Carbohydrate Research, 92 (1981) 9–20
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

STRUCTURE OF DEHYDROISOASCORBIC ACID ISOMERS IN SOLUTION

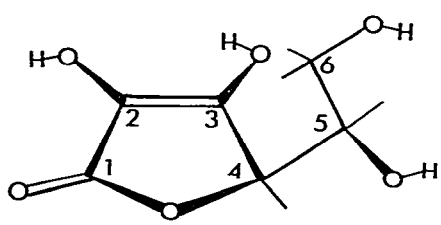
JAN HVOSLEF AND BJØRN PEDERSEN
Department of Chemistry, University of Oslo, Blindern, Oslo 3 (Norway)
(Received September 15th, 1980; accepted for publication, October 20th, 1980)

ABSTRACT

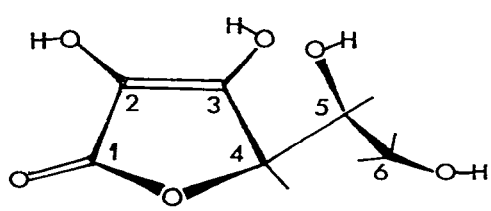
N.m.r. spectroscopy (^1H and ^{13}C) shows that dehydroisoascorbic acid (D-*erythro*-2,3-hexodiulosono-1,4-lactone) in solution is only partially similar to dehydroascorbic acid (L-*threo*-2,3-hexodiulosono-1,4-lactone). In *N*,*N*-dimethylformamide, the preponderant species is an asymmetric dimer, only 7% of the symmetric dimer is present, and other species are also detected. In water, significant differences between dehydroascorbic acid and dehydroisoascorbic acid are observed. In fresh, aqueous solutions, both acids are present as bicyclic lactones, but, with time, dehydroisoascorbic acid is transformed irreversibly into approximately equal amounts of two pyranose anomers. In contrast, dehydroascorbic acid mainly changes into a hydrated 1,4-lactone having a free side-chain. This difference is probably caused by strain in the lactone ring of dehydroisoascorbic acid, because of the proximity of O-4 and O-5 after formation of the furanoid ring. In water, this leads to opening of the lactone ring prior to the furanoid ring, and a simple equilibrium between isoascorbic acid and its primary oxidation product is lost.

INTRODUCTION

D-Isoascorbic acid (**1**) is the only diastereomer of L-ascorbic acid (**2**), and the compounds show similar acidity and reducing power. Biologically, D-isoascorbic acid is much less active (~5%) than vitamin C, but is more active than D-ascorbic acid[1].



1



2

The oxidation product (DHA) of L-ascorbic acid plays an important role in the vitamin's function. It has the same effect as L-ascorbic acid against scurvy, but

0008-6215/81/0000–0000/$ 02.50, © 1981 — Elsevier Scientific Publishing Company

also has biological effects of its own. Presumably a reversible equilibrium exists *in vivo*, with DHA as a storage version of the vitamin[2]. The oxidation product (DHI) of D-isoascorbic acid has never been characterised, but was believed[3] to have structural properties similar to those of DHA. A variety of isomers of DHA exists, particularly in solution[4]. If DHI behaved similarly, one would expect symmetric dimers in

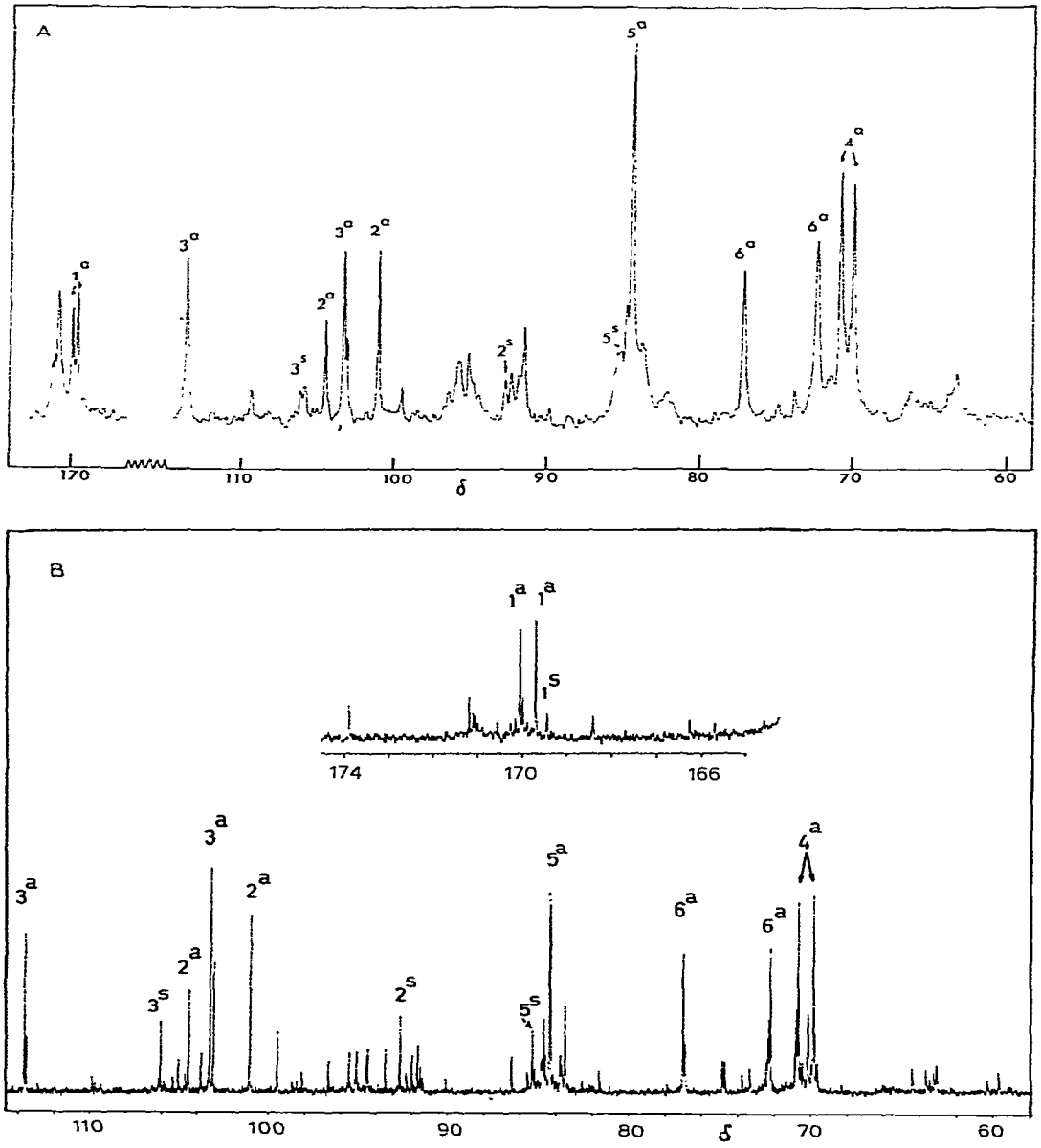
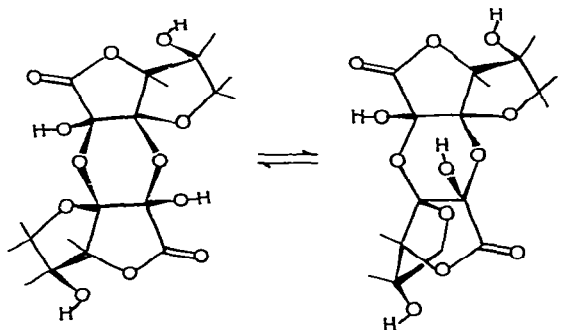


Fig. 1. Proton-decoupled, ^{13}C-n.m.r. spectra of the reaction mixture after the oxidation of D-isoascorbic acid in $HCONMe_2$: A, 15 MHz; B, 100 MHz.

solids, symmetric and asymmetric dimers in inert solvents, and bi- and mono-cyclic γ-lactones in water and alcohols[5]. Unfortunately, no crystals of DHI have been found. We have therefore studied solutions of the compound by means of ^{1}H- and ^{13}C-n.m.r. spectroscopy.

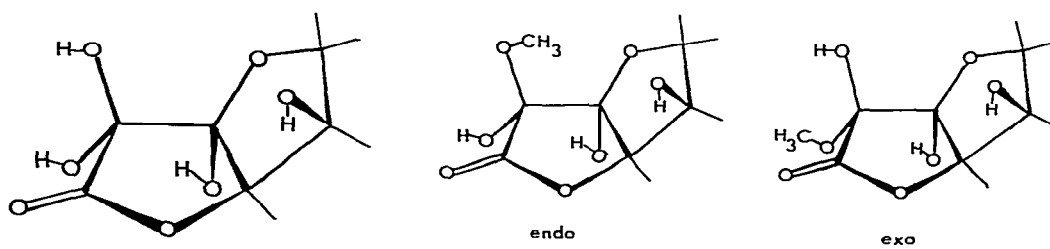
RESULTS

N,N-*Dimethylformamide solution.* — The 15-MHz and 100-MHz ^{13}C-n.m.r. spectra of the reaction mixture formed by oxidation of D-isoascorbic acid in *N,N*-dimethylformamide are shown in Fig. 1. The 15-MHz spectrum differs from and is more complex than that of the reaction mixture formed by oxidation of L-ascorbic acid (Fig. 1*a* in ref. 4). All 18 major peaks in the latter spectrum could be interpreted in terms of a mixture of a symmetric and an asymmetric dehydroascorbic acid (DHA) dimer. The number of observed peaks in Fig. 1 shows that more than two species are formed by oxidation of D-isoascorbic acid. It is possible, however, to identify the peaks from a symmetric (3^{s}) and an asymmetric (3^{a}) DHI dimer (see below).



3^{s} 3^{a}

When water or methanol was added to the reaction mixture, the spectrum simplified during a few days and became closely similar to those observed[5] in corresponding experiments with DHA. All of the peaks can be interpreted on the basis of the species **4** (reaction with water) and **5** (reaction with methanol). It is therefore concluded that, whatever the nature of the species originally formed, they are all converted into the simple forms **4** and **5**, respectively.



4 **5**

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS (δ, INTERNAL Me_4Si) AT 28° FOR COMPOUNDS RELATED TO D-ISOASCORBIC ACID

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*Solvent*
1	170.8	119.6	152.9	77.9	72.1	63.2	$HCONMe_2$
1	173.3	118.3	156.3	77.6	71.2	61.6	Water[b]
3[a]	169.69	101.02	103.28	69.85	84.38	72.20	$HCONMe_2$
	170.04	104.47	113.69	70.68	84.33	77.02	
3[s]	169.44	92.66	106.05	69.9[a]	85.32	72.9[a]	$HCONMe_2$
4	173.9	92.5	105.8	70.5	83.5	72.1	$HCONMe_2$
4	173.7	91.7	105.0	69.7	83.3	72.0	Water
5 I[c]	171.09	95.19	105.90	70.81	83.60	72.14	$HCONMe_2$
5 II	171.80	94.60	106.49	70.45	83.60	72.43	
9, 10 I[d]	171.9	101.0	99.7	81.1	72.9	61.8	Water
9, 10 II	172.1	102.1	99.4	82.5	74.3	61.5	

[a]Estimated as discussed in the text. [b]Methanol internal reference in water (49.3). [c]I is the abundant isomer (I:II 1.3); MeO, I 50.81, II 50.55; excess MeOH 49.54. [d]I is the abundant isomer (I:II 1.4).

The chemical shifts of the DHI dimers (3^s and 3^a) can be estimated by comparison of the data for **4** and **5** for DHI (Table I) and DHA (Table I in ref. 5). Changing the configuration from *threo* (DHA) to *erythro* (DHI) affects the chemical shifts for all of the C atoms, namely, C-1 +0.2, C-2 +0.3, C-3 −0.5, C-4 −3.8, C-5 −5.4, and C-6 −3.9 p.p.m. The shifts of 3^s and 3^a were estimated by adding these values to the chemical shifts observed for the corresponding DHA dimers. All of the large peaks in the spectrum in Fig. 1 are then within 0.9 p.p.m. of the estimated shifts of 3^a, with the exception of C-6. As a C-6 peak splits to a triplet in an off-resonance spectrum, it was easy to identify the missing C-6 peak as the one at 77.02 p.p.m. (estimated 72.9 p.p.m.). The asymmetric dimer is therefore the major form of DHI in *N*,*N*-dimethylformamide solution.

However, minor peaks from the symmetric dimer were also observed. Close to the estimated chemical shifts for C-1,2,3,5, small peaks were found; in the expected region for C-4 and C-6, there were several peaks of the right height. From the relative height of the C-5 peak, the concentration of the 3^s DHI isomer was estimated to be only 7% of that of 3^a. This is a much smaller value than found for DHA, where the concentration is 30%.

Freshly made, aqueous solution. — When D-isoascorbic acid was oxidised in aqueous solution, the ^{13}C-n.m.r. spectrum of fresh solutions contained only peaks for DHI in its monomeric, bicyclic form **4**. To characterise this molecule further, the non-decoupled, 15-MHz ^{13}C-spectrum (Fig. 2) and the 98-MHz ^{1}H-spectrum (Fig. 3) were recorded. Fig. 2 also contains non-decoupled ^{13}C spectra of DHA and D-isoascorbic acid; the corresponding, non-decoupled spectrum of L-ascorbic acid has been published[6,7].

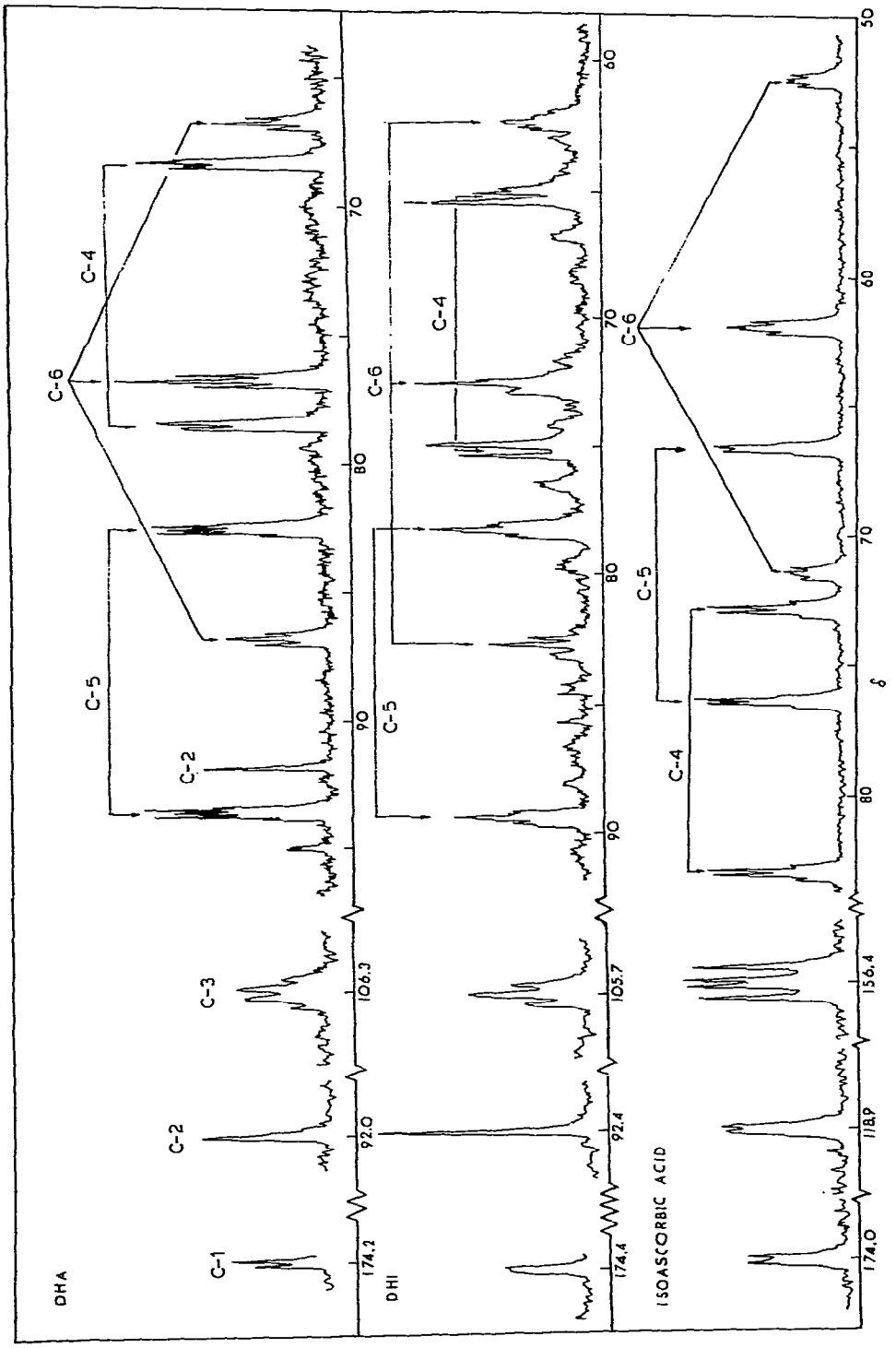


Fig. 2. Non-decoupled, 15-MHz, ^{13}C-n.m.r. spectra (internal MeOH, δ 50.0) at 28° of isoascorbic acid and DHI and DHA in their bicyclic, monomer form (4).

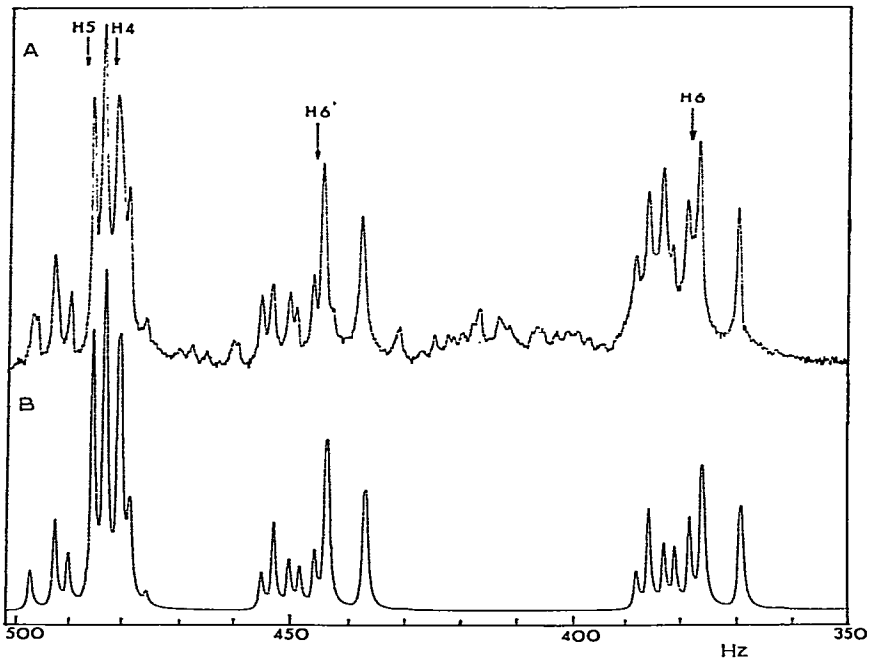


Fig. 3. ^{1}H-N.m.r. spectrum (98 MHz) of DHI in freshly prepared, aqueous solution at 28°: A, observed spectrum; B, calculated spectrum.

The ^{1}H spectrum of **4** could be interpreted in terms of an ABMX spin system. The calculated spectrum, based upon least-squares fitted parameters, is shown in Fig. 3. The final chemical shifts and coupling constants are given in Table III, together with values for DHA. The ^{1}H-n.m.r. spectra of DHA[4] and DHI are markedly different, but the difference is directly related to the change in configuration from *threo* to *erythro*. Thus, H-4 is *cis* to HO-5 in DHA, and the chemical shift is 0.3–0.4 p.p.m. higher than in DHI where the configuration is *trans*[8]. In the same way, the relative shifts of H-6,6′ may be rationalised if it is assumed that H-6 is *endo* and H-6′ is *exo* in each compound. This assignment is consistent with the observed values of the coupling constants (*cis* > *trans*).

Most of the multiplets in the non-decoupled ^{13}C-spectrum shown in Fig. 2 can be interpreted in terms of first-order rules, but because some of the H atoms are strongly coupled, some multiplets deviate from first order. The coupling constants in Table II have been obtained by comparing calculated and observed multiplets.

The data in Table II show that closure of the furanose ring leads to a 22-Hz increase in $J_{C\text{-}5,H\text{-}5}$. Also, the one-bond coupling constant of C-6 increases by 8 Hz. The ring closure is accompanied by large, downfield chemical shifts for C-5 and C-6, and a high field shift for C-4 (Table I), as observed for butanediols[9].

It is remarkable that the C-3 multiplet for DHA is a quartet, whereas it is a doublet of doublets in DHI and in D-isoascorbic acid. Therefore, C-3 in DHA must

TABLE II

OBSERVED $J_{X,Y}$ COUPLING CONSTANTS (Hz) DERIVED FROM AN ANALYSIS OF THE SPECTRA IN FIG. 2 (SPECTRUM OF **2** NOT SHOWN)

X	*Y*															
	H-4				*H-5*				*H-6*				*H-6'*			
	I[a]	*DHI*	*DHA*	*A*	*I*	*DHI*	*DHA*	*A*	*I*	*DHI*	*DHA*	*A*	*I*	*DHI*	*DHA*	*A*
C-1	1.8	0.9	1.5	2.1												
C-2	1.2			1.9												
C-3	5.5	3.1	3.0	5.8	3.7	3.1	3.0	1.4			0[b]				3.0[b]	
C-4	155.6	150.8	155.2	153.2	2.7		4.0	2.3	2.7		1.0	2.3	2.7		1.0	2.3
C-5	2.3	1.0	4.0		146.3	168.5	166.8	145.6	2.3	3.4	3.3	2.5	2.3	3.4	3.3	2.5
C-6	2.6		3.3	1.3	4.0		3.9	5.3	144.1	153.7	151.3	144.3	144.1	153.7	151.3	144.3

[a]Key: I, **1**; DHI, **4**; DHA, bicyclic monomer corresponding to **4**; A, **2**. [b]Can be exchanged.

TABLE III

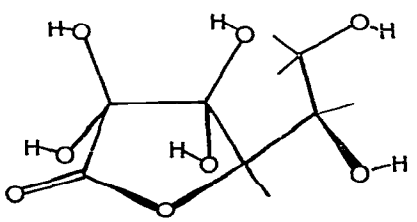
^{1}H-N.M.R. DATA FOR DHI (FIG. 3) AND DHA IN THE BICYCLIC MONOMER FORM 4 IN AQUEOUS SOLUTION AT 28°

	Chemical shifts (δ)			*Coupling constants (Hz)*	
	DHA[a]	*DHI*		*DHA*[a]	*DHI*
H-6	4.269	3.848	$J_{6,6'}$	−10.4	−9.3
H-6′	4.167	4.530	$J_{6,5}$	5.7	7.1
H-5	4.579	4.949	$J_{6,4}$	0.0	0.0
H-4	4.714	4.902	$J_{6',5}$	2.6	7.2
			$J_{6',4}$	0.0	0.0
			$J_{5,4}$	0.9	4.4

[a]Ref. 3.

be coupled not only to H-4 and H-5, but also to H-6 or H-6′ with approximately the same coupling constant.

Stored, aqueous solutions. — We found earlier that DHA is gradually transformed in water from a bicyclic monomer into a γ-lactone having a free side-chain. Under similar conditions, the ^{13}C-n.m.r. spectrum for DHI after one week was completely different, as shown in Fig. 4. A part of this spectrum is compared (Fig. 5) with that for DHA under identical conditions. Fig. 4 shows that, whereas stored DHA gives six peaks corresponding to **6**, the spectrum of stored DHI has twelve resolved peaks. Moreover, D-isoascorbic acid could not be recovered from stored solutions by treatment with H_2S; reduction by H_2S proceeds readily for fresh solutions of DHA or DHI.



6

The integrated intensities and chemical shifts of the twelve peaks in Fig. 4 showed the presence of two species in slightly different concentrations. The structures of these species will be discussed in detail below.

The non-decoupled ^{1}H (98-MHz) spectrum of these compounds is complex and has not been interpreted yet, and the non-decoupled ^{13}C-spectrum is partly unresolved.

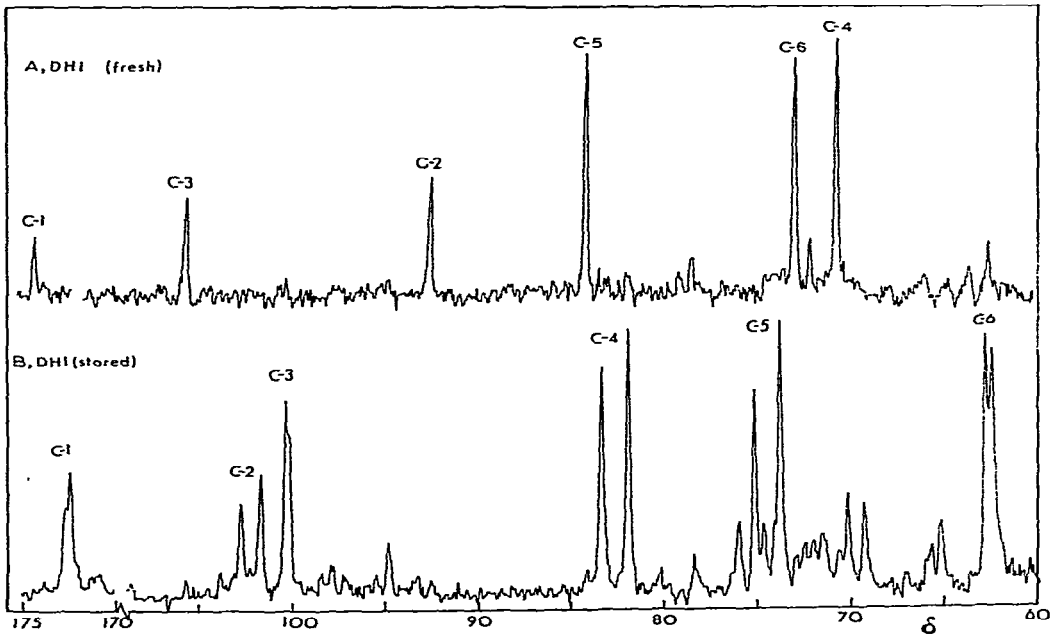


Fig. 4. Proton-decoupled, ^{13}C-n.m.r. spectra (15 MHz) of DHI in H_2O at 28°: A, freshly prepared solution; B, sample stored for 1 week.

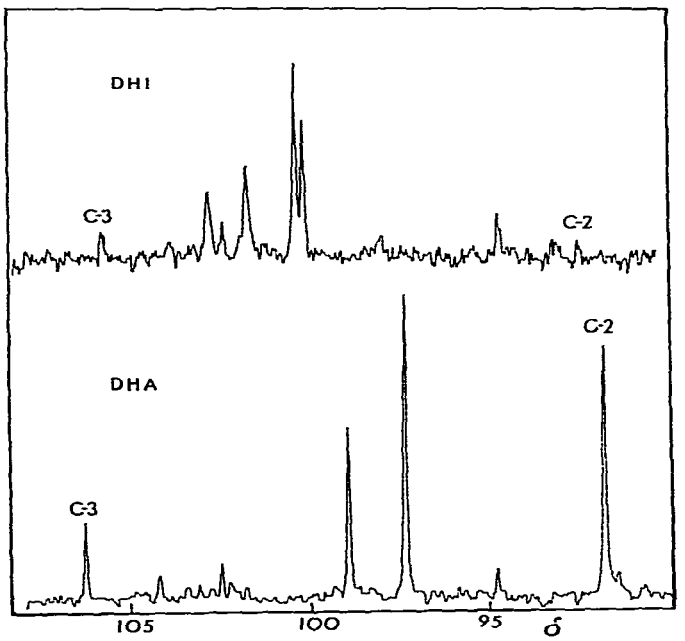


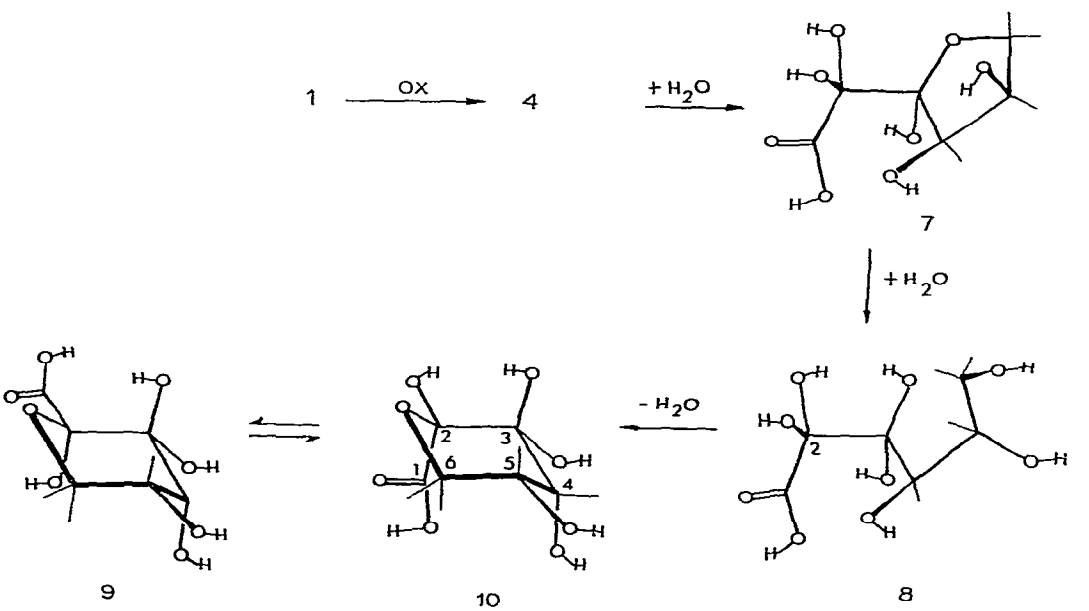
Fig. 5. Part of the proton-decoupled, 15-MHz, ^{13}C-n.m.r. spectra (internal MeOH, δ 50.0) of DHI and DHA.

DISCUSSION

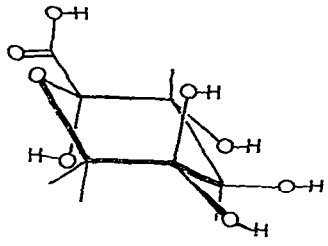
For L-ascorbic acid, no drastic change in the ring or in the configurations at C-4 and C-5 took place during mild oxidation. The γ-lactone with the enediol group became saturated, and mono-, bi-, or poly-cyclic molecules were formed, depending on time and the nature of the solvent.

If oxidation was avoided, the prolonged action of water had little effect on the vitamin, whereas its dehydro forms were attacked at regions of strain. Structural evidence[3] shows that these are located at O–C–C–O systems having non-bonded O···O distances that are less than the van der Waals distance of 2.8 Å. In the symmetric dimer of DHA, the O-3···O-3′ distance across the dioxane ring is 2.57 Å, and it is known that this ring is disrupted prior to the furanose ring, whose oxygen atom is 2.64 Å from O-2. The γ-lactone ring is stable and remains intact during the process.

As regards the two species formed from DHI in water, we share the view that reasonable models can be assessed according to what is mentioned above for DHA. The important feature is that, whereas the configuration of O-4 and O-5 in DHA is *trans*, the bi- or poly-cyclic molecules of DHI must adopt a *cis* conformation as a result of the change of configuration at C-5. This implies a non-bonded O-4···O-5 contact as short as 2.5 Å, *i.e.*, shorter than O-2···O-6 in DHA. Even allowing for conformational lability, this will introduce strain in the lactone ring which exceeds that in the furanose moiety. Thus, the stability of the bicyclic monomer of DHI is impaired in such a manner that, contrary to the DHA case, the prolonged action of water opens the lactone ring prior to the furanose ring. Relief is attained when the



C-4–O-4 bond is broken, leading to two transient molecules (**7** and **8**) of which **7** contains a furanose moiety and **8** is acyclic. Eventually, ring closure takes place by elimination of water from the CH_2OH group and one of the two OH groups on C-2. This produces isomeric pyranose anomers **9** and **10**, which are related to the α and β anomers of D-*threo*-2,5-hexodiulosonic acid[10] (**11**). The transient species **7** and **8** may explain the weak, unassigned peaks in the ^{13}C-n.m.r. spectra shown in Fig. 4. L-*xylo*-2-Hexulosonic acid[11] is a precursor of vitamin C, which can be described as the γ-lactone of the enediolic form of that acid. The proposed pyranose forms of DHI are similarly related to DHI in its lactone forms.



11

It is evident that the n.m.r. data contain significant structural information on the various species. In particular, the vicinal coupling constants found for DHA and DHI in the bicyclic, monomeric form **4** contain information on the conformation of the two fused, five-membered rings. Attempts to analyse the data on the basis of the Karplus equation did not lead to a consistent model, presumably because the furanose rings are not in one, well-defined conformation. This might be expected, as DHA, in the crystalline state, has its furanose ring in an irregular, envelope conformation with C-6 *exo*, whereas the conformation is different and less well-defined in a derivative of the monomer[12].

DHA crystallises from *N*,*N*-dimethylformamide in the symmetric form. The failure to crystallise a symmetric DHI dimer could be due to the low concentration and possibly higher solubility. Crystallisation of DHA or DHI monomers from water has so far been unsuccessful.

The most important consequence of the chemical reactions of DHI in water is that the simple equilibrium between isoascorbic acid and DHI is lost. The different behaviour from that of DHA is probably also the cause of the very different optical-rotatory power of the two isomers[13], and of the relatively strong acidity of aged solutions of dehydroisoascorbic acid.

EXPERIMENTAL

N.m.r. spectroscopy. — The ^{13}C-n.m.r. spectra were recorded on a JEOL FX-60 spectrometer operating at 15.04 MHz. From 1000 to 10,000 FID's were collected

using a 56° tip angle and a 3-s pulse-repetition rate (spectral width, 2.5 kHz, and 8 k or 16 k points).

The ^{13}C-n.m.r. spectrum reproduced in Fig. 1 was recorded on a Bruker WH-400 spectrometer operating at 100.62 MHz. 450 FID's were collected using an 80° tip angle and a 7-s pulse-repetition rate (spectral width, 19 kHz; and 64 k points).

The ^{1}H-n.m.r. spectra were recorded on a Varian HR-100 spectrometer operating at 98 MHz. The simulated ^{1}H spectra were calculated using the programme included in the software for JEOL FX-60. The least-squares iteration was performed on a Nicolet 1180 computer using the program ITRCAL.

Dehydroisoascorbic acid (DHI). — Solutions of DHI in *N,N*-dimethylformamide or water were prepared by oxidising 10 mmol of D-isoascorbic acid (Koch–Light) with a stoichiometric amount of 1,4-benzoquinone in 20 or 10 ml of H_2O (or D_2O) at room temperature.

REFERENCES

1 G. BRUBACHER AND J. P. VUILLEUMIER, *Wiss. Veroeff. Dtsch. Ges. Ernaehr.*, 14 (1964) p. 61 and references therein.
2 E. DEGKWITZ, N. SCHNEIDER, AND H. J. STAUDINGER, *Wiss. Veroeff. Dtsch. Ges. Ernaehr.*, 14 (1964) p. 61 and references therein.
3 J. HVOSLEF, *Acta Crystallogr., Sect. B*, 28 (1972) 916–923.
4 J. HVOSLEF AND B. PEDERSEN, *Acta Chem. Scand., Ser. B*, 33 (1979) 503–511.
5 J. HVOSLEF AND B. PEDERSEN, *Acta Chem. Scand., Ser. B*, 34 (1980) 285–288.
6 R. MATUSCH, *Z. Naturforsch., Teil B*, 32 (1977) 562–568.
7 T. OGAWA, J. UZAWA, AND M. MATSUI, *Carbohydr. Res.*, 59 (1977) c32–c35.
8 T. D. INCH, *Annu. Rev. NMR Spectrosc.*, 2 (1969) p. 71.
9 R. G. S. RITCHIE, N. CYR, B. KORSCH, H. J. KOCH, AND A. S. PERLIN, *Can. J. Chem.*, 53 (1975) 1424–1433.
10 G. C. ANDREWS, B. E. BACON, J. BORDNER, AND G. L. A. HENNESSEE, *Carbohydr. Res.*, 77 (1979) 25–36.
11 B. BERGEN AND J. HVOSLEF, *Acta Crystallogr. Sect. B*, 31 (1975) 697–702.
12 J. HVOSLEF AND S. NORDENSON, *Acta Crystallogr., Sect. B*, 32 (1976) 1665–1669.
13 U. H. D'AVILLA AND J. LEIBOWITZ, *Bull. Res. Counc. Isr., Sect. A*, 8 (1959) 186–187.