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Note

5,6-Anhydro-L-ascorbic acid. A reactive intermediate for the formation of 6-substituted derivatives of L-ascorbic acid

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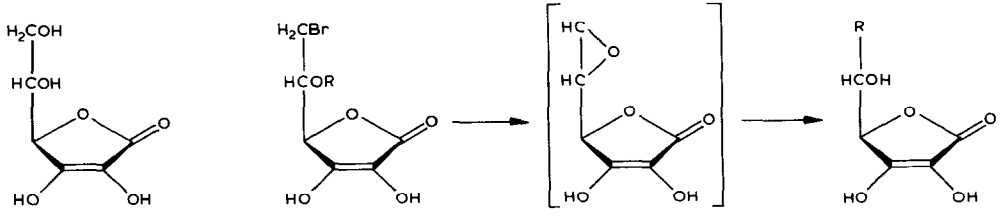
The well-known hydrolytic and oxidative instability[1] of L-ascorbic acid (**1**) has limited its utility in various commercial applications[2]. The implication of OH-6 in the proposed pathway[3] for the acid-catalyzed decomposition has prompted interest in the preparation of derivatives of **1** in which OH-6 is absent or blocked[4]. Recently, the halogenation of **1** with hydrogen bromide–acetic acid has been reported, independently by Berg and Kiss[4], and by Pedersen *et al.*[5], to afford 5-*O*-acetyl-6-bromo-6-deoxy-L-ascorbic acid (**2**). Mild hydrolysis of **2** gave 6-bromo-6-deoxy-L-ascorbic acid (**3**); the 6-deoxy-6-halogeno (bromo, chloro, and fluoro) derivatives were synthesized from a protected derivative of L-*xylo*-2-hexulosonic acid[6]. The ready availability of **3** in good yield suggested its potential as an intermediate in the formation of other 6-substituted derivatives of **1**.

The reaction of nucleophiles with 6-bromo-6-deoxy-L-ascorbic acid (**3**) under mildly basic conditions may occur either *via* direct displacement of the halogeno group or *via* an intermediate 5,6-anhydro compound. In order to determine the pathway involved, the reaction of **3** in aqueous sodium carbonate–sodium



1

2 R = Ac
3 R = H

4

5 R = CH_3
6 R = CH_2OPh
7 R = CH_2N_3
8 R = CH_2NH_2
9 R = CH_2SPh

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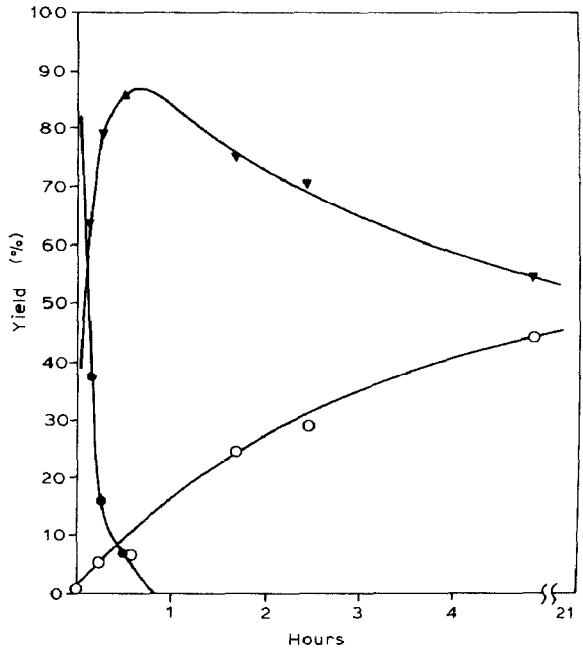

Fig. 1. Formation from 6-bromo-6-deoxy-L-ascorbic acid (**3**), and hydrolysis into L-ascorbic acid (**1**), of 5,6-anhydro-L-ascorbic acid (**4**), at 24° in $NaHCO_3$–Na_2CO_3 buffer, pH 8.0: (—○—○—) **1**, (—●—●—) **3**, and (—▼—▼—), **4**.

hydrogencarbonate buffer at pH 8 was followed by ^{1}H-n.m.r. spectroscopy with respect to time. Under these conditions, the half-life of **3** was only ~10 min (Fig. 1). The rapid formation of 5,6-anhydro-L-ascorbic acid (**4**) was evidenced by the upfield shifts in the ^{1}H-n.m.r. spectrum of the methylene protons of **3** from δ 3.52 to 3.02, and by the ^{13}C-n.m.r. shifts (Table I). At 30 min, epoxide **4** was formed to the extent of 86% (realtive to the internal standard) with < 7% of bromide **3** remaining. Epoxide **4** is itself unstable to hydrolytic ring cleavage, giving L-ascorbic acid (**1**) under these reaction conditions, and had a half-life of ~5 h at 25°. Processing of the reaction mixture after 25 h afforded **1** in 89% yield (m.p. 190–192°). The same reaction with D_2O as solvent afforded no evidence of deuterium incorporation at C-4 by ^{1}H-n.m.r. spectroscopy.

Attempts to isolate epoxide **4** as the free acid from the aqueous reaction mixture by de-ionization with Dowex 50 resin (H^+) were unsuccessful owing to the rapid, acid-catalyzed hydrolysis of **4** to **1**. Although isolation of the impure sodium salt by acetone precipitation was possible, attempted trimethylsilylation of the crude sodium salt with chlorotrimethylsilane–pyridine and isolation of the per(trimethylsilyl)ated derivative afforded only 6-chloro-6-deoxy-2,3,5-tri-*O*-trimethylsilyl-L-ascorbic acid[7]. The lability of **4** to a relatively weak nucleophile, such as the hydroxide ion, prompted us to examine its reactivity with several other nucleophiles. Treatment of epoxide **4** with sodium phenoxide under the conditions

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS OF INTERMEDIATES IN THE FORMATION AND HYDROLYTIC CLEAVAGE OF 5,6-ANHYDRO-L-ASCORBIC ACID (**4**)[a]

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
3	164 (s)	117.9 (s)	154.8 (s)	76.5 (d)	68.8 (d)	32.6 (t)
4	174.3 (s)	112.3 (s)	162.4 (s)	78.3 (d)	52.1 (d)	45.1 (t)
6	173.7 (s)	118.7 (s)	155.8 (s)	77.8 (d)	69.8 (d)	63.0 (t)

[a]Signal of internal standard (MeOH) adjusted to δ 49.3. Letters in parentheses represent multiplicity as determined by off-resonance decoupling.

TABLE II

SPECTRAL SHIFTS OF 6-AMINO6-DEOXY-L-ASCORBIC ACID (**8**) WITH RESPECT TO pH

pH	λ_{max} *(nm)*	$[\alpha]_D^{25}$	^{13}*C-N.m.r. chemical shifts* (δ)					
			C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
1.0	236	−11.7	172.4	117.8	153.9	76.6	64.9	41.7
5.3	258	+92.8	172.7	118.1	154.2	76.8	65.2	41.9
11.0	294	+126	180.4	121.2	169.6	78.5	70.6	43.6

just described afforded, in moderate yield, 6-*O*-phenyl-L-ascorbic acid (**5**) soluble in organic solvents. Attempts to treat **4** with more basic oxygen nucleophiles, such as sodium methoxide–methanol, were unsuccessful. The more nucleophilic sodium thiophenoxide reacted with **4** rapidly to give, in excellent yield, 6-*S*-phenyl-6-thio-L-ascorbic acid (**9**). The ready reaction of **4** with nucleophiles allowed the synthesis of 6-amino-6-deoxy-L-ascorbic acid (**8**). Aside from the potentially interesting physical properties that this analog of 4-aminobutyric acid might possess, **8** has been proposed as a potential substrate for the resin-bound-affinity chromatography of enzymes involved in ascorbic acid metabolism*. The addition of sodium azide to epoxide **4** afforded rapidly 6-azido-6-deoxy-L-ascorbic acid (**7**), as evidenced by ^{1}H-n.m.r. spectroscopy. Azide **7** was not isolated but immediately reduced in the presence of palladium–carbon at ambient temperature to afford, after neutralization, 6-amino-6-deoxy-L-ascorbic acid (**8**), which precipitated as a high-melting, meso-ionic solid. Compound **8** showed large bathochromic shifts in the u.v. spectrum, as well as large changes in optical rotation with increasing pH. This is suggestive of a conformational change due to increasing intramolecular ionic-association (see Table II). The large β-shifts (5.4 p.p.m.) of the C-5 signal in the ^{13}C-n.m.r.

*Compound **8** failed to inhibit GABA (4-aminobutyric acid) binding; see ref. 8.

spectrum of **8** from pH 11 to 5.3, and the relatively minor shift (0.3 p.p.m.) from pH 5.3 to 1.0 is also consistent with the presence of a zwitterion in solution.

Epoxide **4** was also found to be readily hydrogenolyzed in the presence of palladium–carbon at ambient temperature with a stoichiometric uptake of hydrogen to give 6-deoxy-L-ascorbic acid (**5**) in 56% yield*. The material was identical with authentic **5** prepared by the method of Müller and Reichstein[10].

It appears that 5,6-anhydro-L-ascorbic acid (**4**) is formed as a reactive intermediate and serves as a substrate for various moderately basic nucleophiles. This intermediate affords a convenient method for preparing 6-deoxy-6-substituted L-ascorbic acid derivatives having potentially interesting physical and biological properties.

EXPERIMENTAL

General methods. — Melting points are uncorrected. I.r. spectra were recorded with a Perkin–Elmer model 727B spectrometer. ^{1}H-N.m.r. spectra were recorded with a Varian Associates Model EM-360-L spectrometer. Chemical shifts are reported relative to an internal standard of tetramethylsilane or sodium 4,4-dimethyl-1-silapentane-1-sulfonate. ^{13}C-N.m.r. spectra were recorded, in the fast, Fourier-transform (fFt) mode, with a Varian XL-100 A-15 (25.2 MHz) spectrometer equipped with a Nicolet Technology 1080 data system. Complete proton-decoupling was provided by square wave-modulation[11] of the Varian gyrocode, heteronuclear decoupler. The spectra were obtained with a pulse angle of ~30°, an aquisition time of 1.4 s, and a quadrature phase-detection. The upfield frequency-lock was maintained by the deuterium resonance of the solvent in a 5-mm (o.d.) sample-tube. Methanol was used as the internal standard, adjusted to δ 49.3 to provide chemical-shift values relative to the signal of tetramethylsilane. Mass spectral data were obtained with an MS-30 instrument having a PS-50 data system.

6-Bromo-6-deoxy-L-ascorbic acid (**3**). — This derivative was synthesized by the procedure of Berg and Kiss[4] affording, after crystallization from nitromethane, a 38% yield of **3**, m.p. 175–176° (lit.[4] 175–176°); ν_{max}^{KBr} 5.74 (s), 6.02 cm^{-1} (s); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 4.83 (d, 1 H, J 2.0 Hz), 3.93 (m, 1 H), and 3.52 (ABX, 2 H).

5,6-Anhydro-L-ascorbic acid (**4**). — To a solution of Na_2CO_3 (1.04 g, 8.4 mmol) in D_2O (6 mL) was added **3** (1.00 g, 4.2 mmol) in D_2O (4 mL). The reaction was monitored by ^{1}H-n.m.r. spectroscopy with respect to time. After 30 min, the mixture consisted of 86% of **4**; ^{1}H-n.m.r. (D_2O): δ 4.37 (d, 1 H, J 4.8 Hz), 3.39 (m, 1 H), and 3.02 (ABX, 2 H). Attempts to add the reaction mixture to Dowex 50 and isolate the free acid afforded only L-ascorbic acid (**1**). Under the basic reaction conditions, **4** had $t_{0.5}$ 4 h 50 min, and was converted completely into **1** over a 21-h period at pH 9.

L-Ascorbic acid (**1**). — To a solution of Na_2CO_3 (5.2 g, 42 mmol) in water (50

*Longer reaction-times resulted in further reduction of the enonolactone group; see ref. 9.

mL) was added **3** (5.0 g, 21 mmol) in small portions. The mixture was stirred for 25 h at ambient temperature and added to Dowex 50 (H^+; 75 mL) ion-exchange resin. After being stirred for 30 min, the resin was removed by filtration and washed with water (100 mL), and the combined washings and filtrate concentrated to 20 mL. The solution was treated with Darco charcoal and filtered. On removal of solvent *in vacuo,* a white crystalline solid was isolated (3.3 g, 89%) and shown by ^{1}H- and ^{13}C-n.m.r. spectroscopy to be **1**.

*6-Deoxy-*L*-ascorbic acid* (**5**). — To a solution of Na_2CO_3 (10.8 g, 85 mmol) in water (100 mL) was added **3** (10.0 g, 42 mmol), followed by 5% Pd–C (2 g). The mixture was hydrogenated overnight in a Parr shaker-apparatus at 0.21 MPa hydrogen pressure. After the theoretical uptake of hydrogen, the catalyst was removed by filtration through Celite and the filtrate stirred with Dowex 50 (H^+; 100 mL) ion-exchange resin for 1 h. The resin was removed by filtration and washed with water (3 × 100 mL). The combined filtrate and washings were treated with Darco charcoal (0.5 g) and filtered, and the filtrate was evaporated *in vacuo* to give an oil. Trituration with ethyl acetate afforded a white crystalline solid (3.75 g, 56%; 93.3% pure by I_2 titration). Recrystallization from ethyl acetate afforded a sample for analysis, m.p. 162–163° (lit.[10] 167–168°); $[\alpha]_D^{24}$ +39.2° (*c* 1.02, MeOH); ν_{max}^{KBr} 5.80 (s), 6.10 (s); ^{1}H-n.m.r. (D_2O): δ 4.83 (d, 1 H, *J* 2.0 Hz), 4.15 (quart. of d., 1 H, *J* 2.0, 6.8 Hz), and 1.36 (d, 3 H, *J* 6.8 Hz).

Anal. Calc. for $C_6H_8O_5$: C, 45.01; H, 5.04. Found: C, 44.80; H, 4.97.

O-*Phenyl-*L*-ascorbic acid* (**6**). — To a stirred solution of Na_2CO_3 (7.44 g, 60 mmol) in water (25 mL) was added **3** (4.76 g, 20 mmol) in small portions, followed by phenol (3.8 g, 40 mmol). The solution thickened to a white mobile paste, gradually turning yellow after being stirred for 6 h under N_2 atmosphere. The pH of the mixture was adjusted to 0.5 with 6M aqueous HCl, the solution extracted with ethyl acetate (3 × 100 mL), and the ethyl acetate layer washed with water and sodium chloride solution, and dried ($MgSO_4$). After removal of the solvent *in vacuo,* the residue was triturated with benzene, and the resulting crystals collected by filtration and dried *in vacuo* overnight, affording 1.54 g of crude **6**. A sample was obtained for analysis by recrystallization from nitromethane, followed by drying *in vacuo* overnight; m.p. 160–161°, $[\alpha]_D^{24}$ +79.3° (*c* 1.09, MeOH); ν_{max}^{KBr} 5.68 (s), 6.00 cm^{-1} (s); ^{1}H-n.m.r. (D_2O): δ 7.54–6.75 (m, 5 H, arom.), 7.20 (m, 3 H, -C*H*$_2$-O and -C*H*OH), and 4.93 (br. s, 1 H); m.s. (70 eV): *m/z* 252.0563 (parent, $C_{12}H_{12}O_6$), 136 (Ph–O–CH_2–C^+H–OH), 116, 107 (Ph–O^+=CH_2), and 94 (base, Ph–OH).

Anal. Calc. for $C_{12}H_{12}O_6$: C, 57.14; H, 4.80. Found: C, 56.77; H, 4.91.

*6-Amino-6-deoxy-*L*-ascorbic acid* (**8**). — To a solution of Na_2CO_3 (15.6 g, 126 mmol) in water (100 mL) was slowly added **3** (15 g, 63 mmol) in water (20 mL) followed by sodium azide (6.0 g, 92 mmol). The mixture was stirred overnight, Dowex 50 (H^+; 175 mL) ion-exchange resin added, the slurry stirred another h, and the resin removed by filtration. The resin was washed exhaustively with water (4 × 100 mL), the filtrate and washing were concentrated to 150 mL, 5% Pd–C (3 g) was added, and the mixture hydrogenated in a Parr shaker-apparatus at 0.34 MPa

hydrogen pressure. After the theoretical uptake of hydrogen (6 h), the catalyst was removed by filtration and the filtrate evaporated *in vacuo* to afford a white amorphous solid (3.75 g, 95% purity by I_2 titration). A sample was recrystallized from water for analysis and dried overnight *in vacuo*, m.p. 210° (dec.), $[\alpha]_D^{23}$ −11.7° (*c* 0.886, 0.1M HCl), $[\alpha]_D^{23}$ +92.8° (*c* 0.687, water), $[\alpha]_D^{23}$ +126° (*c* 0.920, 0.1M NaOH); λ_{max}^{258} (H_2O) (ε 11.2 × 10^4), λ_{max}^{294} (0.1M NaOH) (ε 3.89 × 10^4); λ_{max}^{236} (0.1M HCl) (ε 1.8 × 10^4); ν_{max}^{KBr} 5.77 (s), 6.25 cm^{-1} (s); ^{1}H-n.m.r. (D_2O, pH 5): δ 4.33 (br. s, 1 H), 3.85 (br. t, 1 H, *J* 7.0 Hz), and 2.79 (br. d, 2 H, *J* 7.0 Hz); ^{1}H-n.m.r. (D_2O, pH 1): δ 4.94 (d, 1 H, *J* 2.0 Hz), 4.28 (octet, 1 H), and 3.28 (ABX, 2 H).

Anal. Calc. for $C_6H_9NO_5$: C, 41.15; H, 5.18; N, 8.00. Found: C, 40.97; H, 5.15; N, 8.07.

*6-S-Phenyl-6-thio-*L*-ascorbic acid* (**9**). — To a solution of Na_2CO_3 (21.6 g, 174 mmol) in 3:1 water–methanol (100 mL) were added sequentially thiophenyl (2.7 mL, 46 mmol) and **3** (10.0 g, 42 mmol). The mixture solidified after being stirred for 15 min and reliquified after an additional h. It was stirred for an additional 30 min and made acid to pH 1 with 6M HCl. The solution was extracted with ethyl acetate (3 × 100 mL) and the combined organic layers were dried (Na_2SO_4). Removal of the solvent *in vacuo* afforded **9** as needles, m.p. 99–100°; ν_{max}^{KBr} 5.71 (s), 6.00 cm^{-1} (s); ^{1}H-n.m.r. [(CD_2)SO]: δ 7.32 (s, 5 H, arom.), 4.75 (d, 1 H, *J* 1.6 Hz), 3.83 (t, 2 H, *J* 7.2 Hz), and 3.08 (d, 2 H, *J* 3.6 Hz); ^{13}C-n.m.r. [$(CD_3)_2$SO]: δ 170.52, 152.75, 136.04, 129.22, 128.23, 125.93, 118.28, 76.13, 66.71, and 35.95; m.s. (70 eV): *m/z* 268.0389 ($C_{12}H_{12}O_5S_1$, parent).

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