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STRUCTURAL DETERMINATION OF ASCORBIC ACID 2-O-PHOSPHATE FORMED VIA ACID HYDROLYSIS OF AN ASCORBIC ACID 3-O-PHOSPHINATE

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Abstract—The synthesis and X-ray structural determination of 3-O-[(bis-morpholino)phosphinyl]-5,6-O-isopropylidene-L-ascorbate (**9**) are described. Acid-catalyzed hydrolysis of **9** afforded the 2-O-phosphate **6**. Definitive structural proof of **6** is based on a study of the pH profile of its UV spectra as compared with those of ascorbic esters, **2** and **9** (Figs. 1–3).

Because of the need for a more stable form of vitamin C, recent literature has been inundated with various methods for the production of sulfate and phosphate acid esters. Many of these esters have been shown to be biologically active or anti-scorbutic.[1] In many cases, it is difficult to make structural assignments of these esters on the basis of spectral and chemical data alone. The reported ascorbic acid 3-O-sulfate **1**, for example, was recently reassigned the 2-O-sulfate structure **2** based on an X-ray single crystal analysis.[2]

Since Cutolo and Larizza's first report on the synthesis of an ascorbic acid phosphate (characterized as its tris-cyclohexylammonium salt),[3] many papers and patents have dealt with the preparative methodology of this phosphate ester.[4–8] Yet, despite the unabated interest in this area, the exact structure of this compound is still in dispute. The original authors as well as Hinkley[5] and Chitose[6] reported this ester as the 3-O-phosphate **3** (or its salt **4**) while others, including Seib,[8] contended it was the 2-O-phosphate **5**. We sought to determine the exact structure of this ester.

Since a 3-O-ascorbic acid ester of definitive structure was unknown for use as a model for comparison studies, we prepared such an ester. Thus alkylation of the thallium 3-ascorbate[9] **10** with bis-morpholinophosphinyl chloride (**11**)[10] afforded the 3-O-ascorbate **9**. The structural assignment of the product was confirmed by X-ray analysis. The same product could also be prepared from **11** and 5,6-O-isopropylidene-L-ascorbic acid (**12**) in the presence of pyridine, though this reaction was much slower. Identity was made on the basis of a comparison of the X-ray powder diffraction patterns of these two products.

Unlike most ascorbic acid derivatives, compound **9** is thermally stable; it melts at 170° without decomposition. It is acidic, being soluble in dilute sodium bicarbonate solution, and, upon acidification, can be recovered unchanged.

When an attempt was made to obtain the 3-O-phosphate derivative **3** via the acid-catalyzed hydrolysis of **9**, two products were observed by tlc analysis. Only one of these products could be isolated as the tris-cyclohexylammonium salt of micro-fine needles (m.p. 173–6°). It eluted as a single component on various paper chromatographic systems, on a DE-23 LC column (triethylammonium carbonate gradient), and on a HPLC PWAX-SO_4^{2-} column (ammonium sulfate gradient). This salt is identical (m.p., ^1H and ^{13}C NMR, UV, and HPLC comparisons) with that prepared by the direct phosphorylation of **12** with phosphorus oxychloride according to the procedure of Cutolo and Larizza.[3]

We demonstrate now that the phosphate salt in question is in fact the 2-O-phosphate **6**, presumably formed through an acid-catalyzed isomerization of the initially formed 3-O-phosphate **3** during the hydrolysis of **9**. This identification is based on comparisons of the salt's pK_a value and of the pH profile of its UV spectra with those of the known 2-O-sulfate **2** and 3-O-phosphinate **9**.

The anion from a 2-O-ester **7** bears a linear conjugated chromaphore, while that of 3-O-ester **8** bears a cross-conjugated chromaphore. A distinction between these isomers might be observed in their pK_a values and UV spectra. Whereas we expect both isomers to show bathochromic shifts (λ_{max} shifts toward longer wavelength) accompanying the ionization due to a pH increase, we would expect definite differences in their absorption intensity. The 2-O-derivative should exhibit a hyperchromic shift and the 3-O-derivative should display a hypochromic shift.

Ascorbic acid (**13**) displays two acidic protons of pK_a values 4.25 and 11.79 for the 3- and 2-hydroxyls, respectively.[11] Sulfation of the 2-OH as in **2**† enhances the acidity of the 3-OH by 1 unit to pK_a of 3.11. On the other hand, a phosphinyl substitution at the 3-O-position as in **9** results in a significant drop in the pK_a value of the 2-OH group to 6.22.[12] A pK_a value of 3.40 for the ascorbic acid phosphate in question is obviously compatible with a 2-O-phosphate ester structure; i.e. **6** (Table 1).[13]

In addition to the evidence drawn from the pK_a value comparison, the pH profile of the UV spectra of the 2-O-sulfate **2** in varying acidic media (Fig. 1) closely resembles that of the 2-O-phosphate **6** (Fig. 2). Both profiles exhibit bathochromic shifts accompanied by hyperchromic effects upon increasing the pH of the solvent. However, the pH profile of the UV spectra of the 3-O-phosphinate ester **9** is different (Fig. 3). Although it also shows a bathochromic shift with increasing basicity, it is accompanied by a hypochromic effect. It is

†We would like to thank Dr. Clark Perry of Hoffmann-La Roche for a supply of dipotassium L-ascorbic acid 2-O-sulfate.

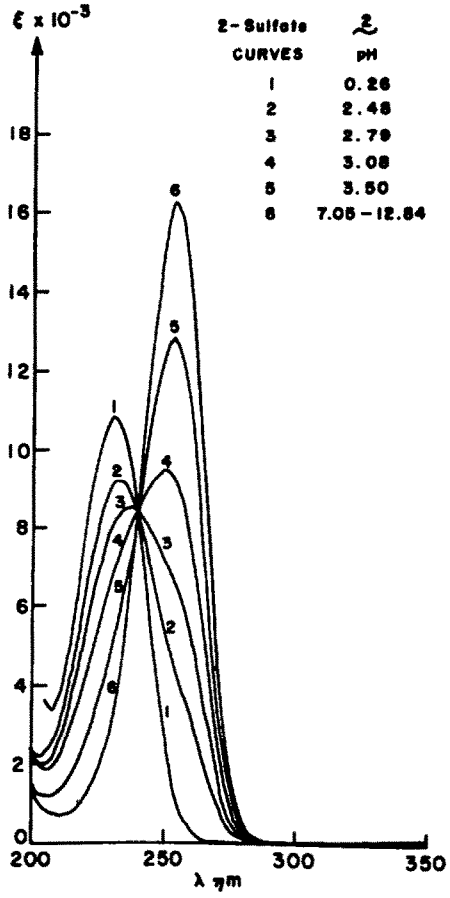


Fig. 1. UV spectra of ascorbic acid 2-O-sulfate 2 at various acidities.

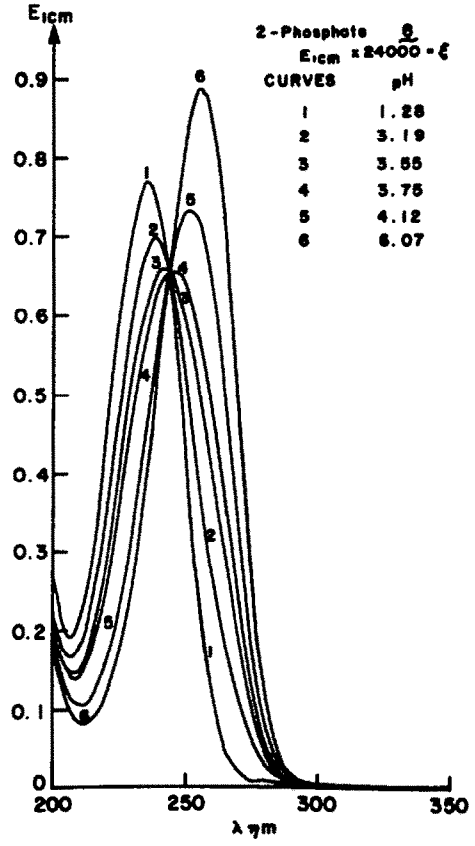
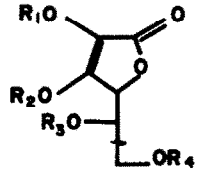
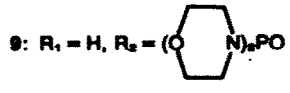


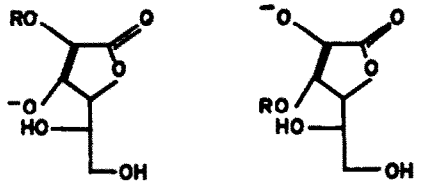
Fig. 2. UV spectra of ascorbic acid 2-O-phosphate 6 at various acidities.



1: $R_1 = K$, $R_2 = KOSO_3$, $R_3 = R_4 = H$
2: $R_1 = KOSO_3$, $R_2 = K$, $R_3 = R_4 = H$
3: $R_1 = R_3 = R_4 = H$, $R_2 = H_2PO_3$
4: $R_1 = C_6H_{11}NH_3^+$
$R^2 = (C_6H_{11}NH_3^+)_2PO_3^-$
$R_3 = R_4 = H$
5: $R_1 = H_2PO_3$, $R_2 = R_3 = R_4 = H$
6: $R_1 = (C_6H_{11}NH_3^+)_2PO_3^-$
$R_2 = C_6H_{11}NH_3^+$, $R_3 = R_4 = H$



9: $R_1 = H$, $R_2 = (O\ N)_2PO$
R_3, $R_4 = C(CH_3)_2$
10: $R_1 = H$, $R_2 = Tl$, R_3, $R_4 = C(CH_3)_2$
12: $R_1 = R_2 = H$, R_3, $R_4 = C(CH_3)_2$
13: $R_1 = R_2 = R_3 = R_4 = H$



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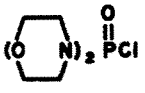

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Table 1. Acidity of ascorbic acid esters[14]

Esters	pKa 2-OH	pKa 3-OH
Ascorbic acid 13	11.79	4.25
2-O-Sulfate 2		3.11
2-O-Phosphate 6		3.40
3-O-Phosphinate 9	6.22	

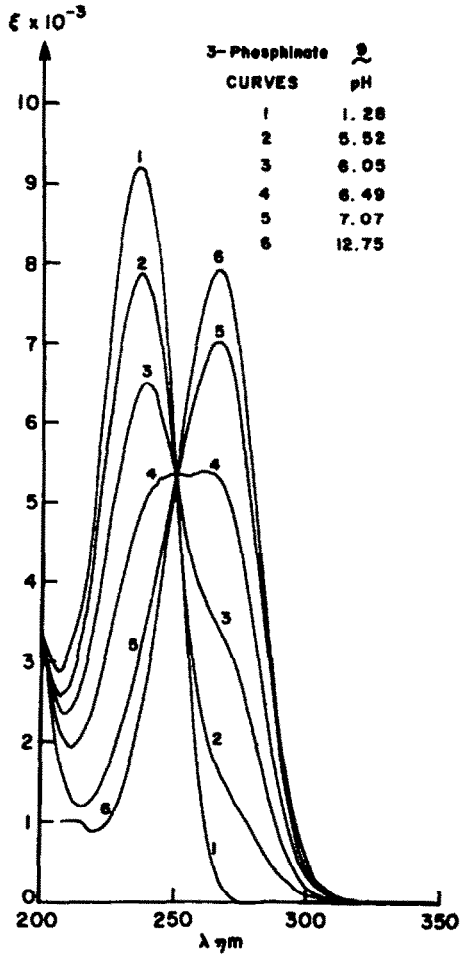

Fig. 3. UV spectra of ascorbic acid 3-O-phosphinate 9 at various acidities.

clear that we are dealing with two different chromaphores, a linear conjugated (2 and 6) versus a cross-conjugated chromaphore (9).

We believe that the 3-O-phosphate 3, formed first from the acid hydrolysis of the phosphinate ester 9, was unstable, and that the product isolated was the more stable 2-O-phosphate salt 6. In addition, based on the evidence presented here, the tris-cyclohexylammonium salt of the ascorbic acid phosphate, first prepared by Cutolo and Larizza and assigned the 3-O-phosphate 4, is in fact the 2-O-phosphate 6.[14]

EXPERIMENTAL

General. M.ps are uncorrected. The IR spectra were recorded on either a Perkin–Elmer 621 or Digilab F.T.S. 14 Beckman IR-9 spectrophotometer; UV spectra were recorded on a Cary Model 14M spectrophotometer; and the NMR spectra, on a HA-100 or XL-100 spectrophotometer using TMS internal standard. The mass spectra were taken with a CEC 21-100 mass spectrometer at 70 eV.

5,6 - O - *Isopropylidene* - L - *ascorbic acid* 3-*thallium salt* (10). The titled compound was prepared from 12 and TlOH according to the procedure of Hughes[9] as an off-white solid (recrystallized from EtOH–H_2O), m.p. 170–180° dec. (Found: C, 25.66; H, 2.54. Calc. for $C_9H_{11}O_6Tl$: C, 25.78; H, 2.64%).

3 - O - [(*Bis* - *morpholino*)*phosphinate*] - 5,6 - O - *isopropylidene*- L - *ascorbate* (9)

A. To a soln of 10 (1.9 g; 4.5 mmol) in 15 ml DMF, 11 (1.3 g; 5.0 mmol) in 15 ml of DMF was added. Within 2 min a voluminous ppt formed, and the mixture became slightly warm (35°C). After stirring for 2 hr, the TlCl was removed by filtration, and the DMF soln was concentrated in vacuum. The resultant semi-crystalline residue was recrystallized from EtOH–EtOAc to yield 1.2 g (63%) of 9, m.p. 169.5–171°. IR(KBr) 1770, 1691 cm^{-1}. UV (EtOH): 235/6 nm (ϵ = 9500). NMR (DMSO-d_6) δ: 1.24 (br. s. CH_3CCH_3); 3.07 (m, 4XNCH_2); 3.50 (m, 4XOCH_2); 3.90 (q, C_4H); 4.11 (t, C_6H); 4.36 (d × t, C_5H); 4.99 (t, C_4H); 10.01 (s, 2-OH). MS: *m/e* 434 (M^+). $[\alpha]_D$: +35.86° (in CH_3OH, c6.721). (Found: C, 46.99; H, 6.33; N, 6.48. Calc. for $C_{17}H_{27}N_2O_9P$: C, 47.00; H, 6.26; N, 6.45%).

B. A mixture of 12 (8.64 g; 40 mmol), 11 (10 g; 40 mmol), pyridine (3.2 g; 40 mmol) in 320 ml acetone was stirred for 15 days. At the end of this period, the mixture was concentrated in vacuum and the oily residue was washed with H_2O, dil. H_2SO_4 and H_2O, and dried to yield 16.5 g (95%) of crude product. Recrystallized as above, this residue afforded 8.2 g of 9, m.p. 165–174°. This product was identical to that prepared by method A based on a comparison of the X-ray powder diffraction patterns.

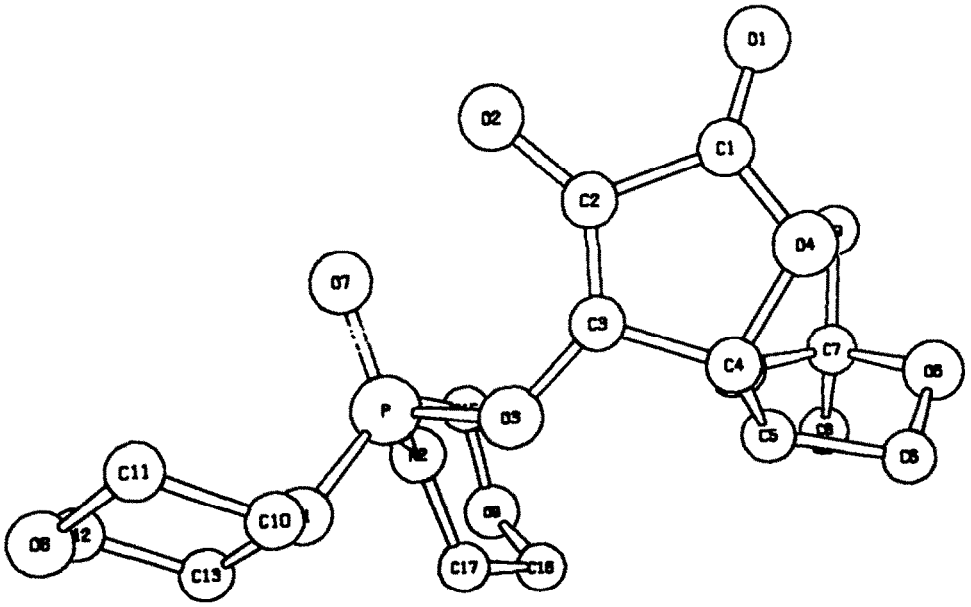

Fig. 4. Stereodrawing showing the conformation of the ascorbate 3-O-phosphinate 9.

Tris-cyclohexammonium L-ascorbate-2-O-phosphate (6) *from the hydrolysis of* 9

A mixture of 9 (449 mg; 1 mmol), Dowex (H^+) 50W-X8 (1.5 ml), acetone (5 ml) and H_2O (5 ml) was heated on the steam bath to reflux for a total of 10 hr. After separating from the resin, the aqueous acetone soln was treated with cyclohexylamine (300 mg; 3 mmol) and concentrated in vacuum to a gel. This was dissolved in EtOH (insolubles filtered off) and, upon the addition of EtOAc and cooling, afforded 136 mg (25%) of the tris-cyclohexylamine salt 6, m.p. 173–6° (lit. m.p. 169–70°).[3] (Found: C, 51.92; H, 8.95; N, 7.63. Calc. for $C_{24}H_{48}N_3O_9P$: C, 52.07; H, 8.74; N, 7.59%).

X-Ray analysis of 9

The intensity data for the X-ray analysis were measured on a Hilger–Watts diffractometer from a crystal which was approximately 0.1 × 0.2 × 0.4 mm in size. Crystal data: $C_{17}H_{27}N_2O_9P$, monoclinic, space group $P2_1$, Z = 2, a = 9.487 (5) A, b = 13.570 (8) A, c = 8.355 (4) A; $d_{calc} = 1.377\ g\ cm^{-3}$, μ(Cu Kα) = 16.2 cm^{-1}.

Of the 2186 accessible reflections with $\theta < 76°$, 1858 had intensities which were significantly greater than background and these reflections were used in the structure analysis. The reflection data were corrected for absorption. The coordinates of the P atom were obtained from a sharpened Patterson map. The first electron density map (Fourier), based on the P atom, showed only the four atoms about the P. Additional Fouriers, based on successively more complete trial structures, slowly revealed the full structure. Seven Fouriers were required to locate all the nonhydrogen atoms. The structure was refined by full-matrix least squares to R = 0.117 (all atoms isotropic). A difference Fourier calculated at this point did not show any additional atoms. Further refinement of the structure (with anisotropic thermal parameters and including the hydrogens) was not done because only the gross structure was required.

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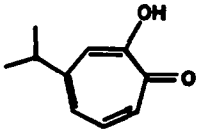
Note that Hinoki (I) exhibits a pK_a value of 7.2.



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[13]The pK_a determinations were made according to the method described in *The Determination of Ionization Constant, A Laboratory Manual* (Edited by A. Albert and E. P. Serjeant), 2nd Edn, Chap. 4. Chapman & Hall, London (1971).
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