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VITAMIN C—A ^{13}C MAGNETIC RESONANCE STUDY

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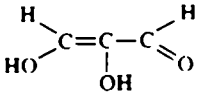
Abstract—The pH dependence of the ^{13}C chemical shifts of ascorbic acid has been measured and interpreted in terms of protonation sites. The transition of the dimer of dehydroascorbic acid into the hydrated monomer form is monitored by ^{13}C spectroscopy.

Despite the general importance of the bio-redox pair ascorbic acid-dehydroascorbic acid[1] there is to our knowledge no systematic study of the ^{13}C magnetic resonance of this system. Only Billman *et al.* have reported on the chemical shifts of ascorbic acid and its monosodium salt.[2] In this work we show a complete pH dependence study of ascorbic acid **1** and report on the analytical problems encountered with its oxidized form, dehydroascorbic acid **8**.

The ^{13}C spectrum of ascorbic acid. In H_2O solution ascorbic acid could possibly exist in four tautomeric forms **1** to **4**.[3] The ^{13}C spectrum of a 1 M H_2O solution shows six signals which under off resonance decoupling split into one triplet at 63.4 ppm, two doublets at 70.1 and 77.4 ppm, whereas the other three signals at 118.8, 157.0 and 174.4 ppm remain as singlets. From this spectrum we can rule out the keto forms **3** and **4** immediately since in these forms one of the low field carbon atoms should split into a doublet under off resonance conditions. The carbon atoms 2 and 3 in **1** resemble an $\alpha\beta$ unsaturated cyclic ketone and this is in accordance with their chemical shifts at 118.8 and 157.0 ppm respectively.

furanosides.[6] Ascorbic acid is a reductone according to structure **1**. It was therefore interesting to compare its chemical shifts with the parent reductone, the threose reductone **5** which was prepared according to the procedure of Eistert.[7]



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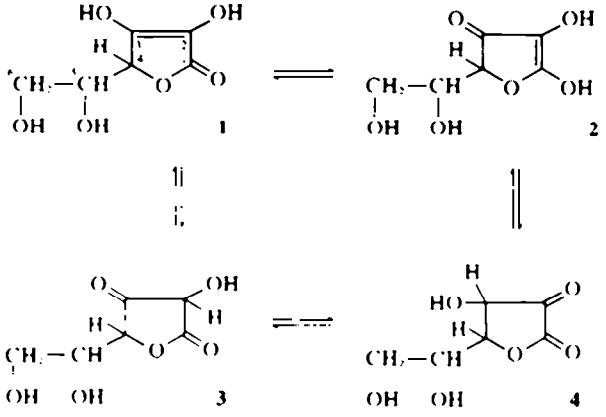
5 gives rise to two signals at 171.1 and 138.0 ppm due to the rapid tautomeric equilibrium, which in **1** apparently must be many times slower. There are no significant chemical shift changes or line broadening for **1** down to −20° in CD_3OD and up to 150° in DMSO-d_6 solution. A tautomeric equilibrium is therefore not detectable.

pH *dependence of ascorbic acid chemical shifts.* Ascorbic acid forms in the first deprotonation step the mesomeric anion **6** which on further deprotonation leads



Whereas in the solid state form **1** is proved by X-ray analysis[4] the literature claims that in solution form **2** should be predominant.[1] This, however, is in contradiction to the result of the proton coupled carbon spectrum which shows a 5.7 Hz geminal proton-carbon coupling constant for C-3 consistent only with form **1**. C-1 and C-2 do show a spin coupling constant of 2 Hz. The shift value of C-1 is typical for a lactone carbonyl[5] and the resonances of the sidechain carbon atoms can be easily assigned (C-4: 77.4 ppm, C-5: 70.1 ppm) by the general principles applicable to ^{13}C spectroscopy of to the mesomeric dianion **7**. The results of the pH dependent measurements are given in Table 1 and shown in Fig. 1. An inspection of the chemical shift titration diagram (Fig. 1) shows immediately that the first deprotonation step affects C-3 in accordance with the X-ray results.[8] Towards higher pH C-1, C-3 and C-4 are deshielded. This deshielding is normally observed for carboxylic acids.[9] The same is true for enolic carbon atoms like the transition phenol-phenolate.[10] C-2, however, is shielded towards higher pH values. Since both its neighbour atoms are deshielded the upfield shift

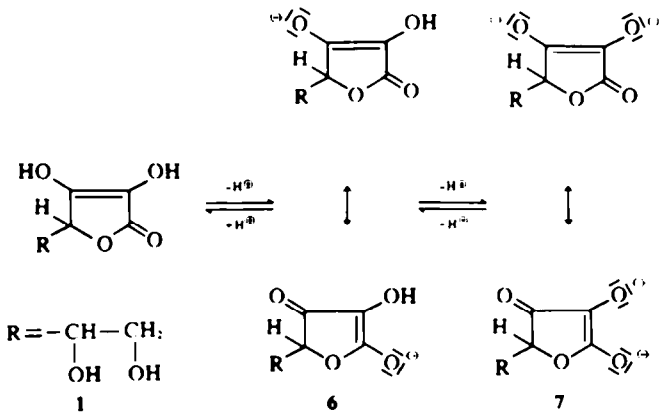


Table 1. pH dependent ^{13}C chemical shifts of ascorbic acid 1

of C-2 is normal and generally observed as a substitution pattern in olefinic systems.[11]

In **6** C-1 and C-3 resonate very close together pointing perhaps to its delocalized structure.[9] On further deprotonation, occuring at C-2, this carbon atom experiences a downfield shift as did C-3 in the first deprotonation step. Accordingly C-3 is now moving upfield. The result for C-1 again is a downfield shift indicating that both ionisations are affecting this carbon atom in the same manner and no ring opening orrurs. The shift changes of carbon atom 4 resemble those of C-3 on a minor scale. The changes in the ppm values of C-5 and C-6 are unimportant.

Oxidation of dehydroascorbic acid (DHA)

If ascorbic acid is oxidized by iodine in aqueous solution the resulting spectrum again shows six signals four of which are close to those of ascorbic acid. A lactone carbonyl atom now resonates at 172.0 ppm, C-4 at 75.2 ppm and the side chain carbon atoms at 68.9 and 63.4 ppm. As expected a drastic change results for C-2 and C-3 which now resonate very close together at 95.6 and 97.4 ppm. These results immediately tell that the dihydrated form **9** of DHA **8** has been obtained, since the chemical shift values for C-2 and C-3 are typical for carbon atoms bearing two oxygen functions.[12] The distinction between C-2 and C-3 could again be carried out by observing the geminal C–H coupling constant of 7 Hz for C-3, C-1 and C-2 do not show a resolvable coupling constant.

If dehydroascorbic acid is formed in anhydrous solution, however, according to the description by Kenyon and Munro[13] a different compound is isolated, later identified as a dimer **10** by Albers *et al.*[14] Recently Hvoslef reported the X-ray analysis of this dimer.[15] If one

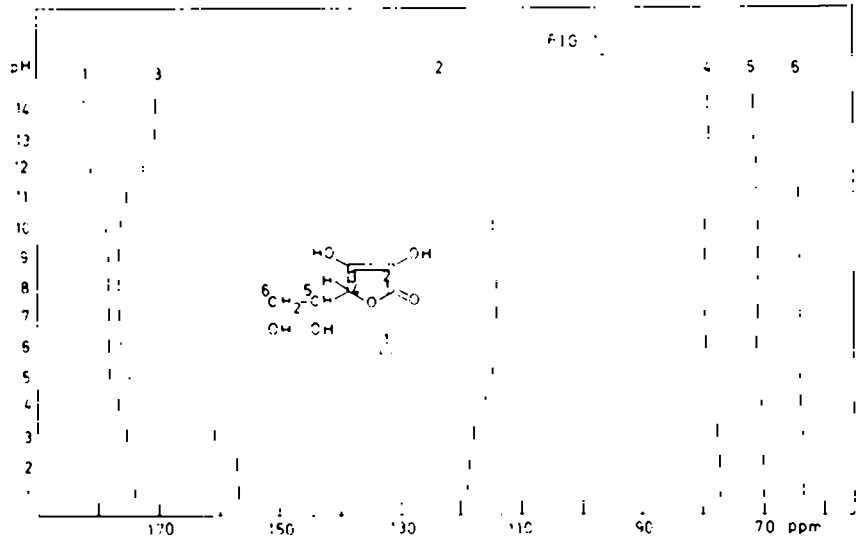
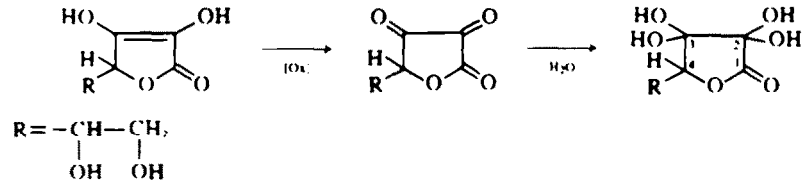


Fig. 1. pH dependent ^{13}C chemical shifts of ascorbic acid 1.



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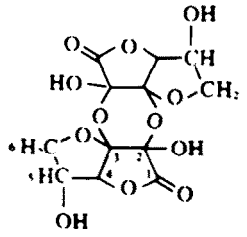
dissolves the compound prepared in this manner or commercially available DHA and measures the chemical shifts directly again a system of six signals is found, which first shows, that the dimer of DHA must be symmetrical. On repeated measurements the original signals decrease and the signals of the hydrated monomer form appear in the spectrum until after 2-3 days the hydrated form **9** is the only present in solution. The signals for the structure **10** can be assigned as follows: again there is one lactone carbonyl carbon atom at 174.2 ppm. The side chain carbon atoms C-6 and C-5 are deshielded by more than 15 ppm since the side chain now forms a fused ring system compared with the monomer form. The methylene group resonates at 76.7 and the CH(OH) group at 88.2 ppm. C-2 and C-3 are now members of a *p*-dioxane ring and resonate at 92.0 and 106.3 ppm. Their individual assignments could be performed with the observation of the geminal CH coupling constant for C-3 of 3 Hz, C-1 and C-2 again show none.

Dimeric DHA can be detected even after reduction in aqueous solution, if one measures the ^{13}C spectrum immediately. The kinetics of this process are currently under investigation.



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The transformation of dimeric DHA into its monomer form has been widely discussed in recent literature.[16-18] Our results support conclusively the work of Müller-Mulot and Dietz.[16,17] It was not possible to monitor the pH dependent chemical shifts of DHA, since contrary to ascorbic acid the solutions were not pH stable. At values higher than pH 5 DHA starts to decompose giving a very complex mixture of compounds. As a conclusion we have shown, that ^{13}C spectroscopy is a very efficient probe to monitor structural changes in systems with many oxygen functions. Especially the pH dependent shifts of poly-hydroxy acids can give direct insight into protonation sites and keto enol equilibria.

EXPERIMENTAL

(1) Carbon chemical shifts were measured on the Varian CFT-20 spectrometer in 10 mm tubes containing a centered 5 mm tube with D_2O and *p*-dioxane as lock solvent and reference. Chemical shifts were recorded at a probe temperature of 38° and calculated with δ_{TMS}-$\delta_{dioxane}$ = 67.4 ppm. Undecoupled carbon spectra were taken on the Varian XL-100 spectrometer with the gated decoupling method.

(2) pH *titrations*. A stock solution of 1 molar ascorbic acid in distilled water was adjusted with drops of concentrated HCl to pH 1 using a Metrohm (Herisau) E 510 pH meter and a EA 121 glass electrode. The chemical shifts of an aliquot of this solution were measured and the pH was checked after the measurements. The solutions showed stable pH. The combined solutions were adjusted to higher pH values with concentrated NaOH.

(3) *Materials*. L-ascorbic acid was purchased from Merck, Darmstadt, dehydroascorbic acid was prepared according to reference 13 and compared with dehydroascorbic acid from Fluka which showed the same results.

REFERENCES

[1] E. Percival, In *Comprehensive Biochemistry* (Edited by M. Florkin and E. H. Stotz), Vol. 5, p. 84 Elsevier, New York (1963).
[2] J. H. Billman, S. A. Sojka and P. R. Taylor, *J. Chem. Soc. Perkin II* 2034 (1972).
[3] R. Hüttenrauch, *Deutsche Apothekerzeitung* **105**, 1621 (1965).
[4] J. Hvoslef, *Acta Cryst. B* **24**, 23 (1968).
[5] J. B. Stothers, *Carbon-13 NMR Spectroscopy*, p. 300. Academic Press, New York (1972).
[6] R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch and A. S. Perlin, *Can. J. Chem.* **53**, 1424 (1975).
[7] H. Euler and B. Eistert, *Chemie und Biochimie der Reduktone und Reduktonate*, p. 53. Ferdinand Enke Verlag Stuttgart (1957).
[8] J. Hvoslef, *Acta Cryst. B* **25**, 2214 (1969).
[9] R. Hagen and J. D. Roberts, *J. Am. Chem. Soc.* **91**, 4504 (1969).
[10] Ref. 5, p. 197.
[11] A. Rieker and S. Berger, *Org. Magn. Res.* **4**, 857 (1972).
[12] E. Breitmaier and W. Völter, *^{13}C NMR Spectrocopy*, p. 223. Verlag Chemie Weinheim (1974).
[13] J. Kenyon and N. Munro, *J. Chem. Soc.* 158 (1948).
[14] H. Albers, E. Müller and H. Dietz, *Z. Phys. Chemie* **334**, 243 (1963).
[15] J. Hvoslef, *Acta Cryst. B* **28**, 916 (1972).
[16] W. Müller-Mulot, *Z. Phys. Chem.* **351**, 56 (1970).
[17] H. Dietz, *Ann.* **738**, 206 (1970).
[18] B. Teichmann and D. Ziebarth, *J. Prakt. Chemie* **33**, 124 (1966).