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Chemical reactions induced by ultrasound and γ-rays in aqueous solutions of L-ascorbic acid

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ABSTRACT

The degradation of L-ascorbic acid in aqueous solutions by ultrasound and ionising radiation was more extensive in the presence than in the absence of air. The primary products were L-*erythro*-2,3-hexodiulosonic acid, *cis*- and *trans*-L-*glycero*-4-hexulos-2-enonic acid; the secondary products were 2-pentulosonic acid, 4-pentulos-2-enonic acid, tetronic acid, and glyceric acid; and the tertiary product was tetraric acid. The formation of products with less than six carbon atoms was especially effective during irradiation in the presence of air. A mechanism for the degradation of L-ascorbic acid by sonolysis and radiolysis is proposed.

1. INTRODUCTION

The redox properties of L-ascorbic acid are responsible for its function as an antioxidant. Ascorbic acid can undergo a two-step oxidation–reduction process with a free-radical intermediate that is relatively stable, reacts slowly with oxygen [1–3], and disproportionates to give ascorbic acid and dehydroascorbic acid. The biological protective properties of ascorbic acid against damage by free radicals are attributed to its efficiency as a radical scavenger. The formation of radicals from ascorbic acid has been studied extensively by ESR spectroscopy. The radicals can originate from enzymic, chemical, photochemical, and radiation-chemical processes [4–6], and involve mainly H-abstraction from HO-2 to give the semidehydroascorbic acid radical, but H-abstraction can also occur [7] at C-4. No evidence for the formation of radicals by abstraction from the side chain of ascorbic acid has been reported. On irradiation of aqueous solutions, another set of radicals originates from the addition of HO$^{\cdot}$ to the double bond of ascorbic acid. Due to the keto-enol tautomerism in ascorbic acid, H-abstraction can

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occur [5,7] at C-1, 2, 3. The corresponding H-adducts were observed only on irradiation of single crystals of ascorbic acid, but not in aqueous solution [7].

In constrast to the numerous ESR studies of the formation of radicals from ascorbic acid by ionising radiation, investigation of products has been restricted to the measurement of the formation of dehydroascorbic acid, hydrogen, and the loss of ascorbic acid [8,9]. A speculative mechanism for the decomposition of ascorbic acid has been proposed, which assumes that, in addition to dehydroascorbic acid, L-gulonolactone should be formed by H-addition.

Both H$^{\cdot}$ and HO$^{\cdot}$ are primary products of the sonolysis and radiolysis of water [10]. In dilute aqueous solutions, these radicals react with the solutes and induce chemical reactions. We have investigated these reactions for L-ascorbic acid and have correlated the reactions with those of ESR spectroscopy given in the literature.

EXPERIMENTAL

Solutions of L-ascorbic acid in bidistilled water were irradiated at 10° with an 800-kHz ultrasound generator (Physikalische Werkstätten, PHYWE GMBH, Göttingen). The transducer had a diameter of 2.5 cm and an average intensity of 2 W/cm^2. γ-Irradiations were performed at 20° using a ^{60}Co source. For irradations in the presence of oxygen, air was bubbled through the solution during irradiation. For irradiation in the absence of oxygen, the glass vial containing the solution was repeatedly degassed by evacuation followed by saturation with argon, and then sealed. GLC–MS was performed with a Varian Model 3700 gas chromatograph and a Finnigan 212 mass spectrometer. The solvent was removed by evaporation in a vacuum centrifuge and the residues were silylated [11] and methoximated [12]. Trimethylsilylated D-mannitol was added as the standard after derivatisation. The appearance of a high molecular weight fraction, on irradiation of solutions of ascorbic acid with γ-rays in the absence of oxygen, was detected by size-exclusion chromatography on Ultrahydrogel 250 (Waters Millipore GmbH) with bidistilled water as the mobile phase.

RESULTS

Irradiation of L-*ascorbic acid and isolation of the products.*—L-Ascorbic acid (2.8 mM) in bidistilled water was irradiated in the presence and absence of air either by 800-kHz ultrasound or by ^{60}Co γ-rays. By using a Fricke–ferrous sulfate dosimeter, a dose rate of 1.2 kGy/h for the latter experiment was found to be equivalent to the dose rate of ultrasound (see ref. 13 for details). Each irradiated solution was evaporated to dryness and the residue was trimethylsilylated prior to GLC–MS. In some experiments, in addition to silylation, the carbonyl groups were methoximated. Due to the fact that methoximation competes with the reduction of methoxylamine by ascorbic acid, the gas chromatograms could not be evaluated

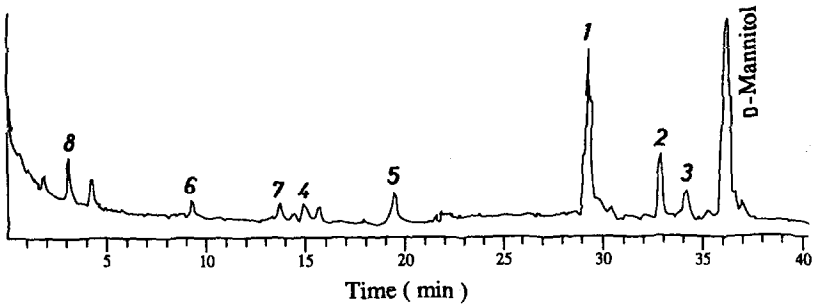


Fig. 1. Gas chromatogram of the silylated products of radiolysis (5 kGy) of L-ascorbic acid in the presence of air (50 m column of Durabond I, detection by mass spectrometry): L-*erythro*-2,3-hexodiulosonic acid (*1*), *cis*- and *trans*-L-*glycero*-4-hexulos-2-enonic acid (*2* and *3*), 2-pentulosonic acid (*4*), 4-pentulos-2-enonic acid (*5*), tetronic acid (*6*), tetraric acid (*7*), and glyceric acid (*8*).

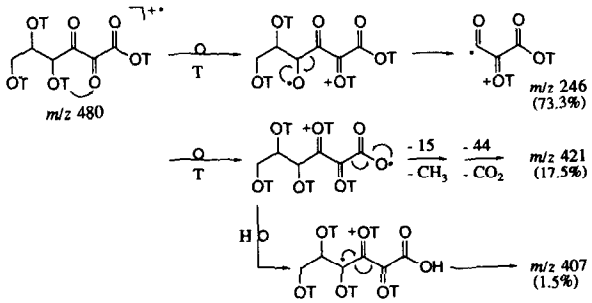
exactly. However, the mass spectra of these derivatives gave information on the structure, especially the location of the carbonyl groups.

Identification of the products.—A gas chromatogram of the silylated products obtained after irradiation of ascorbic acid in the presence of air is shown in Fig. 1. The compounds in peaks *1–8* were identified as follows.

L-erythro-2,3-*Hexodiulosonic acid (1).*—Besides a small parent ion at *m/z* 480 (1.7%), the fragment ions at *m/z* 465 ($M^+ - Me$, 19.5%), 390 ($M^+ - Me_3SiOH$, 1.9%) and 375 ($M^+ - Me - Me_3SiOH$, 8.7%) were present. The series of ions at *m/z* 103, 205, and 307 (27.9%) is characteristic for $CH_2OT–CHOT–CHOT–$ as a structural unit (T = Me_3Si). The other characteristic fragments at *m/z* 246 (73.3%), 421 (17.5%), and 407 (1.5%) are induced by migrations of Me_3Si groups with a subsequent hydrogen transfer (Scheme 1).

cis- *and* trans-L-glycero-*4-Hexulos-2-enonic acid (2 and 3).*—The partial mass spectra of the compounds in peaks *2* and *3* of the gas chromatogram, which are very similar, are presented in Fig. 2.

The parent ion *m/z* 552 is lacking, but the fragment ions at *m/z* 537 ($M^+ - Me$) and 447 ($M^+ - Me_3SiOH$) are present. From the fragment ions pro-



Scheme 1.

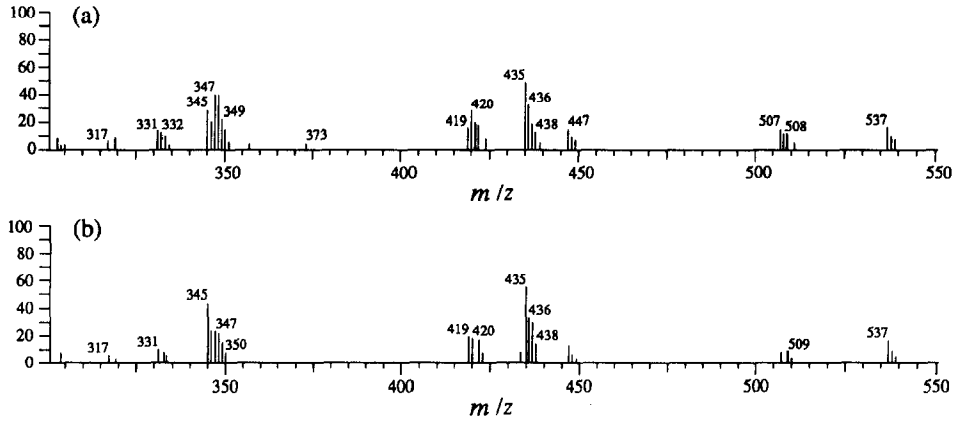
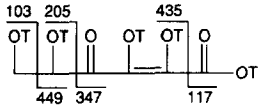
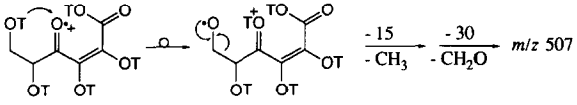


Fig. 2. Partial mass spectra attributed to (a) *cis*- and (b) *trans*-L-*glycero*-4-hexulos-2-enonic acid.

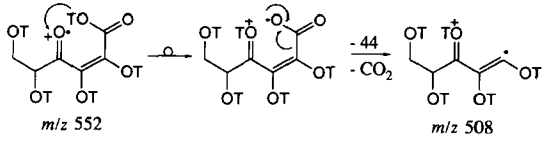
duced by cleavage of the C–C bonds, the characteristic ions at m/z 420 (435-Me), 345 (435-Me_3SiOH), and 257 (347-Me_3SiOH) result (Scheme 2). Due to steric hindrance, there should be differences for the fragmentations preceded by rearrangement. The $6 \rightarrow 4$ migration of a Me_3Si group should be equally possible in the *cis* and *trans* compounds (Scheme 3), but the $1 \rightarrow 4$ migration shown in Scheme 4 is possible only in the *cis* compound.



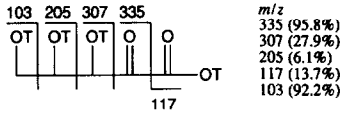
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

Only in the mass spectrum of *2* is this fragment ion at *m/z* 508 present, which confirms the *cis* structure. This conclusion is supported by the retention times in GLC. Models show that the *trans* compound is bulkier than the *cis* isomer, which accords with the higher retention time of the former.

2-Pentulosonic acid (4).—The characteristic fragment ions originating from simple cleavage of a C–C bond are shown in Scheme 5.

In addition, the fragment ion at *m/z* 218 (74.2%), arising from the migration of a Me_3Si group from O-3 to the carboxyl oxygen followed by cleavage of the C-2–C-3 bond, is observed.

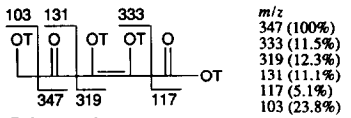
4-Pentulos-2-enonic acid (5).—In the mass spectrum of compound *5*, in addition to a small parent ion at *m/z* 450 (2.1%), the fragment ions at *m/z* 435 ($M^+ - Me$, 7.7%) and 345 ($M^+ - Me - Me_3SiOH$, 21.1%) are present. Simple cleavage of the carbon chain leads to the ions in Scheme 6.

Tetronic (6), tetraric (7), and glyceric (8) acid.—These products were identified by comparison with the mass spectra of authentic samples.

The corresponding GLC peaks can be integrated using the total ion current of the mass spectrometer, but incompletely resolved peaks or small peaks could not be evaluated exactly. Better results are obtained by identification of the compound of the corresponding GLC peak, selection of three intensive characteristic ions, which are not present in the neighbouring GLC peaks, and integration of all peaks in the gas chromatogram, using the following selected three ions: (*1*) *m/z* 246, 307, and 465; (*2*) and (*3*) *m/z* 345, 420, and 435; (*4*) *m/z* 307, 335, and 437; (*5*) *m/z* 319, 347, and 435; (*6*) *m/z* 205, 220, and 292; (*7*) *m/z* 219, 292, and 423; (*8*) *m/z* 292, 307, and 322; D-mannitol *m/z* 307, 319, and 421.

Comparison of the effects of sonolysis and radiolysis.—A dose rate of 1.2 kGy/h was used for radiolysis, which was equivalent to the efficiency of the ultrasound facility. On this basis, the irradiation doses for radiolysis and sonolysis can be expressed in Gy.

In agreement with the results of Rao [8], the decomposition of ascorbic acid was more rapid in the presence than in the absence of air. The doses for complete



Scheme 6.

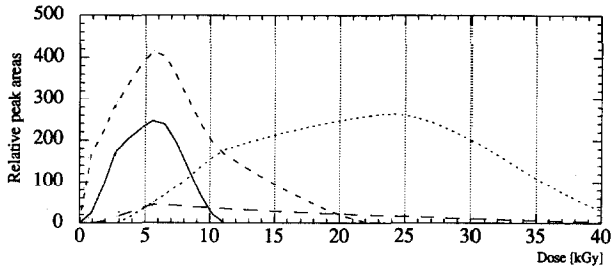


Fig. 3. Formation of primary products on radiolysis of L-ascorbic acid: - - - - - -, L-*erythro*-2,3-hexodiulosonic acid; ———, *cis*- and *trans*-L-*glycero*-4-hexulos-2-enonic acid; · · · · · ·, tetraric acid; — — —, tetronic acid.

consumption of ascorbic acid were as follows, in air: radiolysis, 5 kGy; sonolysis, 10 kGy; in argon: radiolysis, 20 kGy; sonolysis, 60 kGy.

The larger doses needed for the complete decay of ascorbic acid by sonolysis reflect the fact that the nucleation centres, which are necessary for cavitation, are reduced and result in a decreased efficiency. An analogous effect was observed in the sonolysis of sugars [14].

Based on the peak areas in the gas chromatograms standardized by reference to trimethylsilylated D-mannitol, the formation of products as a function of the irradiation dose can be monitored and the effects of radiolysis and sonolysis in the presence and absence of air can be compared.

Thus, L-*erythro*-2,3-hexodiulosonic acid (*1*) together with *cis*- and *trans*-L-*glycero*-4-hexulos-2-enonic acid (*2* and *3*) are the primary products, as shown in Fig. 3, for radiolysis of ascorbic acid in the presence of air. 2-Pentulosonic acid (*4*), 4-pentulos-2-enonic acid (*5*), tetronic acid (*6*), and glyceric acid (*8*) are secondary products, and tetraric acid (*7*) is a tertiary product.

The results for sonolysis and radiolysis in the presence and absence of air are summarised in Fig. 4.

For sonolysis and radiolysis, the maximum yields of the degradation products with less than six carbon atoms were higher for irradiation in the presence of air, but even in an argon atmosphere the yields were higher for sonolysis than for radiolysis. This result reflects the fact that ultrasound, in contrast to γ-rays, produces a small amount of oxygen [15]. Therefore, even in deoxygenated solutions, peroxyl radicals should participate in the formation of degradation products.

Radiolysis in a argon atmosphere gave a lower yield of products than did sonolysis, probably due to the formation of high molecular weight products. By using size-exclusion chromatography, it was found that only for γ-irradiation in the absence of oxygen was a high molecular fraction formed. The lack of such polymeric products after sonolysis reflects the formation of a small amount of oxygen, which inhibits polymerisation. The structure of this polymer fraction is being studied.

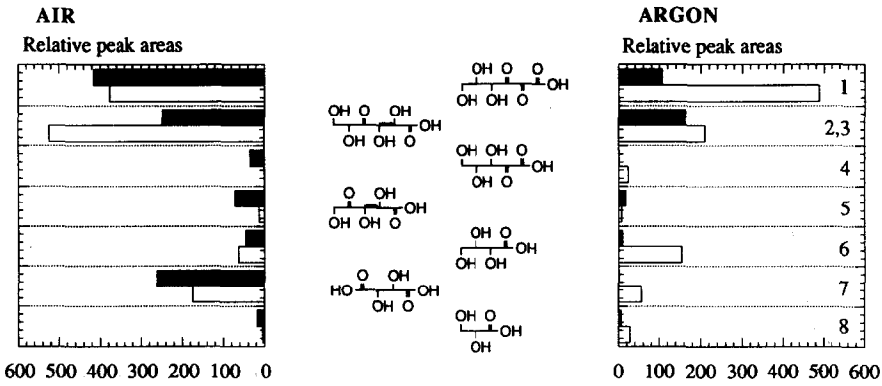
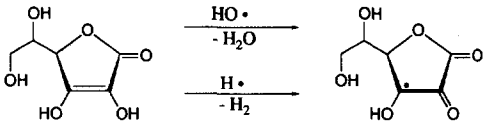


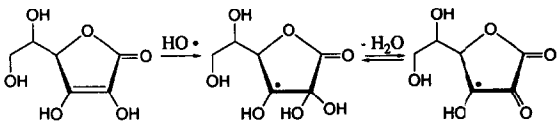
Fig. 4. Maximum formation of products after complete consumption of L-ascorbic acid for sonolysis (□) and radiolysis (■) in the presence and absence of air (numbers refer to peaks in Fig. 1).

Mechanism of product formation.—Reactions caused by ultrasound and ionising radiation in aqueous solutions are known to be induced by H·, HO·, and solvated electrons. At the concentration of ascorbic acid used for irradiations (pH 4), the solvated electrons will be converted into H· ($H^+ + e^-_{aq} \rightarrow H\cdot$). ESR spectroscopy proved the preferred formation of the semidehydroascorbic acid radical as an intermediate [5]. This radical can be formed by two routes: (*a*) abstraction of H· from C–OH by H· or HO· (Scheme 7) and (*b*) addition of HO· followed by elimination of water (Scheme 8).

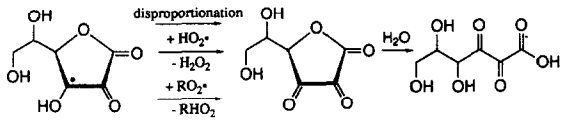
The semidehydroascorbic acid radical disproportionates to give dehydroascorbic acid and ascorbic acid. In the presence of oxygen, H· is converted into $HO_2^\cdot$ and R· (R = the organic solute) into $RO_2^\cdot$. Since the reaction of the semidehydroascorbic acid radical with oxygen is slow [1–3] even in the presence of air, dehydroascorbic acid should be formed by reaction with $HO_2^\cdot$ or $RO_2^\cdot$ by disproportionation [2].



Scheme 7.



Scheme 8.



Scheme 9.

Dehydroascorbic acid was not detected, but it is known that its lactone ring is cleaved easily in aqueous solution at pH 2–4 to give L-*erythro*-2,3-hexodiulosonic acid [16,17], which is the main primary product (*1*) on irradiation in the presence and absence of air (Scheme 9).

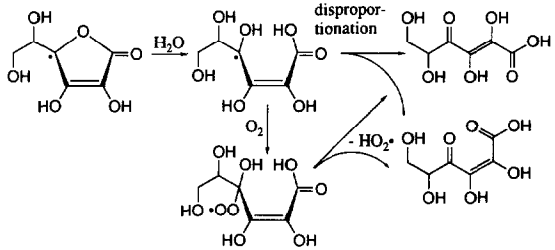
The other primary products, namely *cis*- and *trans*-L-glycero-4-hexulos-2-enonic acid (*2* and *3*) could be formed by successive keto-enol tautomerisations. However, a more probable route involves abstraction of H˙ at C-4 and the existence of such a radical has been proved [7] by ESR spectroscopy. Radicals of this type have high rates for cleavage of the lactone ring [18]. Due to the fact that the planarity of the double bond in the furanoid ring is lost, both the *cis* (*2*) and *trans* (*3*) forms are produced in equivalent amounts (Scheme 10) and the conjugation in the molecules explains the marked tendency for their formation. In contrast, on rearrangement from L-*erythro*-2,3-hexodiulosonic acid, there is no conjugation in the intermediates and no statistical distribution of the isomers is expected.

In the presence of oxygen, the intermediate radical will be converted into a peroxyl radical and such radicals are known to eliminate $HO_2^{\cdot}$ in a monomolecular reaction [19]. This reaction leads to *cis*- and *trans*-L-*glycero*-4-hexulos-2-enonic acid (*2* and *3*) by elimination of $HO_2^{\cdot}$ (Scheme 10).

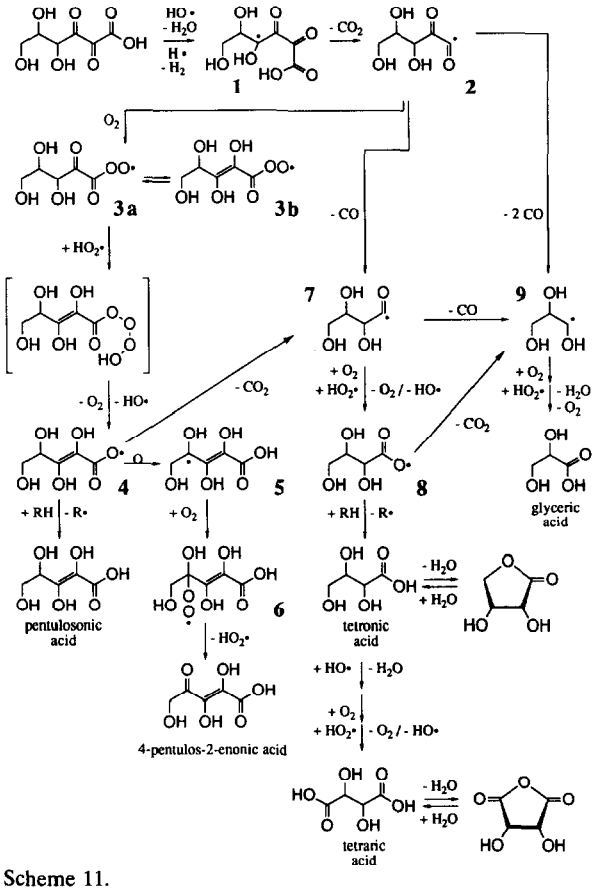
In agreement with the results of ESR spectroscopy [5], no products derived from L-ascorbic acid by abstraction of H˙ from the side chain were observed.

Secondary C_5, C_4, and C_3 products were produced in the presence and absence of air, but their yields were larger under the former conditions. These compounds can be derived only from L-*erythro*-2,3-hexodiulosonic acid by abstraction of H˙ from C-4, which is activated by the adjacent carbonyl group (Scheme 11).

The structure of the resulting radical **1** is similar to the semidehydroascorbic acid radical and its reaction with oxygen should be slow. Therefore, even in the



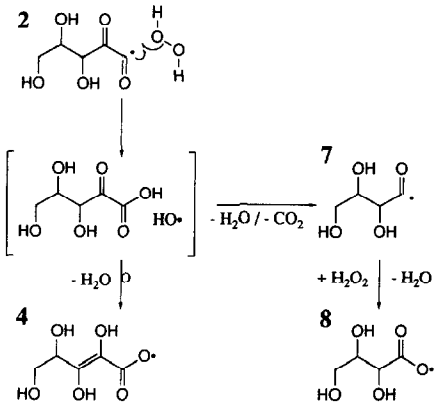
Scheme 10.



Scheme 11.

presence of air, CO_2 should be eliminated from **1** in a concerted reaction leading to radical **2**. Radical **2** will be converted into the acylperoxyl radical **3a** and by reaction with $HO_2^{\cdot}$ or $RO_2^{\cdot}$, by analogy to the Russel mechanism [20], radical **4** should be formed.

For the peroxyl radical **3a**, intramolecular abstraction of $H^{\cdot}$ at C-3 via a six-membered ring would also be feasible [21], but no products resulting from such a reaction were observed. This finding suggests that the radical **3a** is present mainly in the tautomeric form **3b**, which contains no H-atom appropriate for abstraction via a six-membered ring. The radical **4** can react by several routes. (*a*) 2-Pentulosonic acid can be formed by intermolecular abstraction of $H^{\cdot}$. (*b*) Intramolecular abstraction of $H^{\cdot}$ via a six-membered ring will give radical **5**, which will be converted into the peroxyl radical **6** and thence into 4-pentulos-2-enonic acid. (*c*) Radical **7** can be formed by decarboxylation or from radical **2** by elimination of CO. By a sequence of reactions analogous to the formation of 2-pentulosonic acid



Scheme 12.

from radical **2**, tetronic acid can be derived from radical **7** with radical **8** as an intermediate.

In competition with the intermolecular abstraction of H˙ in radical **8**, which leads to tetronic acid, an intramolecular abstraction of H˙ via a six-membered ring should be possible, leading to tetraric acid. However, the dependence of the production of tetraric acid on the dose proves it to be a tertiary product (Fig. 2). Therefore, it is assumed to be formed from the tetronic acid or its lactone by abstraction of H˙ at C-4 and subsequent reactions.

Radical **9**, originating from radical **2** by elimination of two molecules of CO or from radical **7** by elimination of CO, could be the precursor of glyceric acid.

For sonolysis in the absence of air, the yield of degradation products with less than six carbon atoms was low, reflecting the small amount of oxygen produced and the participation of peroxyl radicals in their formation [15]. The formation of small proportions of these products during radiolysis was also observed and a mechanism not involving participation of peroxyl radicals must exist. It is proposed that radical **2** reacts with hydrogen peroxide, which is a product of sonolysis and radiolysis of water. The resulting HO˙ radical, in a cage reaction, then abstracts H˙ from $-COOH$, the resulting formation of water being the driving force for this reaction (Scheme 12).

Such a mechanism is supported by the photodecomposition of H_2O_2. Hunt et al. [22] obtained evidence that some OH˙ radicals react in the cage according to the reaction: $2\ OH\cdot \rightarrow H_2O + O$.

From radical **4**, 2-pentulosonic acid and, by H-rearrangement via a six-membered ring, 4-pentulos-2-enonic acid can be derived with radicals **5** and **6** as intermediates (see Scheme 11). An analogous reaction sequence starting from radical **7** (**4**-CO_2 or **2**-CO) leads to tetronic acid. Elimination of two molecules of CO from radical **2**, followed by disproportionation, should give glyceraldehyde and glycerol, but only glyceric acid was observed, for which no conclusive mechanism can be proposed at present.

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