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Oxidation of L-Ascorbic Acid with Hydrogen Peroxide in Aqueous Solution

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The oxidation of L-ascorbic acid with aqueous H_2O_2 gives CO_2, L-threonic acid and its γ-lactone as the final products in solution. The reaction has been followed quantitatively using ^1H and ^{13}C NMR spectroscopy, and several intermediates have been detected. The concentration of the intermediates depends on the relative concentration of acid and H_2O_2. Some of the species observed have been formed by breaking bonds and some by carbonyl groups reacting with H_2O_2 and H_2O to give diols or C(OOH)OH groups. These groups react further forming intra- and intermolecular –O– or –O–O– bridges giving rings and dimers. Dehydroascorbic acid is the first intermediate detected. The reaction mechanism and structures of the other intermediates are discussed, and experimentally determined rate constants are given.

Jan Hvoslef (1926–86) devoted a large part of his professional life to the study of the crystal and molecular structure of Vitamin C and other ascorbates using neutron and X-ray methods.[1,2] Using NMR methods Hvoslef and Pedersen found that in solution several ascorbates that were present could not be isolated in crystalline form. This was found to be the case when L-ascorbic acid (**1**)[3] and D-isoascorbic acid[4] are oxidized with 1,4-benzoquinone. The situation is similar when iodine is used as the oxidizing agent as shown by Matush[5] and Berger *et al.*[6]

1,4-Benzoquinone (and iodine) is a relatively mild reagent which only carries the process to the dehydroascorbate stage and leaves the molecular backbone unharmed. When stronger oxidants are used, or when ascorbates are metabolized *in vivo*, the reaction proceeds, and the final products are carbon dioxide and various other products depending on oxidizing agent, solvent and, in aqueous solution, pH.

Hvoslef showed that dehydroascorbic acid is in a dimer form in the crystalline state.[7] In aqueous solution, however, the dominating species in a freshly made solution is the bicyclic monomer (**2**) (the 2,3-dihydrate of the γ-lactone of L-*threo*-2,3-hexodiulosonic acid).[3]

We have chosen hydrogen peroxide as the oxidizing agent, because then only ascorbic acid and the oxidation products will give signals in ^1H- and ^{13}C-NMR spectra. Isbell and Frush[8] found that the main product was L-threonic acid. Using ^1H- and ^{13}C NMR spectroscopy we have followed the reaction in more detail, and we have detected several intermediates. In this paper we report on the observed kinetics of the reaction and the structure of the intermediates we have observed. We have also made similar studies on D-isoascorbic acid (to be published later).

Experimental

L-Ascorbic acid was purchased from the Norwegian Medicinal Depot, and a 35% aqueous solution of H_2O_2 (purum) was purchased from Kebo Lab. Both chemicals were used as received.

The ^1H- and ^{13}C-NMR spectra were recorded on a Bruker CXP 200 spectrometer and a Varian XL-300 spectrometer in Oslo. One set of ^{13}C spectra were recorded on a Bruker WM-500 spectrometer in Trondheim. Spectra were recorded of samples at three temperatures: 12, 26 and 34 °C.

The molecular mechanics calculation was done using the program Maximin 2 in the Molecular Modeling Software version 6.0 from Tripos Associates, Inc. (a subsidiary of Evans & Sutherland). The atomic charges were calculated using a method developed by Del Re included in Maximin 2.

Kinetics simulation was done using the program FACSIMILE/CHEKMAT from Harwell Laboratory's Computer Science and Systems Division.

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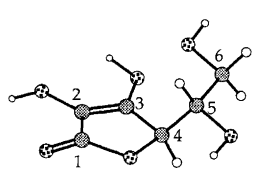

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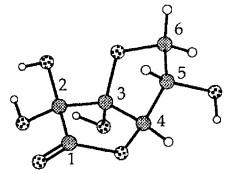

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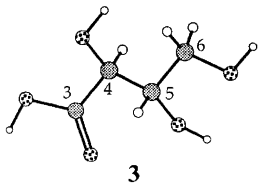

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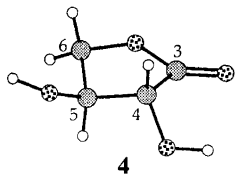

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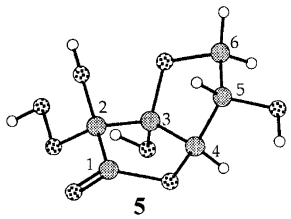

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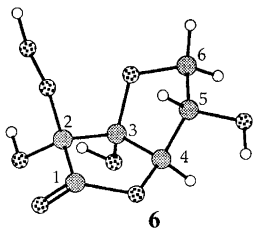

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Results

The end products. We find that in aqueous solution, L-ascorbic acid ($C_6H_8O_6$) is oxidized by hydrogen peroxide to L-threonic acid ($C_4H_8O_5$) and carbon dioxide. The overall reaction can be written:

$$C_6H_8O_6(aq) + 3H_2O_2(aq) \rightarrow C_4H_8O_5(aq) + 2CO_2(aq) + 3H_2O(l)$$

To obtain a sufficiently large signal-to-noise ratio in the ^{13}C NMR spectra the solutions used were 0.3–1.0 M in ascorbic acid and about 10 M in hydrogen peroxide. The solutions were not buffered. pH at 25 °C was found to decrease from 1.6 just after the reaction started to 0.9 after an hour or two. pH was then constant for a period, and then it slowly increased again. pH of 1 M ascorbic acid solution is expected to be 2.1 ($pK_{a1} = 4.17$). The oxidation products must therefore be more acidic than ascorbic acid.

The NMR spectra show that after about 60 h at 25 °C less than 2% ascorbic acid remains, and the only carbon-containing species detected is carbon dioxide and an equilibrium mixture of L-threonic acid and its γ-lactone.

Interpretation of the NMR spectra shows that C(1) and C(2) in **1** have been oxidized to CO_2. The equilibrium concentration of CO_2 at 25 °C and atmospheric pressure in water is 30 mM. We observe a CO_2 peak at $\delta = 124.8$.[9] We observe small amounts of oxalic acid ($pK_{a1} = 1.23$ for oxalic acid), but oxalic acid is oxidized to CO_2 by hydrogen peroxide: $K = 4.3 \times 10^{43}$ at 25 °C for the reaction

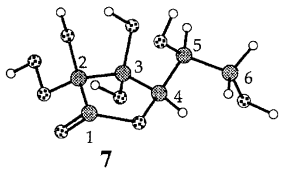
$$(COOH)_2(aq) + H_2O_2(aq) \rightleftarrows 2CO_2(g) + 2H_2O(l)$$

The NMR spectra show that the side chain [C(3)–C(6)] is not oxidized, but two molecules containing this fragment is produced: L-threonic acid [(2*R**,3*S**)-2,3,4-trihydroxybutanoic acid] (**3**) and its γ-lactone [(2*R**,3*S**)-2,3-dihydroxybutyro-1,4-lactone] (**4**).

The acid (T) and the lactone (L) are in equilibrium. [L]/[T] is found to be 0.22 at 25 °C at equilibrium. Attempts to make crystals of the lactone or acid have failed.

The ^1H spectrum of the lactone is strongly coupled even at 500 MHz. Approximate values for the coupling constants and chemical shifts for the four protons were obtained from 2D HETCOR-spectra of the lactone dissolved in DMSO at 300 MHz, and from the changes induced in the spectra by adding DMSO to the aqueous solution. On this basis we could interpret the spectrum in aqueous solution. The final values are given in Table 1, together with the corresponding values for the acid. The ^{13}C chemical shifts of the acid and the lactone are given in Table 2. The observed and calculated ^1H spectra at 300 MHz are shown in Fig. 1.

The kinetics of the reaction qualitatively. We have made a detailed study of the ascorbic acid/hydrogen peroxide reaction at different concentrations and temperatures. Our



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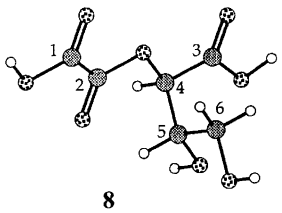

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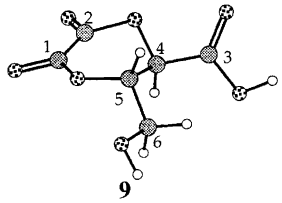

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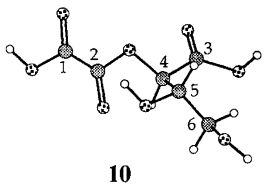

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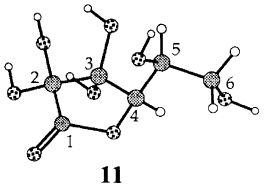

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Table 1. ^{1}H-Chemical shifts and coupling constants (labeled as in the text; δ_4 of compound **3** used as reference).

	Chemical shifts (in ppm)				Coupling constants (in Hz)			
Compound	δ_4	δ_5	δ_6	$\delta_{6'}$	J_{45}	J_{56}	$J_{56'}$	$J_{66'}$
1 (A)	4.9839(4)	4.1044(4)	3.7942(1)	3.7745(1)	1.91(11)	5.8(3)	7.0(4)	−11.6(1)
2 (DHA)	4.7289(1)	4.5592(1)	4.1386(1)	4.2363(1)	0.93(3)	2.92(4)	5.32(4)	−10.48(3)
3 (T)	4.4271(1)	4.12916(4)	3.75504(6)	3.70801(6)	1.98(1)	6.19(2)	7.28(2)	−11.48(1)
4 (L)	4.5409(3)	4.5386(3)	4.5762(3)	4.0896(2)	8.19(10)	7.45(10)	8.53(11)	−9.31(12)
5 (P)	4.7872(1)	4.5599(2)	4.1609(2)	4.2176(2)	0.84(8)	2.20(5)	5.09(5)	−10.42(4)
7 (R)	5.7476(1)	4.9099(2)	4.7253(2)	4.2553(1)	7.78(4)	7.76(4)	7.79(4)	−9.32(4)
8 (V)	5.37408(5)	4.35337(5)	3.7557(1)	3.7354(2)	2.46(2)	7.09(4)	6.17(4)	−9.89(3)
9 (W)	4.6455(2)	5.4846(2)	3.9214(8)	3.9373(9)	2.47(5)	7.7(3)	4.8(3)	−11.61(6)
10 (X)	4.4963(1)	4.4224(2)	4.4191(2)	4.4450(3)	2.40(1)	7.7(3)	4.8(2)	−11.3(2)

Table 2. ^{13}C-chemical shifts (in ppm) (labeled as in the text), with C(4) of **1** as reference.

Compound	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
1 (A)	173.77	117.96	156.06	76.50	69.18	62.51
2 (DHA)	173.47	91.33	105.65	87.35	72.90	76.35
3 (T)			176.38	72.52	70.96	62.45
4 (L)			177.68	73.34	72.48	69.54
5 (P)	169.75	97.89	104.86	87.39	72.65	76.50
6 (Q)	169.50	97.23	106.24	88.12	72.52	76.39
7 (R)	169.14	99.51	102.02	79.73	69.54	61.91
8 (V)	160.67	161.09	171.5	74.4	71.2	61.85
9 (W)	160.5	161.3	174.96	76.9	69.4	60.28
10 (X)	161.0	161.4	175.72	69.66	70.96	66.85

main goals have been to detect as many intermediates as possible and to determine the structure of the intermediates.

We found it difficult to get a set of spectra with the same quality for runs lasting of the order of 100 h. The signals can be poor at the start of the reaction, improve,

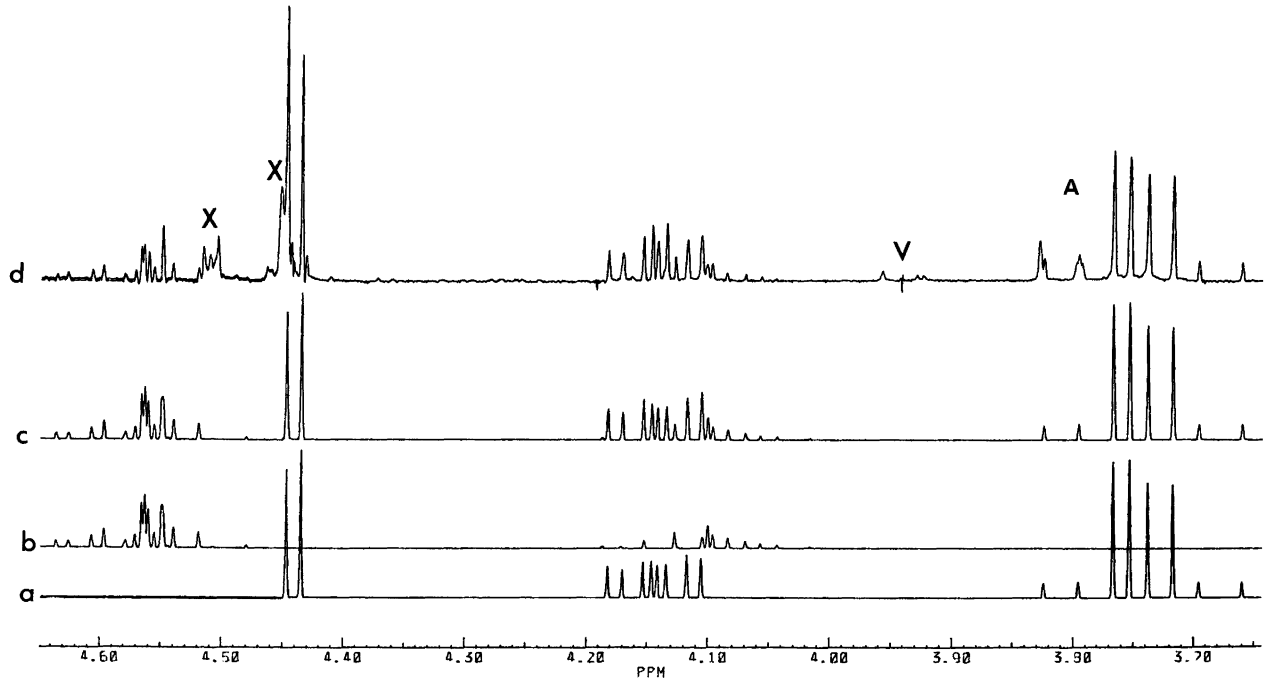


Fig. 1. Observed and calculated ^{1}H NMR spectra of the reaction mixture obtained after 30 h at 12 °C when 0.33 M L-ascorbic acid was dissolved in 10 M H_2O_2(aq): (a) calculated spectrum of threonic acid (T; **3**); (b) calculated spectrum of threonic acid lactone (L; **4**); (c) sum of spectrum a and b; (d) observed spectrum. A marks peaks from L-ascorbic acid, V and X mark peaks from intermediates as explained in the text.

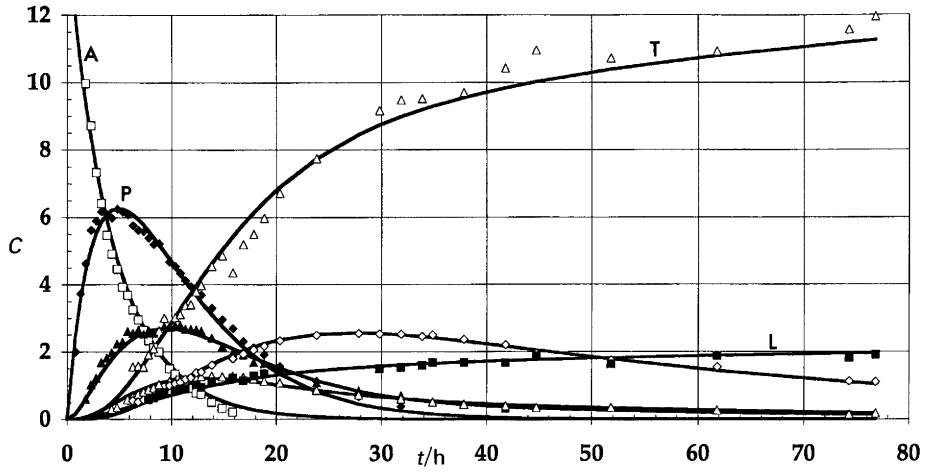


Fig. 2. The concentration of the different species observed as a function of time when 0.33 M L-ascorbic acid was dissolved in 10 M H_2O_2(aq) at 12 °C. The curves have been calculated from the proposed reaction mechanism as explained in the text.

and then become poorer again. The poor initial quality may be due to free radicals even though we have not observed any CIDNP effects.[10] The poor signals appearing after some hours may be due to bubbles formed by carbon dioxide. In some runs the bubbles did not appear, and the solution is then probably supersaturated.

We base the discussion here on a run performed at 12 °C with an ascorbic acid (A) concentration of 0.33 M and a H_2O_2 concentration of 10 M. The concentration of the different species were determined from selected peaks in the ^{1}H-spectra by measuring peak heights. With a large surplus of H_2O_2 the concentration of H_2O_2 is regarded as constant. The concentration of ascorbic acid decreases roughly according to first-order kinetics with a half-life of about 5 h at this temperature and H_2O_2 concentration. As the peaks from A decrease in intensity a series of peaks grow up in the ^{1}H- and ^{13}C-spectra. We ascribe the peaks on the basis of six major intermediates (P, R, Q, V, W and X). In Figs. 2 and 3 the time dependence of some of these intermediates are shown, and in Table 2 are given their ^{13}C chemical shifts. The assignments have been made on the basis of non-decoupled spectra, SEFT spectra and by comparing with observed chemical shifts in related compounds. The chemical shift varies somewhat with time in the reaction mixture due to solvent effects. The ^{1}H chemical shifts given are from the analyzed spectrum of each compound, so that the values of the chemical shifts and coupling constants are the fitted values and therefore constitute a consistent set.

From a reaction mechanism to be presented below we have calculated the time dependence of the different species. The results are presented in Figs. 2 and 3 as the fully drawn curves (observed values as points).

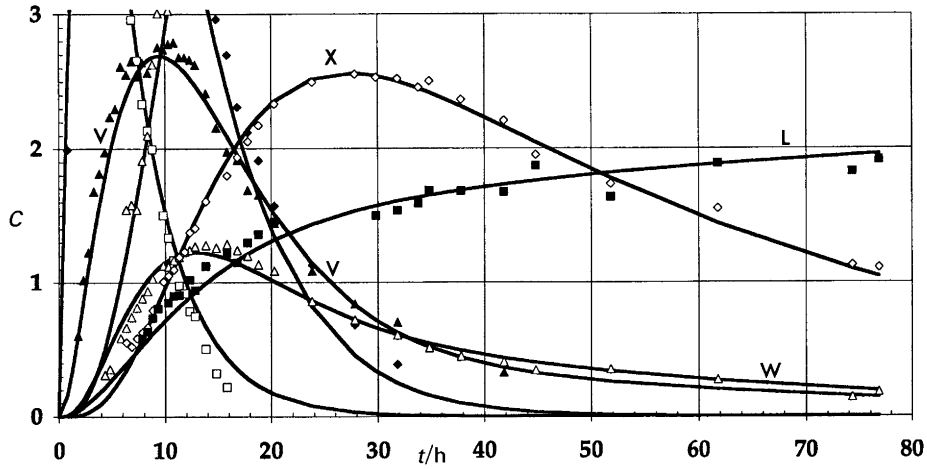
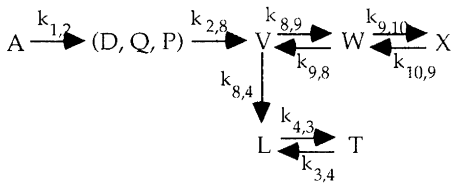


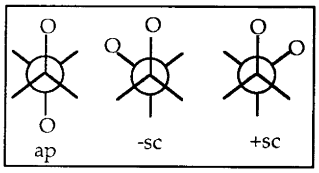
Fig. 3. The same data as in Fig. 2, but drawn with a scale to show the concentration of the species with smaller concentrations (X, V, W).

The kinetics of the reaction quantitatively. We have fitted the following reaction mechanism by the method of least squares to the observed time dependence of the concentration of the different species using the program FACSIMILE.



The rate constants obtained at three temperatures are given in Table 3, which also shows the activation energies calculated from Arrhenius plots. This reaction mechanism is the basis for the calculated time dependence of the concentration of the different species given in Figs. 2 and 3.

The structure of L-ascorbic acid and the end products. At *ca.* pH 1 L-ascorbic acid is not dissociated, and the ^{13}C chemical shifts observed are in agreement with the values found by Matush[5] and Berger.[11] (Their values are 0.6–0.7 ppm larger than ours. The difference is due to the difference in the choice of reference. We did not use an internal reference to avoid interference with the reaction.) Small differences in chemical shifts could be due to solvent effects (difference in hydrogen bonding between H_2O and H_2O_2 to L-ascorbic acid).



The ^1H NMR spectrum of all three compounds (**1**, **3** and **4**) is of the ABMX or ABCD type. The coupling constants J_{45}, J_{56} and $J_{56'}$ contain information about the conformation. We can discuss the conformation about each bond on the basis of three conformers *ap* (*anti periplanar*), $-sc$ (*syn clinal*) and $+sc$.

Table 3. Measured rate constants $k_{x\,y}$ (in $10^3\ h^{-1}$) and calculated activation energies E_a (in kJ mol^{-1})

Rate constant	12 °C	26 °C	34 °C	E_a
$k_{1,2}$	2.634(7)	6.39(3)	9.23(5)	41
$k_{2,8}$	164.7(4)	443(2)	713(4)	49
$k_{8,9}$	217.2(5)	835(4)	1621(10)	67
$k_{9,8}$	300.5(7)	1069(5)	1747(10)	59
$k_{9,10}$	207.7(5)	813(4)	799(5)	48
$k_{10,9}$	60.0(2)	173.2(7)	195(2)	41
$k_{8,4}$	208.4(5)	486(2)	680(4)	40
$k_{4,3}$	372.7(9)	565(3)	745(5)	23
$k_{3,4}$	50.52(11)	65.5(3)	69.0(4)	11

In solid L-ascorbic acid Hvoslef found that the conformation of the side chain is $-sc$[O(4)–O(5)] and *ap*[O(5)–O(6)]. In solution J_{45} is small [1.91(11) Hz], indicating that the dominating conformation in solution is also $-sc$[O(4)–O(5)]. J_{56} [5.8(3) Hz] is not much different from $J_{56'}$ [7.0(4) Hz], indicating that all three possible staggered conformers about the C(5)–C(6) bond are present in solution. Guilleme *et al.*[12] interpreted the value of the coupling constant on the basis of the generalized Karplus equation. They found 35% *ap*, 51% $-sc$ and 14% $+sc$ for the conformations about the C(5)–C(6) bond.

The crystal structure of threonic acid is not known. J_{45} is small [1.98(1) Hz], indicating that the dominating conformer in solution is $-sc$[O(4)–O(5)] as in ascorbic acid. J_{56} [6.19(2) Hz] is not much different from $J_{56'}$ [7.28(2) Hz], indicating that all three possible staggered conformers about the C(5)–C(6) bond are present in solution.

The crystal structure of the γ-lactone of threonic acid is not known. The ring is expected to be in the envelope conformation. J_{45} is large [8.19(10) Hz], indicating that H(4) and H(5) are *anti periplanar*, making the conformation $+sc$ about the C(4)–C(5) bond. Both J_{56} [7.45(10) Hz] and $J_{56'}$ [8.53(11) Hz] are large, showing that the corresponding two groups are nearly staggered. A molecular mechanics calculation shows that the two envelope conformations with C(5) out of the plane have the lowest energies. The energy depends on the torsional angle involving the hydroxyl groups [C(3)–C(4)–O(4)–H(4) and C(4)–C(5)–O(5)–H(5)]. However, the variation is small (only *ca.* 6 kJ mol^{-1}). The geometry of the rest of the molecule is fairly insensitive to the changes in the orientation of the hydroxyl groups. The calculated torsional angles are given in Table 4.

In solution the NMR parameters will be an average of the two conformers. The weight of each conformer will depend on the energy difference which will depend on the interaction with the solvent (hydrogen bonding). The values of the coupling constant are calculated from the simple Karplus equation $J = 10\ \cos^2\phi$, where ϕ is the torsional angle. The values of the observed coupling constant indicate that the conformer **4A** dominates in aqueous solution. (We have also tried the generalized Karplus equation, but we do not regard the gain as sufficient to warrant the use of a more complex model.)

The structure of the intermediates. The first intermediate detected is dehydroascorbic acid in its dicyclic monomeric form (**2**). The ^{13}C chemical shifts in Table 2 are in agreement with values published earlier[3] and by others,[6] except for the interchange of the assignments of C(4) and C(5) as pointed out by Tolbert and Ward.[13] The ^1H chemical shifts and coupling constants given in Table 1 are more accurate than the values published earlier.[3]

Table 4. Calculated torsional angles and coupling constants of two conformers of the γ-lactone of threonic acid (**4**)

Torsional angle	**4A**	**4B**	J_{calc} (A)	J_{calc} (B)	J_{obs} (H_2O)	J_{obs} (DMSO)
H(4)–C(4)–C(5)–H(5)	−164	−87	9.2	0.0	8.19(10)	7.02(10)
H(5)–C(5)–C(6)–H(6)	46	−44	4.8	5.2	7.45(10)	6.86(10)
H(5)–C(5)–C(6)–H(6′)	167	79	9.5	0.4	8.53(11)	7.16(11)

The dihydroxy groups on C(2) are in a dynamic equilibrium with the keto form:

$$>C(OH)_2 \rightleftarrows >C=O + H_2O$$

Also hydrogen peroxide can react with the keto form:

$$>C=O + H_2O_2 \rightleftarrows >C(OH)(OOH)$$

Owing to the symmetry of the molecule the >C(OH)(OOH) group gives rise to two different molecules that we interpret as P and Q. This interpretation is not inconsistent with the observed chemical shifts. The dominating species (P) is probably the conformer with the bulky –OOH group *trans* to the furanose ring: *exo*-perhydroxy-DHA (**5**). The concentration of P is much higher in the beginning and with time [P]/[Q] reach a constant value of 4.5. The concentration of P (and Q) is much higher than to be expected from the concentration of H_2O_2 molecules. The solution contains about 3.5 H_2O molecules per H_2O_2 molecule, but [P]/[DHA] > 10. This shows that the peroxycompounds are more stable than DHA in solution. The structures of the other intermediates are more speculative: R (**7**), V (**8**), W (**9**) and X (**10**).

It is known that in aqueous solution **11** is slowly formed from **2** by opening of the furanose ring.[3,6] We have not observed **11** in the reaction mixture. The concentration of R is small and not included in Figs. 2 and 3. The observed ^{13}C chemical shifts of C(4), C(5) and C(6) indicate that the furanose ring is open in R as in **11** (and in L-ascorbic acid itself). On the other hand, the chemical shifts of C(1), C(2) and C(3) observed for R are closer to those observed for **11** [δ = 172.6, 95.8 and 97.8 (the same shift reference as in this paper)]. We therefore propose that R is formed from P as **11** is formed from **2** (DHA), and that it has the structure **7**.

In the proposed reaction mechanism V follows from P and Q. We suggest that V has the structure **8**: an oxalic acid ester of the lactone. In **8** both rings in DHA have been opened. The ^{13}C chemical shift of oxalic acid is 160.1 which is a low value for a carboxylic acid. In V we observe a peak at 160.67 and 161.09 interpreted as peaks from the oxalic acid part of **8**. The remaining peaks can be interpreted as peaks from the threonic part of **8** somewhat shifted, as expected, from the signals of free acid. The ^1H parameters also give support to this interpretation.

The structure of W and X is more uncertain. We propose that W has the structure **9** and X the structure **10**. Both are oxalic acid esters of threonic acid.

The reaction mechanism. The NMR spectra only show the major species present. The proposed reaction mechanism only includes the species we have observed and the mechanism is therefore incomplete. More detailed reaction mechanisms covering the initial stages have been proposed by others (see in Ref. 1). We will return to a discussion of the reaction mechanism after completing a similar study of D-isoascorbic acid.

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