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# ON THE STABILITY OF SYMMETRIC DIMERS OF DEHYDROASCORBIC ACID: A STUDY OF THE ESTERS IN THE CRYSTALLINE AND THE SOLUTE STATE

JAN HVOSLEF*,
*Department of Chemistry, University of Oslo, P.O. Box 1033, 0315 Oslo 3 (Norway)*

HÅKON HOPE, AND BRENDAN D. MURRAY
*Department of Chemistry, University of California, Davis, CA 95616 (U.S.A.)*
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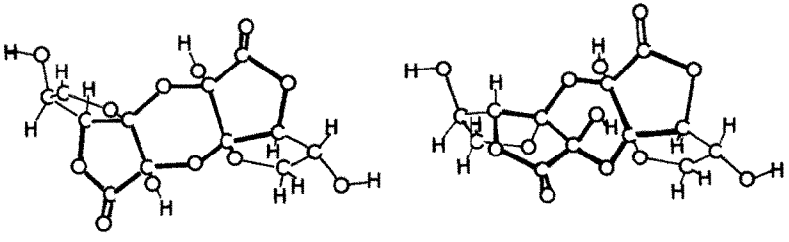
ABSTRACT

Acetylated and benzoylated dimeric dehydroascorbic acid have the same molecular structure as the crystalline parent compound. X-Ray analysis of the tetra-acetate reveals only moderate deviation from two-fold symmetry, caused, presumably, by the packing requirements of the acetate groups. The central dioxane ring is stabilised by the conversion of OH into OAc or OBz. In methyl sulfoxide or *N,N*-dimethylformamide solutions, no anomerisation occurs as is found with dehydroascorbic acid.

## INTRODUCTION

The usual crystalline form of dehydroascorbic acid is a dimer with internal two-fold symmetry[1]. As a solute, however, the structure depends on time and the nature of the solvent[2]. In some solvents, particularly non-hydroxylic, two anomeric forms, $\mathbf{1}^{s}$ and $\mathbf{1}^{a}$, of the dimer are in equilibrium. These dimers are generated by a rearrangement of the C-O bonds associated with C-2, which is one of the two carbon atoms that join the lactone and dioxane rings. In fresh aqueous solutions,



$\mathbf{1}^{s}$ $\mathbf{1}^{a}$

*To whom correspondence should be addressed.

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an unstable bicyclic monomer is formed which is gradually transformed into "2,3-diketogulonic acid"[3,4]. Neither the monomer nor the "2,3-diketogulonic acid" have been studied by X-ray diffraction methods because of crystallisation difficulties.

The X-ray investigation of the crystalline dimeric dehydroascorbic acid was encumbered by the unusually small crystals of the compound. However, the tetra-acetate was suitable for such a study, and the hypothetical molecular asymmetry prompted an investigation of the compound as a solid and a solute.

## RESULTS AND DISCUSSION

*X-Ray analysis.* — A colorless prismatic crystal (0.42 × 1.40 × 0.60 mm) of the tetra-acetate of dehydroascorbic acid was mounted on a computer-controlled Nicolet $P2_1$ diffractometer, and MoK$\alpha$ ($\lambda$ 0.71069 Å) graphite-monochromated radiation was used. The cell constants of the orthorhombic unit-cell ($P2_12_12_1$) were determined from 13 individual reflections by least-squares refinements. Further details are given in Table I. For the collection of intensities, the $\omega$-scan mode with a scan speed of 10 deg.min$^{-1}$ was used. Two standard reflections were measured with intervals of 198 observations. The scan ranges were 1.1 degrees, with a background-to-scan time-ratio of 1.0. 2890 unique reflections were collected at 140 K and below 55° in $2\theta$. For the structural analysis, the number of data was restricted to 2350 by excluding reflections with $F$ values smaller than $6\sigma(F)$.

The standard reflections were stable during the experiment, and the data were reduced according to the standard procedure in the Nicolet software. No corrections for absorption were applied. The atomic scattering factors, including anomalous dispersion effects, were taken from the International Tables for X-ray Crystallography[5]. All calculations were performed on a Data General ECLIPSE computer, using the SHELXTL[6] programme. The structure, which proved to be unusually hard to solve, was elucidated by using carefully adjusted parameters in the instruction file in the direct-methods programme. A first attempt to solve this structure using a slightly inferior set of data from a smaller crystal failed. Apparently, the quality of the experimental data and their precise absolute values were highly critical. The solution ensued only when the improved data were used and also drastically excluded reflections having no less than excellent standard deviations in $F$ (using OMIT = 12) from the phase-determining process. Also, the data statistics procedure defining $E$ values had to be changed from the default

TABLE I

CRYSTAL DATA FOR DEHYDROASCORBIC TETRA-ACETATE

$C_{20}H_{20}O_{16}$; mol wt. 516.37; orthorhombic $P2_12_12_1$
Lattice constants: $a$ = 11.133(2), $b$ = 12.965(4), $c$ = 15.300(5) Å
$U$ = 2208.29(1.05) Å$^3$, $Z$ = 4, $D_x$ = 1.546 g cm$^{-3}$
$\lambda$(MoK$\alpha$) = 0.71069 Å, $\mu_{(MoK\alpha)}$ = 1.29 mm$^{-1}$
T = 140 K, $F(000)$ = 1072, $R$ = 3.09%

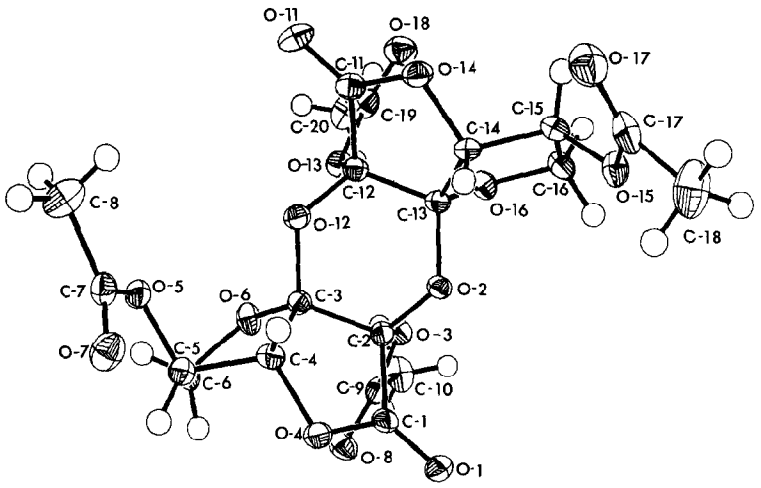


Fig. 1. Dehydroascorbic tetra-acetate with atomic labelling and thermal ellipsoids at 50% probability.

values in the SHELXTL programme, and MERG = 3, 10, 0.001, and 25 were used. Once the gross structure was known, refinements by least-squares calculations converged to $R = 3.09\%$ ($R_w = 3.49\%$) with $S = 0.99$. The weighthing scheme used in the final stage was

$$w = 1/(\sigma^2(F) + 0.001\,F^2),$$

and the function minimised was

$$\Sigma w(|F_o| - |F_c|)^2.$$

The heavy atoms were determined by using anisotropic thermal parameters, whereas the H atoms were included with the isotropic values.

A perspective of dehydroascorbic acid tetra-acetate is shown in Fig. 1. The positional coordinates and thermal parameters are given in Table II, and selected interatomic distances, bond angles, and torsion angles in Table III*.

The dehydroascorbate moiety of the molecule has a structure similar to that of the parent compound although the space-group symmetry requirement is absent. The moderate deviations from two-fold symmetry are apparently caused by packing only, since no hydrogen bonds are present. A stereo view of the molecule is given in Fig. 2.

The crucial aspect of the tetra-acetate relates to the central dioxane ring

*Lists of anisotropic temperature-factors, hydrogen co-ordinates, torsion angles, and observed and calculated structure factors have been deposited with, and may be obtained from, Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/330/*Carbohydr. Res.*, 147 (1986) 11–19.

TABLE II

FRACTIONAL ATOMIC CO-ORDINATES ($\times 10^4$) AND EQUIVALENT ISOTROPIC TEMPERATURE FACTORS ($Å^2 \times 10^4$)

| *Atom* | x | y | z | U[a] |
|---|---|---|---|---|
| O-1 | 8394(2) | −710(1) | −440(1) | 23(1) |
| O-2 | 6421(1) | 748(1) | −629(1) | 19(1) |
| O-3 | 5669(1) | −849(1) | −351(1) | 20(1) |
| O-4 | 8134(1) | −23(1) | 891(1) | 22(1) |
| O-5 | 6377(2) | 1135(1) | 2649(1) | 23(1) |
| O-6 | 5521(2) | −411(1) | 1405(1) | 23(1) |
| O-7 | 8101(2) | 1928(2) | 2999(1) | 30(1) |
| O-8 | 6648(2) | −2084(1) | 404(1) | 29(1) |
| O-11 | 3563(2) | 2870(1) | 706(1) | 30(1) |
| O-12 | 5306(1) | 1239(1) | 851(1) | 20(1) |
| O-13 | 3614(1) | 623(1) | 185(1) | 23(1) |
| O-14 | 4715(2) | 3017(1) | −489(1) | 24(1) |
| O-15 | 6510(2) | 2650(1) | −2391(1) | 26(1) |
| O-16 | 4626(2) | 869(1) | −1407(1) | 22(1) |
| O-17 | 6408(2) | 4291(1) | −1922(1) | 38(1) |
| O-18 | 2410(2) | 1612(1) | −622(1) | 32(1) |
| C-1 | 7754(2) | −337(2) | 93(2) | 20(1) |
| C-2 | 6413(2) | −56(2) | −20(1) | 18(1) |
| C-3 | 6050(2) | 350(2) | 889(1) | 17(1) |
| C-4 | 7256(2) | 630(2) | 1307(2) | 20(1) |
| C-5 | 7071(2) | 322(2) | 2253(2) | 24(1) |
| C-6 | 6252(3) | −607(2) | 2177(2) | 28(1) |
| C-7 | 7026(2) | 1925(2) | 2987(2) | 25(1) |
| C-8 | 6236(3) | 2772(2) | 3296(2) | 42(1) |
| C-9 | 5864(2) | −1845(2) | −93(2) | 22(1) |
| C-10 | 4975(3) | −2535(2) | −525(2) | 32(1) |
| C-11 | 4182(2) | 2483(2) | 159(2) | 23(1) |
| C-12 | 4571(2) | 1340(2) | 119(2) | 19(1) |
| C-13 | 5302(2) | 1270(2) | −732(1) | 18(1) |
| C-14 | 5583(2) | 2399(2) | −960(2) | 19(1) |
| C-15 | 5389(2) | 2463(2) | −1949(2) | 22(1) |
| C-16 | 4945(2) | 1388(2) | −2212(2) | 25(1) |
| C-17 | 6944(3) | 3630(2) | −2307(2) | 32(1) |
| C-18 | 8165(3) | 3730(3) | −2707(2) | 45(1) |
| C-19 | 2562(2) | 831(2) | −258(2) | 25(1) |
| C-20 | 1685(3) | −22(2) | −124(2) | 35(1) |

[a]Equivalent isotropic $U$ defined as one third of the trace of the orthogonalised $U_{ij}$ tensor. Estimated standard deviations are given in parentheses.

which adopts a twisted-boat conformation. As in dehydroascorbic acid, there is one short and one long C–O bond associated with each oxygen atom in this ring. This finding corresponds to the observation of Fodor *et al.*[7] for the compound formed when dehydroascorbic acid was condensed with acrolein. However, in that compound, the tetrahydropyran ring adopted a flattened chair conformation.

Replacement of the axial OH groups on the dioxane ring of dehydroascorbic acid by OAc significantly lengthens the C–O(H) bonds (1.417 and 1.414 Å *versus*

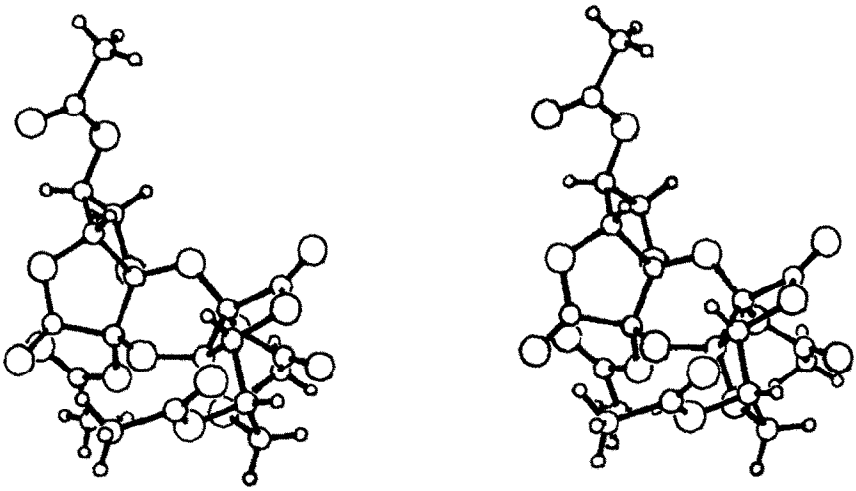

Fig. 2. Stereo-view of the dehydroascorbic tetra-acetate molecule.

1.352 Å). Also, there is a lengthening of the C-2–C(=O) and a shortening of the C-2–O(ring) bonds. This change stabilises the dioxane ring, as only one isomer was found in the solutions mentioned below. In all the cited compounds, an eclipsed conformation of the substituents at C-2 and C-3, C-12 and C-13, respectively, is avoided by a rotation about the C–C bond, resulting in individual dihedral angles that span the range 10.7–31.4°. The values are governed by the properties of the substituents at C-2 and C-12. See also Table IV.

The lactone rings in the two halves of the molecule are similar and have slightly irregular envelope conformations with C-4 and C-14 out of the plane. Except for the bonds and angles related to C-2 and C-12, they compare well with the bonds and angles in dehydroascorbic acid.

The two hemiacetal rings are less regular and have distinctly different sequences of ring torsion angles, but their interatomic bonds and angles differ only slightly from those in dehydroascorbic acid. Their conformational differences may be caused by packing of the peripheral acetate groups.

All the acetate groups are planar and strikingly equal except for the bridging C–O bonds, which are significantly shorter for the axially bonded groups at the central dioxane rings. The C–O–C angles also differ.

*N.m.r. spectroscopy.* In contrast to the complicated $^{13}$C-n.m.r. spectrum of dehydroascorbic acid solutions in *N,N*-dimethylformamide and methyl sulfoxide[2], the spectra of the tetra-acetate and tetrabenzoate were simple and revealed the symmetrical dimer to be the exclusive product. Only one peak was found for each carbon atom, as shown in Fig. 3.

The signals for C-1 and $CH_3CO$ could not be assigned unambiguously due to their close proximity and weak proton-coupling. Similar problems also arose for the assignments of benzoate groups, and no attempts were made to distinguish

TABLE III

SELECTED MOLECULAR PARAMETERS OF DEHYDROASCORBIC ACID TETRA-ACETATE[a]

| *Bond* | *Bond distance* | *Bond* | *Bond distance* |
|---|---|---|---|
| O-1–C-1 | 1.186(3) | O-2–C-2 | 1.398(3) |
| O-2–C-13 | 1.426(3) | O-3–C-2 | 1.414(3) |
| O-3–C-9 | 1.368(3) | O-4–C-1 | 1.354(3) |
| O-4–C-4 | 1.442(3) | O-5–C-5 | 1.440(3) |
| O-5–C-7 | 1.356(3) | O-6–C-3 | 1.394(3) |
| O-6–C-6 | 1.458(3) | O-7–C-7 | 1.197(3) |
| O-8–C-9 | 1.198(3) | O-11–C-11 | 1.194(3) |
| O-12–C-3 | 1.420(3) | O-12–C-12 | 1.393(3) |
| O-13–C-12 | 1.417(3) | O-13–C-19 | 1.381(3) |
| O-14–C-11 | 1.347(3) | O-14–C-14 | 1.447(3) |
| O-15–C-15 | 1.440(3) | O-15–C-17 | 1.365(3) |
| O-16–C-13 | 1.380(3) | O-16–C-16 | 1.447(3) |
| O-17–C-17 | 1.199(3) | O-18–C-19 | 1.198(3) |
| C-1–C-2 | 1.548(3) | C-2–C-3 | 1.541(3) |
| C-3–C-4 | 1.532(3) | C-4–C-5 | 1.515(3) |
| C-5–C-6 | 1.515(4) | C-7–C-8 | 1.484(4) |
| C-9–C-10 | 1.488(4) | C-11–C-12 | 1.545(3) |
| C-12–C-13 | 1.537(3) | C-13–C-14 | 1.537(3) |
| C-14–C-15 | 1.530(3) | C-15–C-16 | 1.533(3) |
| C-17–C-18 | 1.496(5) | C-19–C-20 | 1.489(4) |

| *Bonds* | *Bond angle* | *Bonds* | *Bond angle* |
|---|---|---|---|
| C-2–O-2–C-13 | 114.9(2) | C-2–O-3–C-9 | 119.3(2) |
| C-1–O-4–C-4 | 111.3(2) | C-5–O-5–C-7 | 115.3(2) |
| C-3–O-6–C-6 | 110.3(2) | C-3–O-12–C-12 | 116.9(2) |
| C-12–O-13–C-19 | 118.4(2) | C-11–O-14–C-14 | 112.1(2) |
| C-15–O-15–C-17 | 114.8(2) | C-13–O-16–C-16 | 109.2(2) |
| O-1–C-1–O-4 | 123.7(2) | O-1–C-1–C-2 | 126.8(2) |
| O-4–C-1–C-2 | 109.3(2) | O-2–C-2–O-3 | 107.9(2) |
| O-2–C-2–C-1 | 104.1(2) | O-3–C-2–C-1 | 115.7(2) |
| O-2–C-2–C-3 | 110.4(2) | O-3–C-2–C-3 | 114.7(2) |
| C-1–C-2–C-3 | 103.5(2) | O-6–C-3–O-12 | 110.5(2) |
| O-6–C-3–C-2 | 112.4(2) | O-12–C-3–C-2 | 113.1(2) |
| O-6–C-3–C-4 | 107.5(2) | O-12–C-3–C-4 | 109.6(2) |
| C-2–C-3–C-4 | 103.2(2) | O-4–C-4–C-3 | 105.7(2) |
| O-4–C-4–C-5 | 111.1(2) | C-3–C-4–C-5 | 102.5(2) |
| O-5–C-5–C-4 | 106.4(2) | O-5–C-5–C-6 | 107.0(2) |
| C-4–C-5–C-6 | 102.6(2) | O-6–C-6–C-5 | 105.0(2) |
| O-5–C-7–O-7 | 122.7(2) | O-5–C-7–C-8 | 111.4(2) |
| O-7–C-7–C-8 | 125.8(2) | O-3–C-9–O-8 | 122.9(2) |
| O-3–C-9–C-10 | 109.5(2) | O-8–C-9–C-10 | 127.6(2) |
| O-11–C-11–O-14 | 123.7(2) | O-11–C-11–C-12 | 126.3(2) |
| O-14–C-11–C-12 | 109.8(2) | O-12–C-12–O-13 | 108.8(2) |
| O-12–C-12–C-11 | 102.9(2) | O-13–C-12–C-11 | 114.5(2) |
| O-12–C-12–C-13 | 111.4(2) | O-13–C-12–C-13 | 114.8(2) |
| C-11–C-12–C-13 | 103.8(2) | O-2–C-13–O-16 | 112.4(2) |
| O-2–C-13–C-12 | 113.4(2) | O-16–C-13–C-12 | 111.6(2) |
| O-2–C-13–C-14 | 107.4(2) | O-16–C-13–C-14 | 107.4(2) |
| C-12–C-13–C-14 | 104.1(2) | O-14–C-14–C-13 | 106.1(2) |
| O-14–C-14–C-15 | 111.6(2) | C-13–C-14–C-15 | 104.3(2) |

TABLE III *(continued)*

| *Bonds* | *Bond angle* | *Bonds* | *Bond angle* |
|---|---|---|---|
| O-15–C-15–C-14 | 110.6(2) | O-15–C-15–C-16 | 108.1(2) |
| C-14–C-15–C-16 | 104.8(2) | O-16–C-16–C-15 | 106.2(2) |
| O-15–C-17–O-17 | 122.3(3) | O-15–C-17–C-18 | 111.3(2) |
| O-17–C-17–C-18 | 126.3(3) | O-13–C-19–O-18 | 122.5(2) |
| O-13–C-19–C-20 | 110.1(2) | O-18–C-19–C-20 | 127.3(2) |

[a]Bond lengths in Å, angles in degrees. Estimated standard deviations in parentheses.

TABLE IV

SELECTED TORSIONAL ANGLES (°)[a]

| | | | |
|---|---|---|---|
| O-2–C-2–C-3–O-12 | 27.4 | O-12–C-12–C-13–O-2 | 22.3 |
| O-3–C-2–C-3–O-6 | 31.3 | O-13–C-12–C-13–O-16 | 26.2 |
| O-1–C-1–C-2–O-2 | −67.0 | O-11–C-11–C-12–O-12 | −66.1 |
| O-4–C-1–C-2–O-3 | −133.1 | O-14–C-11–C-12–O-13 | −132.7 |
| C-4–O-4–C-1–O-1 | 165.1 | C-14–O-14–C-11–O-11 | 169.3 |
| C-1–O-4–C-4–C-3 | 23.9 | C-11–O-14–C-14–C-13 | 17.0 |
| O-6–C-3–C-4–C-5 | −23.9 | O-16–C-13–C-14–C-15 | −19.6 |
| O-4–C-4–C-5–O-5 | 168.7 | O-14–C-14–C-15–O-15 | 132.7 |
| C-3–C-4–C-5–O-5 | −78.8 | C-13–C-14–C-15–O-15 | −113.2 |
| C-4–C-5–C-6–O-6 | −31.9 | C-14–C-15–C-16–O-16 | 13.5 |
| C-3–O-6–C-6–C-5 | 17.7 | C-13–O-16–C-16–C-15 | −27.3 |
| C-5–O-5–C-7–O-7 | −3.9 | C-15–O-15–C-17–O-17 | −2.6 |
| C-9–O-3–C-2–C-1 | 37.8 | C-19–O-13–C-12–C-11 | 41.1 |

[a]Standard deviations are 0.2° for all torsional angles.

TABLE V

$^{13}$C CHEMICAL SHIFT (p.p.m.) DATA FOR THE TETRA-ACETATE AND TETRABENZOATE OF DEHYDROASCORBIC ACID

| | *C(Me)* | *C-1/C-10* | *C-2* | *C-3* | *C-4* | *C-5* | *C-6* |
|---|---|---|---|---|---|---|---|
| Acetate | 20.55<br>20.21 | 169.51 ?<br>168.45 ? | 91.79 | 104.85 | 87.78 | 74.04 | 74.89 |
| Benzoate[a] | | 158–165 | — ? | 105.400 | 87.753 | 74.695 | 75.400 |

[a]Data for carbon atoms in benzoate groups are omitted.

these signals. The assignments of most ascorbate carbon atoms in the two compounds are given in Table V.

The following coupling constants for the tetra-acetate were obtained by performing the $^{13}$C experiments without proton decoupling:

$J_{C\text{-}5,H\text{-}5}$ 161.42, $J_{C\text{-}4,H\text{-}4}$ 172.6, $J_{C\text{-}6,H\text{-}6A,6B}$ 152.9, and $J_{C(Me),H(Me)}$ 130.7 Hz.

That the assignments for C-4 and C-5 in the $^{13}$C-n.m.r. spectra of dehydro-

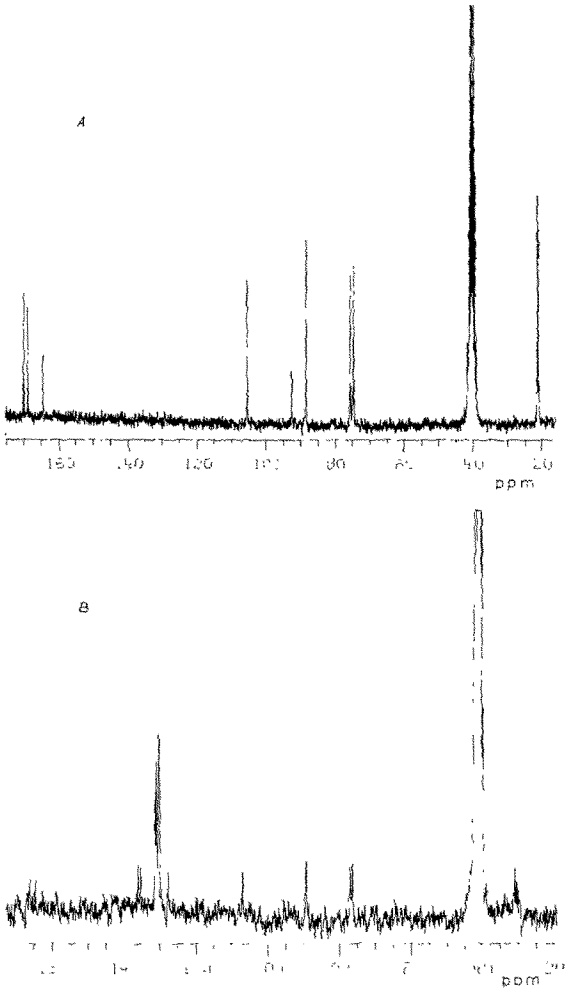


Fig. 3. Proton-decoupled $^{13}C$-n.m.r. spectra (50.3 MHz) in methyl sulfoxide of *A*, dehydroascorbic tetra-acetate; *B*, dehydroascorbic tetrabenzoate.

ascorbic acid[2] should be interchanged, as claimed by Tolbert and Ward[4], has now been confirmed by using two-dimensional n.m.r. techniques, *i.e.*, by $^{1}H$–$^{13}C$ chemical-shift correlation[8]. The new assignments are also valid for the present compounds and for other compounds related to dehydroascorbic acid.

## EXPERIMENTAL

*General methods.* — $^{13}C$- (50.3 MHz) and $^{1}H$-n.m.r. (200 MHz) spectra were recorded with a Nicolet NT-200 spectrometer, and $^{1}H$-n.m.r. spectra (360 MHz)

were recorded with a Nicolet NT-360 instrument. All samples were freshly recrystallised, and spectra were determined for solutions in $CDCl_3$ ($^1H$) or $Me_2SO$ ($^{13}C$) with and without proton decoupling. The $^1H$–$^{13}C$ shift-correlation experiment was performed on a Varian XL 300 spectrometer.

*Dehydroascorbic acid tetra-acetate.* — A solution of crystalline, dimeric dehydroascorbic acid[1] in acetic anhydride was heated[9] to 60° with a drop of sulfuric acid for 30 min. The tetra-acetate was precipitated by the addition of water and recrystallised from acetic acid. It had m.p. 251°, $[\alpha]_D$ −38.4° (chloroform).

*Dehydroascorbic acid tetrabenzoate.* — The tetrabenzoate was made by adding benzoyl chloride to a stirred solution of dimeric dehydroascorbic acid in dry pyridine[9]. The compound was precipitated by the addition of water and recrystallised from ethanol. It had m.p. 281°, $[\alpha]_D$ +26.0° (chloroform).

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