

The Structure of the “Methanol Complex” of Dehydroascorbic Acid

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The so-called methanol complex of dehydroascorbic acid (DHA), formed by dissolving DHA in boiling methanol, is shown from ^1H and ^{13}C NMR spectra to be a mixture of two isomers. These are 2-methoxy derivatives of DHA in its bicyclic, hydrated form, *i.e.* either *exo*- or *endo*-2-methoxy-3,6-furanosido-*L*-threo-hexuronic acid- γ -lactone. Orthorhombic crystals, probably of one of the isomers, have been isolated, but the quality of the crystals was too poor for a crystal structure determination. The same two isomers are formed, but in slightly different proportions by adding methanol to a solution of DHA in dimethylformamide. Other alcohols (like ethanol and benzylalcohol) and water are found to form similar isomers under the same conditions.

The methanol complex of dehydroascorbic acid $\text{C}_6\text{H}_6\text{O}_6 \cdot \text{CH}_3\text{OH}$ can be isolated from the methanolic reaction mixture formed by the oxidation of *L*-ascorbic acid, or from the solution obtained by dissolving crystalline dehydroascorbic acid (DHA) I^s in boiling methanol under reflux.¹ The crystals are colourless, narrow platelets with $a=20.1$, $b=6.7$, $c=6.2$ Å and with four molecules in the orthorhombic unit cell.

The constitution of the title compound, both as

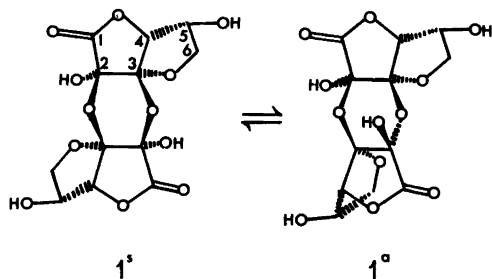
a solid and in solution, has been uncertain. Several models are discussed in the literature,^{2,3} but we have noted that the symmetric crystalline DHA can be formed from the methanol complex by heating the latter to 118 °C.¹ This property has interesting relevance to the behaviour of the various forms of dehydroascorbic acid as recently reported by us.⁴

As shown below, the ^1H and ^{13}C NMR spectra of a methanolic solution of DHA give clear evidence of two isomers in detectable amounts. The same two isomers are formed by adding methanol to a solution of DHA in *N,N*-dimethylformamide (DMF) at room temperature. We have also found that other alcohols form similar isomers under the same conditions.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded on a JEOL FX-60 NMR spectrometer operating at 15 MHz. The ^1H NMR spectra were recorded on a VARIAN HR-100 NMR spectrometer operating at 98 MHz. The simulated ^1H spectra were calculated using the simulation program in the software for JEOL FX-60. The least squares iteration was performed on a NICOLET computer using the program ITRCAL (from Nicolet Inst. Corp.).

The methanol complex is difficult to crystallize, and we could not find a good crystal. Our data were obtained from a specimen that turned out to consist of misoriented crystallites. They had the direction of the *c* axis in common, but the other axes were misaligned by several degrees. Weissenberg diagrams disclosed considerable thermal motion and possibly disorder. A more extensive structural determination with these data was abandoned.



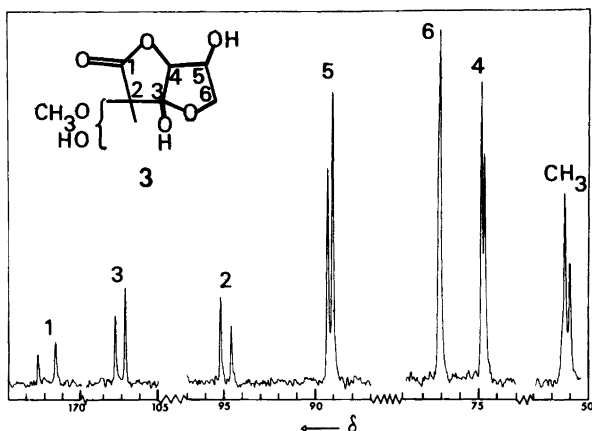


Fig. 1. 15 MHz proton decoupled ^{13}C NMR spectrum of crystalline dehydroascorbic acid dissolved in boiling methanol.

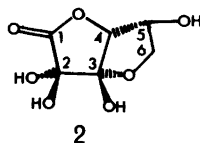
INTERPRETATION OF THE SPECTRA

Crystalline DHA dissolves slowly in boiling methanol. The ^{13}C NMR spectrum of this solution (Fig. 1) is similar to the ^{13}C NMR spectrum of DHA dissolved in DMF one day after the addition of 10 % methanol. Both spectra consist of seven pairs of peaks. In Fig. 1 the two peaks in each pair have somewhat different intensity, but in the spectrum of the DMF solution the intensities of the two peaks in each pair were equal. We conclude that the solutions contain two isomers. In boiling methanol the isomers are in different amounts and in DMF at room temperature in equal amounts.

The ^1H NMR spectrum of the methanol solution (Fig. 2) can also be interpreted as a composite spectrum from two isomers. From the intensity distribution we find that the relative amounts of the two isomers are 1.32 ± 0.10 .

Thin-layer chromatography confirms that the methanol solution of DHA contains two isomers, although the two are not clearly separated.

From ^{13}C NMR spectra taken at different times we have followed the reaction between DHA and water reported earlier.⁴ (The half-life of the DHA dimers in DMF is about 3 h at room temperature in the presence of water or methanol). The isomers are



therefore probably closely related to DHA in its bicyclic, hydrated form 2. The observed ^{13}C and ^1H chemical shifts are given in Tables 1 and 2 and indicate that the "methanol complex" 3 has a

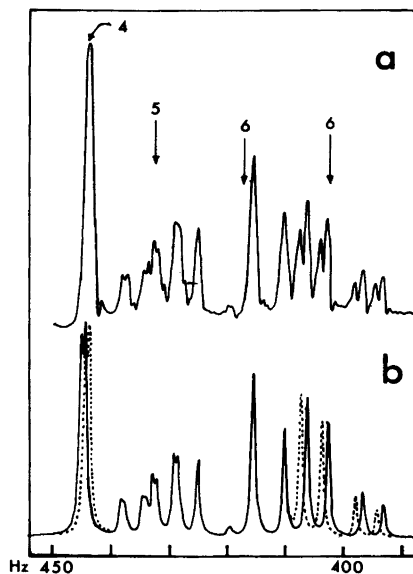


Fig. 2. Part of the 98 MHz ^1H NMR spectrum of crystalline dehydroascorbic acid dissolved in boiling methanol. (a) Observed spectrum. (b) Calculated spectrum. Fully drawn line from isomer I and dotted line from isomer II. The intensities are not adjusted to the relative amounts of I and II found in the observed spectrum.

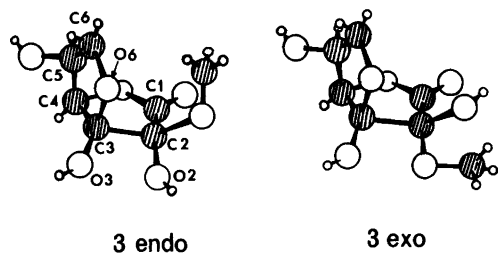


Fig. 3. Perspective drawings of the two isomers of the methanol complex of dehydroascorbic acid. *Endo* and *exo* refer to the orientation of CH_3O relative to the furanose ring C3–C4–C5–C6–O6.

methoxy group attached to C(2) in either the *endo* or the *exo* position relative to the furanose ring as shown in Fig. 3. The signal from C(2) is shifted 2–3 ppm to low field and C(3) is unshifted. The low field chemical shifts of C(5) and C(6) and the large difference between the chemical shifts of H(6) and H(6') show that C(5) and C(6) are parts of a furanose ring and not in a free side chain.⁴

The ^1H NMR spectra of the two isomers are almost identical. The chemical shifts of the methoxy groups are different: 3.44 *versus* 3.59. The spectrum of the H(6) H(6') H(5) H(4) moiety was interpreted on the basis of two superimposing ABMX spectra. The only significant difference

between the two isomers is found for H(6') in the two isomers: 4.077 *versus* 4.107. This chemical shift difference gives rise to the doublet structure of the two peaks in the H(6') part of the spectrum (see Fig. 2). This part was used to determine the relative amounts of the two isomers.

In order to reduce the effect of the strong solvent signal the methanol was substituted by deuterated methanol before the ^1H NMR spectrum was recorded. However, then the intensity of the methoxy peaks from the fresh solution was only 20% of the expected intensity. On the other hand, a peak from CH_3OD at 3.36 could be detected close to the solvent peak from CHD_2OD at 3.32. After 24 h the methoxy peaks had vanished and the CH_3OD peak had increased in intensity. This shows that the lifetime of a methoxy group on a particular molecule is only a few hours and that it is exchanged by a completely deuterated methoxy group from the solvent.

The ^{13}C NMR spectra show that similar alkoxy isomers are formed when ethanol or benzyl alcohol is added to a solution of DHA in DMF. The chemical shifts are given in Table 1. Benzyl alcohol reacted much slower than the other alcohols and the reaction was not completed within one month at room temperature.

Table 1. Observed ^{13}C chemical shifts in ppm referred to internal TMS.

Compound	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	Solvent
2 (Ref. 4)	173.54	92.20	106.36	74.14	88.62	76.02	DMF
3	I ^a	171.43	95.18	106.85	74.79	89.11	methanol
	II	172.41	94.60	107.37	74.62	89.37	
3 ^b		171.56	94.89	106.85	74.49	89.18	DMF
		170.78	94.31	106.43	74.40	88.95	
3/ethanol ^c		171.59	94.70	106.82	74.33	89.08	DMF
		171.14	94.24	106.39	74.33	88.82	
3/benzyl alcohol ^d		171.78	94.78	106.80	74.32	89.04	DMF
		170.98	94.36	106.42	74.32	88.95	

^a I is the abundant isomer. CH_3O : I: 51.33; II: 51.07; excess CH_3OH : 50.0. ^b CH_3O : 50.71, 50.52; excess CH_3OH : 49.57. ^c $\text{CH}_3\text{CH}_2\text{O}$ groups replace CH_3O groups in 3. CH_3 : 15.76, 15.69; CH_2O : 59.13, 58.74; excess $\text{CH}_3\text{CH}_2\text{OH}$: 18.71, 57.34. ^d $\phi\text{-CH}_2\text{O}$ groups replace CH_3O groups in 3. $\phi\text{-CH}_2\text{O}$: 65.28, 65.07; C(1'): 139.18, 138.86; C(2'): 127.07, C(3'): 128.50; C(4'): 127.97; excess $\phi\text{-CH}_2\text{OH}$ in same order: 64.16, 143.23, 126.93, 128.69, 127.84.

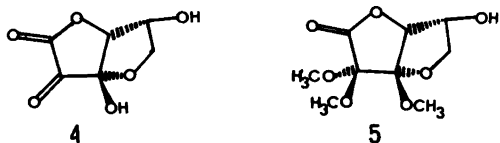
Table 2. Observed ^1H chemical shifts in ppm and coupling constants in Hz.

Compound	H(6)	H(6')	H(5)	H(4)	$J(6,6')$	$J(6,5)$	$J(6',5)$	$J(5,4)$	Solvent	
2 ^a	4.269	4.167	4.579	4.714	-10.4	5.7	2.6	0.9	D ₂ O	
3	I ^b	4.249	4.099	4.409	4.531	-9.7	5.8	3.5	0.7	CD ₃ OD
	II	4.248	4.107	4.409	4.526	-9.7	5.9	3.4	0.7	

^a Ref. 4. ^b I is the abundant isomer. Intensity ratio I/II = 1.32 ± 0.10 CH₃O: I 3.59 (s), II 3.44 (s).

DISCUSSION

Crystalline DHA is composed of symmetric dimers 1^a, but in DMF solution about 3/4 of the substance converts to asymmetric dimers 1^a. The result is an equilibrium of two anomers.⁴ When water is added to this solution, both anomers react to form the bicyclic, hydrated monomer 2. Our spectra show that when an alcohol R-OH is added two alkoxy isomers are formed. This can be understood as examples of a general reaction if we assume that a small, but undetected amount of dehydroascorbic acid is present in the solution in a bicyclic and dehydrated form 4. C(2) in 4 will react with R-OH to form 2 when R=H and to form the two isomers in Fig. 3 when R=CH₃.



It is difficult from models to predict preference for either the *endo* or the *exo* isomer. During the reaction at C(2) either isomer seems equally probable because of similar approach of O(3) and O(6) to the methoxy group. In Table 1 the most abundant isomer is called I, but it is not possible to decide whether this refers to the *endo* or the *exo* isomer.

Egge⁵ has reported the isolation of compound 5 in crystalline form by treating the solution of DHA in methanol with diazomethane. The ^1H NMR spectrum of the 5-acetyl derivative of 5 was analyzed in detail by Egge and the reported coupling constants are similar to the values given in Table 2. For 5 the chemical shift of H(4) is found to be 4.63 and the three methoxy groups are at 3.58, 3.53 and 3.47. These values are also similar to the corresponding values in Table 1.

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Received October 1, 1979.