESR spectral parameters in aprotic solvents of the radicals derived from not only L-ascorbic acid but also five of its analogs are reported and compared with those found in aqueous solutions.

L-Ascorbic acid was obtained from Aldrich Chemical Co. and D-araboascorbic acid from Eastman Chemical Co. Other analogs of L-ascorbic acid were prepared by the methods described previously.[6] Dimethylsulfoxide (DMSO) was dried over calcium hydride and distilled under a reduced pressure. Other chemicals used were of the highest grade available. ESR measurements were performed with an X-band spectrometer constructed in these Laboratories.[10] Except where otherwise noted, radicals were generated by dissolving the compounds in deoxygenated DMSO containing potassium tert-butoxide[11] and ESR measurements were carried out in a static system. Also used was an in situ photolysis method coupled with a flow system.[12]



I-VI

I R = $CH(OH)CH_2OH$ (L)
II R = $CH(OH)CH_2OH$ (D)
III R = $CH(CH_2)(O\text{-}C(CH_3)_2\text{-}O)$
IV R = $CH(OH)CH(OH)CH_2OH$
V R = H
VI R = CH_3

A DMSO solution of 5 mM L-ascorbic acid and 5 mM potassium tert-butoxide gave the well resolved second derivative ESR spectrum shown in Fig. 1. It indicates that the radical species generated is radical I, the same as that present in alkaline aqueous solutions.[7a] Significant changes in ESR parameters, however, are observed. The g factor is considerably larger compared to that in an aqueous system. Among the changes in proton hyperfine coupling constants, the most marked is that which leads to magnetic nonequivalence of the two C_6 protons in this system. This observation is similar to that by Steenken and Olbrich.[9,13] With higher concentrations and under conditions of higher spectrometer gain and larger modulation amplitude the species containing ^{13}C at the natural

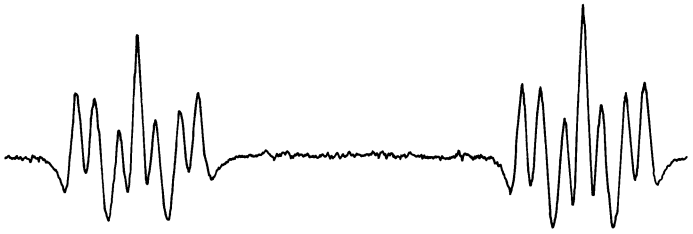


Fig. 1. The second derivative ESR spectrum of radical I from L-ascorbic acid in DMSO. Spectra are displayed with the magnetic field increasing from left to right throughout this paper.

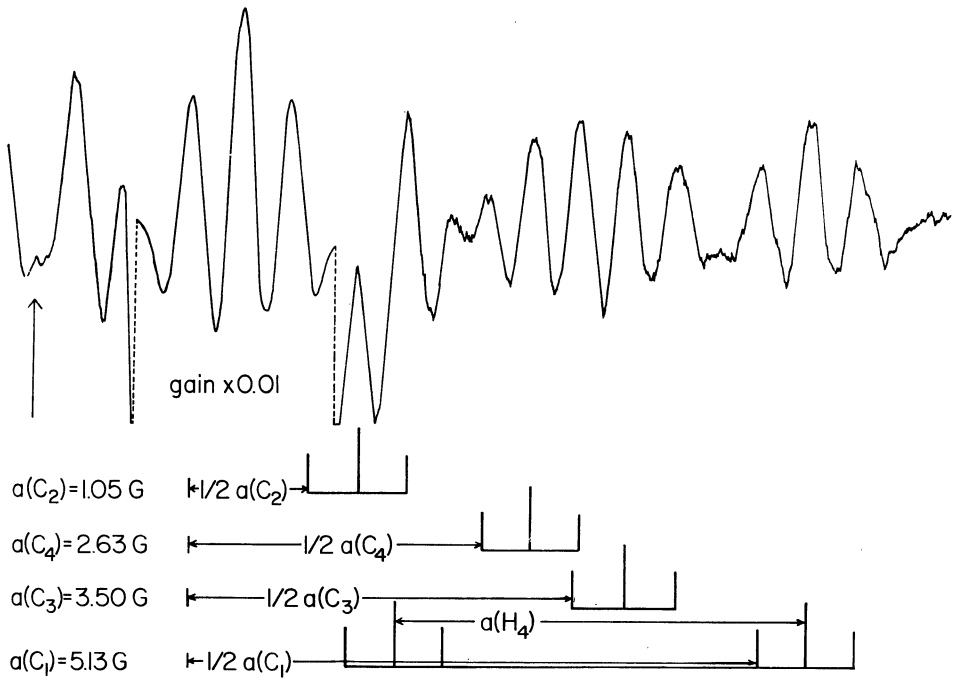


Fig. 2. High field portion of the ESR spectrum of ^{13}C-containing radicals from L-ascorbic acid in DMSO. The entire spectrum is symmetrical with respect to the arrow.

abundance level were also observed. As shown in Fig. 2, four ^{13}C-containing radicals are present. Their coupling constants were confirmed by simulation and assigned by analogy to the results of Laroff et al.[7a]

Since the experimental conditions employed are quite similar to those[14] where the cis form of the semidione anion radical from acetoin exists as an ion-pair with alkali metal cation, ion-pair formation was at first considered to be responsible for the change in the ESR spectrum in the present system from that in aqueous solutions. The ESR parameters, however, have been found to be independent of the change of counter cation ranging over Li^+, Na^+, K^+ and $(CH_3)_4N^+$ and of the concentration of solutions while hyperfine coupling constants have varied with solvent as shown in Table I. These results suggest that the differences between aqueous and non-aqueous systems are not due to ion-pairing effect but to solvent effects and it has been evidenced further by the fact that photoirradiation of L-ascorbic acid in a DMSO solution containing no additives gives rise to spectrum identical to that of Fig. 1.

Figure 3 shows the spectrum derived from D-araboascorbic acid, the C_5-epimer of L-ascorbic acid. The intensity ratio of the triplet is exactly 1:2:1 indicating that, in contrast to the L-ascorbic acid case, two protons which were nonequivalent in aqueous solutions[7a] become magnetically equivalent in DMSO.

The radical III from 5,6-isopropylidene-L-ascorbic acid gives a spectrum very similar to that from L-ascorbic acid in both of aqueous and DMSO solutions although the differences in ESR parameters between the two radicals are larger in DMSO than in aqueous solutions.

Comment should be made on the ESR spectrum from 5,6-isopropylidene-L-ascorbic acid in an aqueous solution. It was described in the past[6b,8] to be the same as that from L-ascorbic acid, and there was a doubt that the 5,6-isopropylidene-L-ascorbic acid may have hydrolyzed rapidly in an aqueous solution. However, in the present study it was shown that the spectra of the two radicals are not identical but definitely different from each other, although the difference is extremely

Fig. 3. The ESR spectrum of radical II from D-arabo-ascorbic acid in DMSO.

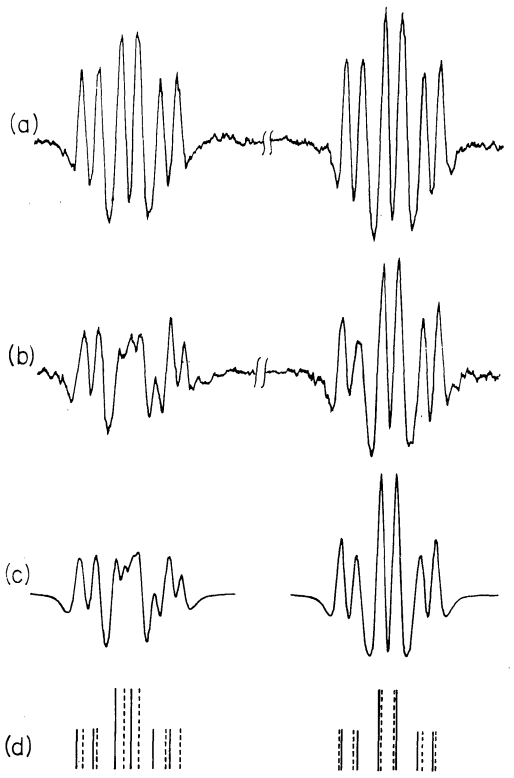


Fig. 4. ESR spectra of the radicals present during the UV-irradiation of 1 mM aqueous solutions of (a) 5,6-isopropylidene-L-ascorbic acid and (b) a 1:1 mixture of L-ascorbic and 5,6-isopropylidene-L-ascorbic acid. (c) Spectrum of the mixture synthesized from the parameters given in text with Lorentzian lines of width 53 mG. (d) Stick spectra of the radicals from 5,6-isopropylidene-L-ascorbic (——) and L-ascorbic (-----) acid.

small. The difference is very clearly illustrated in Fig. 4. A 1:1 mixture of two radicals shows the spectrum of Fig. 4b which is the superposition of the spectra of each radical. The difference between the high and low field groups of lines is clearly the result of a difference of 0.00001 in the g factors. The ESR parameters, in aqueous solutions, of the radical III were determined to be $g = 2.00520$, $a(H_4) = 1.795$ G, $a(H_5) = 0.074$ G, $a(H_6) = 0.176$ G by comparison of the observed and calculated spectra on the basis that those for the radical I are $g = 2.00519$, $a(H_4) = 1.760$ G, $a(H_5) = 0.067$ G, $a(H_6) = a(H_6) = 0.190$ G. The differences in the two sets of parameters are known very accurately from comparison of the observed spectrum with the synthetic pattern.

In Table I are summarized all the data mentioned above, together with the results on other ascorbic acid analogs, i.e., α-hydroxytetronic, γ-methyl-α-hydroxytetronic, and D-glucoascorbic acid. In all cases a considerable increase in the g factors is observed in DMSO. Similar changes in g factor with the composition of solutions have been already reported for acetoin radical anion.[15] The increase in the g factors indicates that in DMSO a substantially greater amount of unpaired electron density has been transferred from the ring atoms to the carbonyl oxygen atoms, as has been pointed out to be the case with trioxopyrrolidine radicals.[16] This transfer may also be responsible for changes in the ^{13}C hyperfine constants, particularly the appreciable decreases noted for the $^{13}C_1$ coupling constant. However, it is not clear by what mechanism the solvation

Table I. ESR Spectral Parameters of the Radicals from Ascorbic Acids[a]

Radical	Parent Molecule	Solvent	g factor	Hyperfine Coupling Constants (G)								Note
				H_4	H_5	H_6	H_6	$^{13}C_1$[f]	$^{13}C_3$[f]	$^{13}C_4$	$^{13}C_2$	
I	L-ascorbic acid	DMSO	2.00558	1.86	0.08	0.18	0.25	5.13	3.50	2.63	1.05	
		DMSO-THF	2.00557	2.03	0.07	0.17	0.24					
		DMF	2.00561	1.77	0.08	0.18	0.27					
		H_2O	2.00518	1.76	0.07	0.19	0.19	5.74	3.62	2.78	0.96	b
II	D-araboascorbic acid	DMSO	2.00558	1.99	< 0.03	0.15	0.15	5.16	3.44	2.70	1.11	
		DMSO-THF	2.00558	2.14	< 0.03	0.14	0.14					
		H_2O	2.00519	1.84	< 0.04	0.17	0.21	5.70	3.72	2.62	0.92	b
III	5,6-isopropylidene-L-ascorbic acid	DMSO	2.00563	1.99	0.06	0.12	0.16					
		H_2O	2.00520	1.80	0.07	0.18	0.18					c
IV	D-glucoascorbic acid	DMSO	2.00556	1.84	0.07	0.19	-					
		H_2O	2.00523	1.79	0.09	0.19	-					d
V	α-hydroxytetronic acid	DMSO	2.00563	2.29	-	-	-					
		H_2O	2.00519	2.32	-	-	-	5.72	3.65	2.85	1.03	b
VI	γ-methyl-α-hydroxy-tetronic acid	DMSO	2.00562	1.69	< 0.04	-	-	5.18	3.54	2.71	1.07	
		H_2O	2.00519	1.84	0.05	-	-	5.69	3.54	2.76	0.91	e

a) The hyperfine coupling constants and the g values are accurate to within ± 0.02 G and ± 0.00003 respectively.
b) Ref. 7a.
c) See text and Fig. 4
d) These values are more accurate than those reported in ref. 6b.
e) Ref. 7b.
f) Assignment to the C_1 and C_3 could be reversed; see ref. 7a.

perturbs the electronic and conformational structure of the radicals, especially with respect to the equivalence or nonequivalence of two C_6 protons of radicals I and II. The assumption of a restricted rotation of the side chain by intramolecular hydrogen bonding, proposed by Steenken and Olbrich,[9] does not seem to explain the results on radical III because the radical does not have hydroxyl groups in the side chain available for hydrogen bonding.

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