Synthesis of 2-Deoxy-L-ascorbic Acid

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Received July 2, 1996

Ascorbic acid (vitamin C) functions as an electron donor and antioxidant for several enzymes and is implicated in host defense mechanisms, endocrine function, and other biological processes.[1] Recent research on the biochemistry of ascorbic acid has been focused on the mechanisms of action of the vitamin as well as on concentrations required for its optimal function.[2] For example, recent clinical studies have shown that the recommended dietary allowance (RDA) for ascorbic acid may be too low.[3]

Recent kinetic measurements of transport inhibition by 6-deoxy-6-haloascorbic acid analogues established the presence of separate pathways for the translocation of ascorbic acid and dehydroascorbic acid,[4] suggesting also that the 6-OH group of ascorbic acid is not critical for transport and, presumably, for other functions. In order to extend these studies to an investigation of the importance of the 2-hydroxyl group for ascorbic acid transport, we have prepared 2-deoxy-L-ascorbic acid **1**. Compound **1** could also be a versatile intermediate for the preparation of other 2-deoxyascorbic acids, e.g., their as yet unreported 2-halo or 2-alkyl derivatives.

Dieckmann condensations have been used effectively for the preparation of tetronic acids[5,6] and should provide an efficient route to **1**, with the particular advantage that chiral starting materials are commercially available. Accordingly, methyl 3,4-*O*-isopropylidene-L-threonate (**2**) was acetylated to give the 2-*O*-acetyl derivative **3**. However, attempts to cyclize the acetyl ester **3** (LHMDS, THF) failed to give the desired ring system. We suspected that deprotonation at C-2 caused elimination of the functional group at position 3 (Scheme 1) in a process similar to that reported by Brandänge and co-workers during attempted cyclization of the di-*O*-acetate of dimethyl (*R,R*)-tartrate.[6] In that report, the contrasting successful LHMDS-mediated cylization of the monoacetate of dimethyl (*R,R*)-tartarate was attributed to an inhibition of C-2 deprotonation by an ionized 3-hydroxy group. Similarly, our acetonide **3** was deprotected to 2-*O*-acetyl L-threonate **5** followed by cyclization with LHMDS to give the target compound **1**. However, the yield was very low (Scheme 1) and separation of the water-soluble **1** from the amine salt quite tedious.

Nucleophilic addition of the 4-hydroxy group to the carbonyl, blocking further nucleophilic attack (Scheme 1), is a likely explanation for the low yield. To thwart this addition, the 4-hydroxy group was protected as the trityl ether. The decreased water solubility, thus of the product also, facilitates its isolation. However, when compound **5** was treated with TrCl in the presence of DMAP, we found that in addition to the desired methyl 2-*O*-acetyl-4-*O*-trityl-L-threonate **7**, methyl 3-*O*-acetyl-4-*O*-trityl-L-threonate **6** was produced by an acetyl migration (Scheme 2). Monitoring the progress of the reaction by ^{1}H NMR and TLC showed **7** to be the initial product. However, **6** was isolated as the major product after workup. Cyclization of **6** gave the δ-lactone **8** along with a small amount of the γ-lactone **9**. The formation of **9** was favored by low reaction temperature. The formation of **9** from **6** demonstrates that the acetyl group also migrates during Dieckmann cyclization. Indeed, cyclization of **7** also gave **8** as the major product.

Structural assignments of the δ-lactone **8** and the γ-lactone **9** are based primarily on their ^{1}H NMR spectra. Comparison of **8**, **9**, L-ascorbic acid, and the tetronic acid **10**[6] reveals significant differences in the spectrum of **8** relative to the spectra of the others in the series, whereas the spectrum of **9** is clearly consistent with that of a γ-lactone (Table 1). The facts that the enolic hydroxy group of **8** is seen 1 ppm upfield and the 5-H resonance is a doublet of triplets, coupled to the 4-H with a coupling constant of 7.5 Hz, strongly support δ-lactone assignment. The γ-lactonization of **9** and the δ-lactonization of **8** are also evident from the downfield shifts of their 4-CH and 5-CH resonances, respectively (Table 1).

The propensity for δ-lactone formation at first appeared to represent a serious obstacle to our proposed synthesis. However, we were gratified to discover that deprotection of lactone **8** in HCl afforded compound **1** in good yield, identical to the material produced by cyclization of compound **5**. Thus, under acid-catalyzed equilibrium conditions, 2-deoxy-L-ascorbic acid was formed as the sole, isolated product. The structure of compound **1** was based on combustion analysis and ^{1}H- and ^{13}C-NMR data and confirmed by X-ray analysis.[7]

Experimental Section

General Methods. Melting points were determined in open-end capillary tubes and are uncorrected. Proton and carbon-13 NMR were recorded on a 300 MHz spectrometer, and chemical shifts are reported in ppm relative to tetramethylsilane. CI mass spectra were performed by the staff of the Laboratory of Analytical Chemistry, NIDDK. Optical rotations were determined on a Perkin-Elmer polarimeter 341. 3,4-*O*-Isopropylidene-L-threonate was purchased from Aldrich Chemical Co. Compound **10** was synthesized according to ref 6 and fully characterized. Solvents and other reagents were purchased from Aldrich or Fluka. Elemental analyses were performed by Atlantic Microlab, Inc.

Methyl 2-*O*-Acetyl-3,4-*O*-isopropylidene-L-threonate (3). To a solution of methyl 3,4-*O*-isopropylidene-L-threonate (**2**) (12 g, 63 mmol) in dry CH_2Cl_2 (150 mL) and pyridine (80 mL) was added dropwise AcCl (7 mL, 98 mmol) with stirring at 0 °C. A white solid formed. After 2 h, water (20 mL) was added. The separated aqueous layer was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed with H_2O (20 mL) and brine and were dried (Na_2SO_4). Removal of the solvent gave a light yellow liquid that was distilled to produce **3** as a colorless liquid (13.5 g, 92%): bp 135−8 °C/14 mm; ^{1}H NMR ($CDCl_3$) δ ppm 1.35 (s, 3H), 1.45 (s, 3H), 2.19 (s, 3H), 3.78 (s, 3H), 3.95 (dd, 1H, J = 5.9 and 8.9 Hz), 4.09 (t, 1H, J = 8.9 Hz), 4.52 (q,

(1) For example, see: Gaby, S. K.; Sigh, V. N. Vitamin C. In *Vitamin Intake and Health*; Gaby, S. K., Bendich, A., Sigh, V. N., Machlin, L. J., Eds.; Dekker: New York, 1991; pp 103−161.

(2) For example, see: Bergsten, P.; Moura, A. S.; Atwater, I.; Levine, M. *J. Biol. Chem.* **1994**, *269*, 1041. Washko, P.; Hartzell, W. O.; Levine, M. *J. Biol. Chem.* **1989**, *264*, 15404.

(3) Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R. W.; Washko, P. W.; Dhariwal, K. R.; Park, J. B.; Lazarev, A.; Graumlich, J. F.; King, J.; Cantilena, L. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 3704.

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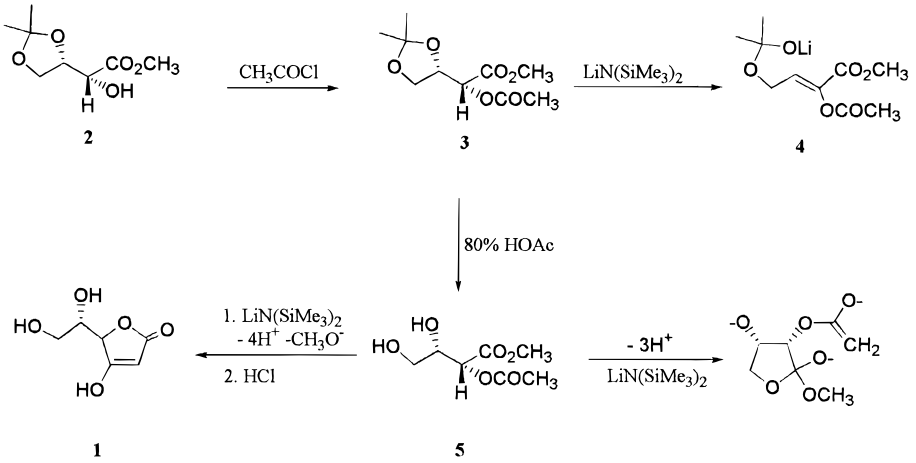
(5) Ireland, R. E.; Thompson, W. J. *J. Org. Chem.***1979**, *44*, 3041.

(6) Brandänge, S.; Flodman, L. Norberg, Å. *J. Org. Chem.* **1984**, *49*, 928.

(7) Morkawa, H.; Kato, K.; Kimoto, H.; Ge, P.; Kirk, K. L. *Anal. Sci.* **1996**, *12*, 825.

S0022-3263(96)01247-9

Scheme 1



Scheme 2

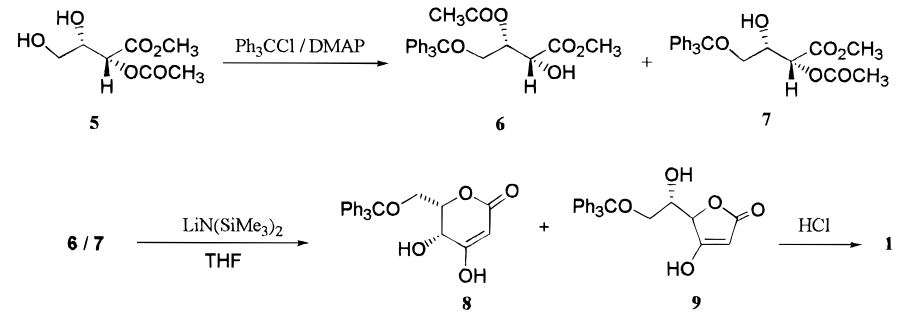
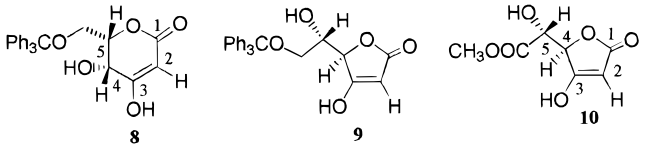


Table 1. ^{1}H NMR Data Support the δ-Lactone Structure for Compound 8[a]



compd	2-H	3-OH	4-H	5-H
8	4.96	11.50	3.92	4.44 (dt)
9	4.90	12.51	4.98 (s)	3.94
10	4.95	12.60	5.20 (s)	4.45
L-ascorbic acid		11.02	4.70 (s)	3.70

[a] Determined in DMSO-d_6 at room temperature.

1H, J = 6.1 Hz), 5.10 (d, 1H, J = 5.1 Hz); ^{13}C NMR ($CDCl_3$) δ ppm 20.87, 25.60, 26.37, 52.82, 65.75, 72.57, 74.55, 110.43, 168.00, 170.50; $[\alpha]^{25}_D = +37.4$ (c 0.5, CH_3OH). Anal. Calcd for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95. Found: C, 51.61; H, 6.96.

Methyl 2-*O*-Acetyl-L-threonate (5). A solution of compound **3** (7 g, 30 mmol) in 80% AcOH (70 mL) was stirred (rt) for 24 h. Evaporation of the solvent below 30 °C gave a colorless oil. This was dissolved in EtOAc (200 mL), and the solution was carefully washed with 5% $NaHCO_3$ until neutral (pH 6−7). The EtOAc layer was separated, washed with H_2O (20 mL) and brine, and dried (Na_2SO_4). Solvent was removed to give a colorless oil that became crystalline on storage. Recrystallization from Et_2O gave **5** as white crystals (4.9 g, 83.3%): mp 66−67 °C; ^{1}H NMR ($CDCl_3$) δ ppm 1.90 (br s, 1H), 2.15 (s, 3H), 2.54 (d, 1H, J = 6 Hz), 3.70 (m, 2H), 3.80 (s, 3H), 4.15 (m, 1H), 5.20 (d, 1H, J = 3.3 Hz); ^{13}C NMR ($CDCl_3$) δ ppm 20.62, 52.90, 63.0, 71.45, 72.76, 169.38, 170.7; $[\alpha]^{25}_D = +36.2$ (c 0.5, CH_3OH). Anal. Calcd for $C_7H_{12}O_6$: C, 43.75; H, 6.29. Found: C, 43.83; H, 6.24.

Methyl 3-*O*-Acetyl-4-*O*-trityl-L-threonate (6) and Methyl 2-*O*-Acetyl-4-*O*-trityl-L-threonate (7). To a solution of compound **5** (1.92 g, 10 mmol) in CH_2Cl_2 (20 mL) was added a solution of TrCl (3.36 g, 11.8 mmol) and DMAP (1.4 g, 11.5 mmol) in CH_2Cl_2 (20 mL). The solution was stirred at rt for 20 h, after which time additional portions of TrCl (1.12 g, 4.0 mmol) and DMAP (0.47 g, 3.85 mmol) in CH_2Cl_2 (15 mL) were added. The mixture was stirred for an additional 8 h and then poured into ice−water. The CH_2Cl_2 layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (20 mL). The combined CH_2Cl_2 layers were thoroughly washed with H_2O (25 mL × 4) and were dried (Na_2SO_4). Removal of the solvent gave a thick oily residue (4.23 g). This was purified by column chromatography (SiO_2, 25% EtOAc in petroleum ether) to give **6** as a viscous oil (2.67 g) and **7** as a semisolid (0.49 g). Compound **6**: ^{1}H NMR ($CDCl_3$) δ ppm 2.04 (s, 3H), 2.86 (d, 1H, J = 7.7 Hz), 3.34 (dd, 1H, J = 9.5, 6.7 Hz), 3.40 (dd, 1H, J = 9.3, 6.3 Hz), 3.77 (s, 3H), 4.47 (dd, 1H, J = 7.7, 1.9 Hz), 5.33 (dt, 1H, J = 2.1, 6.6 Hz), 7.20−7.45 (m, 15H). Anal. Calcd for $C_{26}H_{26}O_6 \cdot 0.25H_2O$: C, 71.14; H, 6.08. Found: C, 71.24; H, 6.21. Compound **7**: ^{1}H NMR ($CDCl_3$) δ ppm 2.03 (s, 3H), 2.25 (d, 1H, J = 7.7 Hz), 3.17 (m, 1H), 3.35 (m, 1H), 3.77 (s, 3H), 4.29 (m, 1H), 5.35 (d, 1H), 7.25−7.45 (m, 15H). Anal. Calcd for $C_{26}H_{26}O_6 \cdot 0.25H_2O$: C, 71.14; H, 6.08. Found: C, 71.12; H, 6.17.

Lactone 8 and 2-Deoxy-6-*O*-(triphenylmethyl)ascorbic Acid (9). To a solution of LHMDS in THF (1 N, 10 mL, 10 mmol) cooled to −78 °C was added dropwise a solution of **7** (1.1 g, 2.53 mmol) in dry THF (10 mL). The resulting light brown solution warmed gradually to rt, was stirred (3 h), was cooled to 0 °C, was acidified by dropwise addition of HCl (2.5 N, 8 mL, 20 mmol), and was extracted with EtOAc (40 × 2 mL). The combined organic layers were washed with H_2O (10 mL) and dried (Na_2SO_4). Removal of solvent gave a light yellow powder (1.5 g) that was purified by column chromatography (SiO_2, 50% EtOAc in petroleum ether) to afford compound **8** as a white powder (0.91 g, 90%), mp 62 °C, after resolidifying mp 143 °C; MS-EI m/z 402 (M^+); ^{1}H NMR (DMSO-d_6) δ ppm 3.35 (m, 2H), 3.92 (m, 1H), 4.44 (dt, 1H), 4.96 (s, 1H), 5.76 (d, 1H), 7.25−7.45 (m, 15H), 11.50 (s, 1H); ^{1}H NMR ($CDCl_3$) δ ppm 3.54 (m, 2H), 3.78 (s, 1H), 3.88 (s, 1H), 4.50 (m, 1H, [collapses to a doublet after deuterium oxide exchange, J = 7.6 Hz]), 4.74 (dt, 1H, J = 7.5, 2.1 Hz), 7.30 (m, 15H); ^{13}C NMR ($CDCl_3$) δ ppm 44.42, 59.92, 71.01, 89.65, 127.68, 128.27, 128.87, 142.78, 167.06, 200.53; $[\alpha]^{25}_D = +63$ (c 0.5, CH_3OH). Anal. Calcd for $C_{25}H_{22}O_5 \cdot 0.5H_2O$: C, 72.97; H, 5.63. Found. C, 72.73; H, 5.71. Further elution of the column gave **9** as a light yellow powder (45 mg, 5%): mp 139−141 °C; MS-EI m/z 402 (M^+); ^{1}H NMR

(DMSO-d_6) δ ppm 2.97 (t, 1H, J = 8.4 Hz), 3.08 (dd, 1H, J = 6.2, 2.6 Hz), 3.94 (m, 1H), 4.90 (s, 1H), 4.98 (s, 1H), 5.18 (br, 1H), 7.24−7.40 (m, 15H), 12.51 (s, 1H). Anal. Calcd for $C_{25}H_{22}O_5 \cdot H_2O$: C, 71.41; H, 5.75. Found: C, 71.09; H, 5.87.

2-Deoxy-L-ascorbic Acid (1) (from 5). To a stirred solution of LHMDS in THF (1 N, 32 mL, 32 mmol) cooled to −78 °C was added dropwise a solution of compound **5** (1.34 g, 7 mmol) in dry THF (20 mL). The resulting light brown solution was stirred (10 min), diluted with THF (50 mL), and warmed gradually to rt. During this period a solid formed. The mixture was stirred for 48 h, was cooled to −78 °C, and was poured into HCl (2.5 N, 29 mL, 72.5 mmol). The THF layer was separated. The aqueous layer was extracted with EtOAc (10 mL). The combined organic layers were washed with H_2O (10 mL). The combined two aqueous layers were extracted with THF until only trace amounts of product could be detected in the aqueous solution. THF was removed. The residue was redissolved in H_2O (10 mL). The solution was extracted twice with EtOAc (5 × 2 mL). The aqueous layer was separated and evaporated to dryness to give a light brown foam that was dissolved in MeOH and absorbed on SiO_2. Column chromatography (SiO_2,$CHCl_3$/MeOH/HOAc 100/5/1) gave the product as colorless crystals (210 mg, 18%): mp 172−174 °C; ^{1}H NMR (DMSO-d_6) δ ppm 3.41 (m, 2H), 3.73 (t, 1H, J = 7.2 Hz), 4.81 (s, 1H), 4.89 (s, 1H), 12.40 (s, 1H); ^{1}H NMR (D_2O) δ ppm 3.75 (d, 1H, J = 6.6 Hz), 4.10 (dt, 1H, J = 1.9, 6.6 Hz), 5.06 (d, 1H, J = 1.9 Hz); ^{13}C NMR (D_2O) δ ppm 63.12, 69.93, 80.88, 92.14, 179.25, 182.40; MS-EI m/e 160 (6, M^+), 100 (100); $[\alpha]^{25}_D$ = +38.8 (c 0.25, CH_3OH). Anal. Calcd for $C_6H_8O_5$: C, 45.00; H, 5.03. Found: C, 44.72; H, 5.05.

2-Deoxy-L-ascorbic Acid (1) (from 8). Compound **8** (400 mg, 1.0 mmol) was stirred (3 h) in THF (2 mL) and HCl (2.5 N, 2 mL) at room temperature. THF was evaporated, and H_2O (5 mL) was added. The mixture was extracted twice with EtOAc (2.5 × 2 mL) to remove TrOH. The aqueous layer was evaporated to a small volume (0.4 mL). Crystals were filtered off and were recrystallized from EtOH to give **1** (120 mg, 75%): mp 173−174 °C; $[\alpha]^{25}_D$ = +39.6 (c 0.25, CH_3OH). The ^{1}H NMR (DMSO-d_6) was identical to the spectrum of **1** prepared from **5**.

JO961247A