**Notes**

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# X-Ray Analysis of L-Ascorbic Acid 2-*o*-Phosphate

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L-Ascorbic acid 2-*O*-phosphate, synthesized from 5,6-*O*-isopropylidene L-ascorbic acid by reaction with phosphoryl chloride in alkaline water–pyridine solution, crystallized as colorless plates of the dipiperazinium salt trihydrate, $C_6H_9O_9P \cdot 2C_4H_{10}N_2 \cdot 3H_2O$. Its molecular structure was determined by X-ray analysis.

**Keywords**——Piperazinium L-ascorbic acid 2-*O*-phosphate; Magnesium L-ascorbic acid 2-*O*-phosphate; X-ray analysis; structure determination; CMR

A number of derivatives of L-ascorbic acid have been synthesized with the aim of obtaining those which have vitamin C activity but are resistant to air oxidation. One of the most promising derivatives is L-ascorbic acid enol phosphate, which has marked stability to thermal and oxidative degradation¹⁾ as well as antiscorbutic activity.²⁾

The synthesis of L-ascorbic acid phosphate has been described in many papers³⁻⁶⁾ and patents⁷⁻¹²⁾ since Cutolo and Lariza's first report.³⁾ Early synthetic procedures all required aprotic solvents, *e.g.* acetone, for the phosphorylation of L-ascorbic acid or its 5,6-*O*-isopropylidene derivative,³,⁴,⁷⁻⁹⁾ and, under this condition, usually gave a complex mixture of structurally similar products including enol monophosphates, diphosphate and bis(ascorbic acid)-phosphate.⁴,⁵⁾ We have reported the separation of these phosphates by column chromatography on ion exchange resin⁴⁾ and further demonstrated an effective procedure for readily preparing L-ascorbic acid 2-*O*-phosphate using water as the solvent together with pyridine and phosphoryl chloride.⁶,¹⁰⁾ We found that in aqueous alkaline solutions at low temperature, phosphorylation of one of the two enol groups of L-ascorbic acid precedes the hydrolysis of phosphoryl chloride and progresses smoothly under selective control, giving L-ascorbic acid 2-*O*-phosphate in high yield.¹⁰⁾ An analogous procedure was reported later.⁵,¹¹⁾

L-Ascorbic acid 2-*O*-phosphate was initially assigned the 3-*O*-phosphate structure on the basis of spectral data³,⁴⁾ and chemical degradation studies.⁴⁾ A number of publications³,⁴,⁶⁻¹⁰⁾ have reported this ester as the 3-*O*-phosphate. However, this structure assignment was questioned¹³⁾ after the structure of L-ascorbic acid enol sulfate was confirmed by X-ray analysis to be the 2-*O*-sulfate.¹⁴⁾ Radford *et al.*¹⁵⁾ proposed a revision of the structure to L-ascorbic acid 2-*O*-phosphate, on the basis of $C^{13}$ nuclear magnetic resonance (CMR) comparison of the L-ascorbic acid phosphate with the 2-*O*-sulfate. Subsequently, Jernow *et al.*¹⁶⁾ reported X-ray analysis of the 3-*O*-(bis-morpholino)phosphinyl ester of 5,6-*O*-isopropylidene-L-ascorbic acid, which was synthesized from thallium 3-(5,6-*O*-isopropylidene)-L-ascorbate by treatment with (bis-morpholino)phosphinyl chloride. Acid-catalyzed deprotection of the morpholino group yielded not the corresponding 3-*O*-phosphate but the 2-*O*-phosphate, which was presumably formed by phosphate group migration in the initially formed L-ascorbic acid 3-*O*-phosphate. The structure determination of the 2-*O*-phosphate was based on comparison of its physicochemical properties with those of the 3-*O*-phosphinyl ester and the 2-*O*-sulfate.¹⁴⁾

Although these studies appear to be well-grounded, there still remains some room for question since the analytical methods used were all indirect. For a direct and unambiguous determination of the structure, we concentrated our efforts on crystallization of the L-ascorbic acid phosphate as a salt by using a wide variety of amine and inorganic cations. We finally succeeded in preparing single crystals of the piperazinium salt suitable for X-ray analysis. This is the first report of direct X-ray structure determination of dipiperazinium L-ascorbic acid 2-*O*-phosphate.

## Experimental

**Compounds**——The magnesium salt of L-ascorbic acid phosphate used in this study was bulk-manufactured in Takeda Chemical Industries by a method similar to that described in the literature.[6] The salt (5 g) was dissolved in 50 ml of water and desalted with Dowex 50W-X12 ($H^+$). After the solution had been concentrated *in vacuo*, about 3.5 g of piperazine hexahydrate was added to make it alkaline (pH 8.3). Addition of ethanol to the solution and subsequent concentration gave crude crystals of the dipiperazinium salt of L-ascorbic acid phosphate. They were dissolved in a small amount of water, and the solution was placed in a desiccator saturated with ethanol vapor. After being left for a couple of days, the solution gave colorless plates of trihydrate, $C_6H_9O_9P \cdot 2C_4H_{10}N_2 \cdot 3H_2O$. A single crystal with the dimensions 0.1×0.3×0.5 mm was used for the X-ray analysis.

**Structure Determination**——A Rigaku Denki automatic four-circle diffractometer was used for data collection with graphite-monochromated Mo$K\alpha$ radiation ($\lambda = 0.7107$ Å). Among 2280 independent reflections measured in the range $3° \leqq 2\theta \leqq 50°$, 2037 satisfied the condition $F_0 \geqq 3\sigma(F_0)$ and were used in the following calculations. Both the direct method[17] and the heavy atom method were used to determine the coordinates of 31 nonhydrogen atoms. Several steps of refinement by the least-squares method[18] applying anisotropic thermal parameters to these atoms revealed 33 hydrogen atoms on the differential Fourier map. The hydrogens of one water molecule could not be determined owing to its large thermal vibration. Further refinement with isotropic thermal parameters for the hydrogen atoms converged to the final value of $R = 0.048$.

CMR spectra were recorded on a Varian XL-100-12 Fourier transform spectrometer operating at 25.2 MHz with proton noise decoupling and off-resonance decoupling techniques. The spectral width was 5000 Hz and 8192 data points were used for 10000—20000 accumulations.

## Results and Discussion

Single crystals of L-ascorbic acid enol phosphate suitable for X-ray diffraction were prepared as the dipiperazinium salt. The crystal data are given in Table I. Atomic coordinates of nonhydrogen atoms and hydrogen atoms are listed in Tables II and III, respectively. Figure 1 shows the molecular conformation together with the bond lengths and angles of ascorbic acid phosphate. As seen in Fig. 1, the location of the phosphate group was confirmed to be at the 2 position of L-ascorbic acid. Figure 2 shows the packing diagram. The phosphate group and the hydroxyl group at the 3 position are dissociated making one of the piperazine molecules protonated and the other doubly protonated.

To exclude the possibility that the magnesium salt of the phosphate had rearranged to the 2-*O*-phosphate during the salt exchange process, CMR spectra of the original magnesium salt, the potassium salt and the piperazinium salt were measured in $D_2O$ under the same condi-

Table I. Crystal Data of Dipiperazinium L-Ascorbic Acid 2-*O*-Phosphate Trihydrate

| | |
|---|---|
| Formula | $C_6H_9O_9P \cdot 2C_4H_{10}N_2 \cdot 3H_2O$ |
| Formula weight | 482.4 |
| Crystal system | Monoclinic |
| Cell dimensions | $a = 14.469(4)$ Å<br>$b = 8.546(2)$ Å<br>$c = 9.360(2)$ Å<br>$\beta = 102.50(2)°$ |
| Cell volume | 1130.0 Å$^3$ |
| Number of formulas in the unit cell | 2 |
| Calculated density | 1.42 gcm$^{-3}$ |
| Systematic absences | $0k0$, $k$ odd |
| Space group | $P2_1$ |

Table II. Fractional Coordinates ($\times 10^4$) of the Nonhydrogen Atoms

| Atom | $x$ | $y$ | $z$ |
|---|---|---|---|
| P | 5124(1) | 8027 (2) | 2751(1) |
| O ( 1) | 7547(3) | 5615( 6) | 2987(6) |
| O ( 2) | 5954(3) | 7188( 5) | 3992(4) |
| O ( 3) | 6832(3) | 10172( 5) | 5316(5) |
| O ( 4) | 8380(3) | 7755( 5) | 3815(5) |
| O ( 5) | 7492(3) | 10092( 6) | 1879(5) |
| O ( 6) | 9775(3) | 11325( 7) | 3967(6) |
| O ( 7) | 4468(3) | 8807( 5) | 3591(4) |
| O ( 8) | 4654(3) | 6653( 5) | 1830(4) |
| O ( 9) | 5613(3) | 9132( 5) | 1880(4) |
| C ( 1) | 7565(4) | 6880( 7) | 3589(7) |
| C ( 2) | 6861(4) | 7712( 7) | 4132(6) |
| C ( 3) | 7219(4) | 9133( 8) | 4736(6) |
| O ( 4) | 8190(4) | 9260( 7) | 4388(7) |
| C ( 5) | 8151(5) | 10516( 8) | 3185(7) |
| C ( 6) | 9109(5) | 10713( 9) | 2765(8) |
| N (11) | 1613(5) | 9915( 8) | 999(7) |
| N (14) | 2664(4) | 7641( 7) | 2924(6) |
| C (12) | 1963(5) | 8673( 9) | 3423(7) |
| C (13) | 1869(6) | 188( 9) | 2592(9) |
| C (15) | 2309(6) | 8903(11) | 532(8) |
| C (16) | 2377(5) | 7340( 9) | 1296(8) |
| N (21) | 5107(4) | 2006( 6) | 937(5) |
| N (24) | 4875(5) | 3875( 6) | 3397(5) |
| C (22) | 4149(4) | 2583( 7) | 1070(6) |
| C (23) | 4122(4) | 2773( 7) | 2689(6) |
| C (25) | 5833(4) | 3308( 8) | 3269(7) |
| C (26) | 5862(4) | 3097( 8) | 1661(6) |
| O 101 | 9407(4) | 4485( 7) | 3653(7) |
| O 102 | 1901(5) | 3306( 8) | 448(8) |
| O 103 | 219(5) | 4570(12) | 989(9) |

Table III. Fractional Coordinates ($\times 10^3$) of the Hydrogen Atoms

| Atom | $x$ | $y$ | $z$ |
|---|---|---|---|
| H(C4) | 133(5) | 442(10) | 465(8) |
| H(C5) | 795(5) | 151(10) | 366(8) |
| H(O5) | 686(5) | 965(10) | 193(8) |
| H(C6a) | 941(5) | 970(10) | 255(8) |
| H(C6b) | 904(5) | 147(10) | 190(8) |
| H(O6) | 965(5) | 238(11) | 390(8) |
| H(N11a) | 322(5) | 806(11) | 306(7) |
| H(N11b) | 279(5) | 687(10) | 339(8) |
| H(C12a) | 217(5) | 884(10) | 442(8) |
| H(C12b) | 122(5) | 810(10) | 299(8) |
| H(C13a) | 135(5) | 94(10) | 291(8) |
| H(C13b) | 241(5) | 78(10) | 299(8) |
| H(N14 | 155(5) | 79(11) | 37(8) |
| H(C15a) | 794(5) | 373(11) | 60(8) |
| H(C15b) | 305(5) | 943(10) | 89(8) |
| H(C16a) | 282(5) | 670(10) | 91(8) |
| H(C16b) | 176(5) | 682(10) | 123(8) |
| H(N21a) | 489(6) | 687(10) | 5(9) |
| H(N21b) | 518(5) | 109(10) | 127(8) |
| H(C22a) | 394(5) | 349(10) | 47(8) |
| H(C22b) | 369(5) | 185(10) | 60(8) |
| H(C23a) | 346(5) | 306(10) | 275(7) |

| Atom | $x$ | $y$ | $z$ |
|---|---|---|---|
| H(C23b) | 422(5) | 174(10) | 322(8) |
| H(N24a) | 472(5) | 485(10) | 307(8) |
| H(N24b) | 497(5) | 384(10) | 437(7) |
| H(C25a) | 630(5) | 407(10) | 373(8) |
| H(C25b) | 597(5) | 231(10) | 388(8) |
| H(C26a) | 646(5) | 254(10) | 155(8) |
| H(C26b) | 578(5) | 407(10) | 118(8) |
| H(O 101a) | 978(5) | 513(11) | 442(8) |
| H(O 101b) | 879(5) | 490( 9) | 316(8) |
| H(O 102a) | 118(5) | 332(11) | 14(8) |
| H(O 102b) | 794(5) | 871(11) | 29(8) |

TABLE IV. $^{13}$C-NMR Chemical Shifts of Magnesium, Piperazinium and Potassium Salts of L-Ascorbic Acid Phosphate in $D_2O$ at pH 7.3 with Dioxane as an Internal Reference

| | Magnesium salt | Piperazinium salt | Potassium salt |
|---|---|---|---|
| C-1 | 176.5 | 176.6[a] | 176.6 |
| C-2 | 113.3, 113.1[b] | 113.6, 113.3[b] | 113.2, 113.0[b] |
| C-3 | 177.8 | 175.0[a] | 177.7 |
| C-4 | 79.4 | 78.8 | 79.4 |
| C-5 | 70.5 | 70.3 | 70.4 |
| C-6 | 63.3 | 63.3 | 63.4 |
| N$\underline{C}H_2$ | | 42.6 | |

a) These assignments may be reversed.
b) This multiplicity results from $^{13}$C-$^{31}$P coupling.

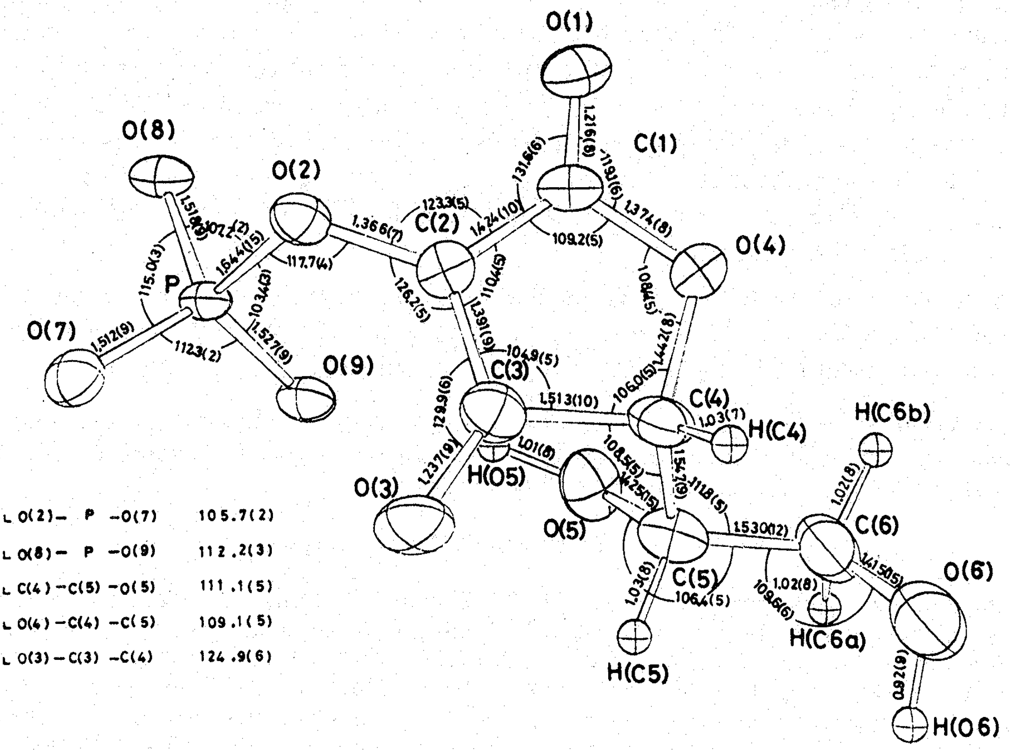

Fig. 1. Perspective Drawing of the Configuration and Numbering Scheme of Atoms of L-Ascorbic Acid 2-*O*-Phosphate

Bond lengths (Å) and angles (°) are shown with their e.s.d.'s in parentheses.

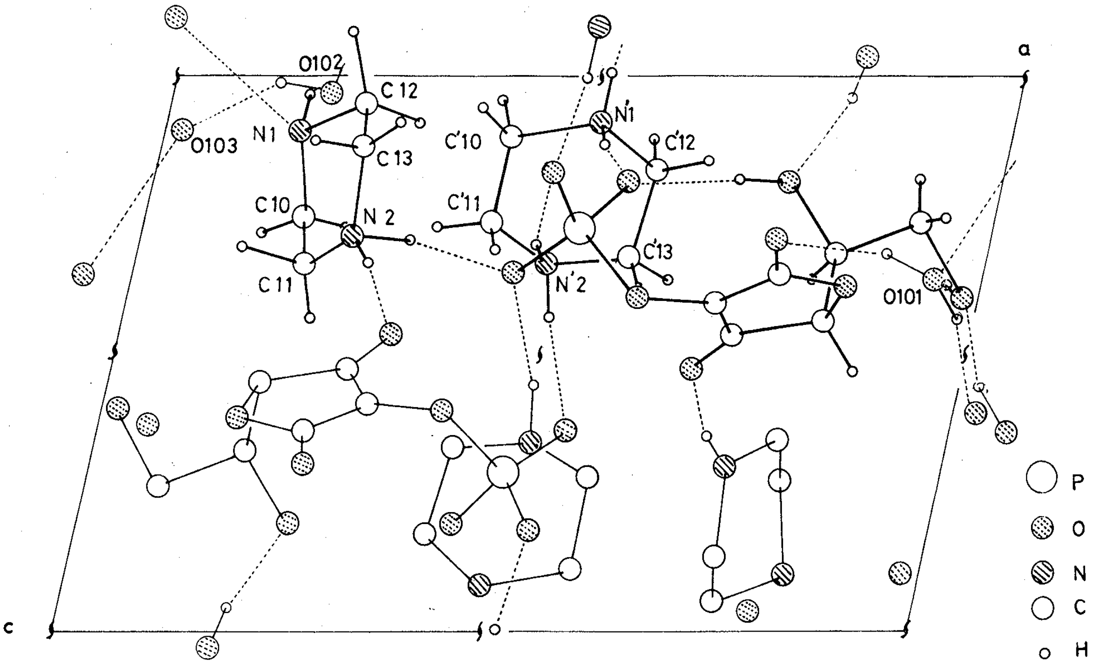

Fig. 2. Packing Diagram of Dipiperazinium L-Ascorbic Acid 2-*O*-Phosphate Trihydrate looking down the *b*-Axis.

Dotted lines represent the hydrogen bonds.

tions (pH=7.3). The latter two were prepared in the same way from the magnesium salt by an ion exchange process using an ion exchange resin, Dowex 50W-X12($H^+$), followed by the addition of the appropriate base.

Table IV[19] shows that there is a complete overlap of the chemical shifts of the magnesium salt with those of the potassium salt, indicating that no phosphate migration occurs during the salt exchange process. The very slight deviation of the chemical shifts observed in the piperazinium salt appears to be due to the difference in the cation structure and the resulting hydrogen bonding (Fig. 2). Further, $^{1}$H nuclear magnetic resonance (PMR) was measured immediately after the dissolution of the magnesium salt and after the measurement of CMR so as to show that no phosphate migration had occurred during the CMR measurement. Exactly the same PMR data were obtained and therefore all three salts were confirmed to be the same in phosphate structure.

Based on the above results, L-ascorbic acid phosphate synthesized by the phosphorylation of 5,6-*O*-isopropylidene-L-ascorbic acid with phosphorus oxychloride in an alkaline protic solvent[6] was unambiguously determined to be L-ascorbic acid 2-*O*-phosphate.

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