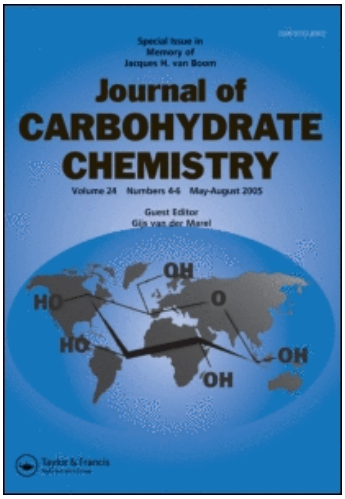

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Liang, Yun Teh S. , Liu, Xue Sheng and Seib, Paul A.(1990) 'Synthesis of D-Erythroascorbic Acid', Journal of Carbohydrate Chemistry, 9: 1, 75 — 84

To link to this Article: DOI: 10.1080/07328309008545798

URL: http://dx.doi.org/10.1080/07328309008545798

J. CARBOHYDRATE CHEMISTRY, 9(1), 75-84 (1990)

SYNTHESIS OF D-ERYTHROASCORBIC ACID

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Received July 1, 1989 - Final Form September 26, 1989

ABSTRACT

D-Erythroascorbic acid (**4**) was synthesized from D-glucose in four steps. Compound (**4**) and L-ascorbic acid (**5**) were lost at approximately equal rates from aqueous solution at pH 7 in the presence and absence of added cupric ion.

INTRODUCTION

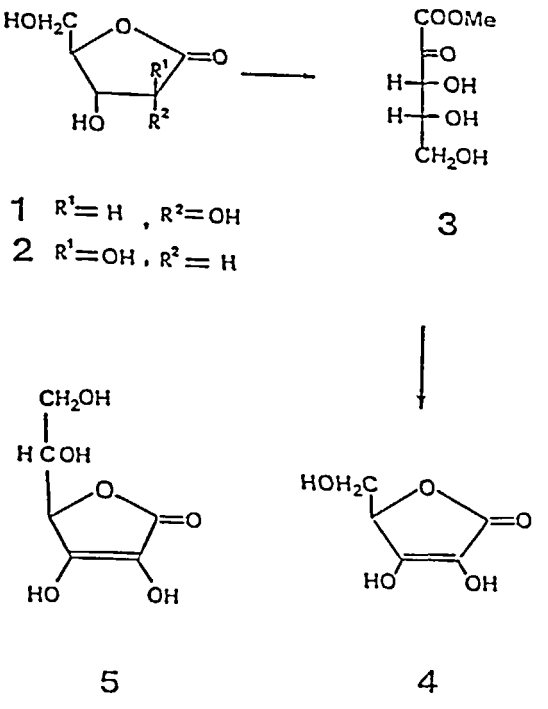
D-Erythroascorbic acid (**4**), which is the trivial name for D-*glycero*-2-pentenono-1,4-lactone, is closely related in structure to L-ascorbic acid (**5**). D-Erythroascorbic acid is found in the imperfect fungus *Candida*[1] and in the broth of *Serratia marcescens*[2]. The enantiomer, L-erythroascorbic acid, is formed during the catabolism[3] of **5** by purple sulfur bacteria and *in vitro* during the oxidative degradation[4] of **5**. One of the two enantiomers is prevalent[5-8] in ascomycete fungi, including *Saccharomyces, Lipomyces,* and *Neurospora*. Those yeasts contain little, if

any, L-ascorbic acid. Loewus[6,9] speculated that oxalate in fungi arises from the catabolism of erythroascorbate.

The synthesis of the L-enantiomer of **4** has been achieved by chemical and biochemical means. In 1934, Reichstein[10] converted L-*erythro*-2-pentulose in two steps to its 3,4-acetonated methyl glycoside. After permanganate oxidation of the primary alcohol to a carboxyl group, the blocked 2-keto-pentonic acid was heated in mild aqueous acid to give crystalline L-erythroascorbic acid. In 1961, Ashwell et al.[11] treated L-xylono-1,4-lactone-1-^{14}C with a suspension of rat liver microsomes at pH 7.5 in a pure O_2-atmosphere. The L-gulonolactone oxidase in the microsomes[12] catalyzed production of L-erythroascorbic acid, which was isolated by co-crystallization with chemically synthesized material. Finally in 1969, Yasuda[13] reported almost quantitative microbial fermentation (*Pseudomonas mildenbergii*) of D-xylose to D-*threo*-2-pentulosonic acid, which, upon heating to 100 °C in concentrated hydrochloric acid under nitrogen, gave a 50% yield of solid **4**. The objective of this investigation was to chemically synthesize the D-enantiomer (**4**) starting from D-glucose.

RESULTS AND DISCUSSION

A four-step synthesis (Scheme) starting from D-glucose was used to prepare D-erythroascorbic acid (**4**). D-Glucose was oxidized[14] to potassium D-arabinonate (78%) using oxygen in potassium hydroxide solution. After the potassium salt was acidified and lactonized, the lactone



SCHEME

(2) was converted in one step, albeit in a low yield (27%), to the 2-keto methyl ester (3). Finally, the ester (3) was tautomerized in hot methanolic sodium acetate, and upon removal of sodium, gave 50% D-erythroascorbic acid (4) as a crystalline solid.

The structure of the intermediate ester (3) was confirmed by ^{13}C-NMR. In methanol solution, 3 gave no detectable keto form but instead a 6:4 mixture of α - and β -furanoses calculated from the intensities of the C1 signals. Using the chemical shifts[15] of α - and β-D-psico-furanoses, it was possible to assign the signals of C-2, C-

3, and C-4 in the anomeric furanoses of 3. The signals of C-5 (α and β) were assigned by a DEPT experiment.

Any D-pentonolactone, of course, can be used in the Scheme to produce 4. We obtained the same yield of 4 starting from D-arabinono- and D-ribono-1,4-lactone (1 and 2). The availability of isotopically labeled D-glucose assures an array of labeled forms of 4 using the Scheme.

The D-enantiomer (4) crystallized from acetonitrile in two polymorphic forms with mp 135 °C and 162 °C, which are identical to the two polymorphs[10] of L-erythroascorbic acid. Compound (4) gave the expected specific rotation,[10] UV properties,[16] ionization constant,[17] and ^{13}C- and ^{1}H-NMR spectra.[18] In addition, Frank A. Loewus at Washington State University examined the pertrimethylsilylated ether of 4 by gas chromatography/mass spectroscopy and found that the major fragments (m/e 73, 103, 133, 147, 215-216, 257, 332, 347, and 362-molecular ion) were precisely the same as those observed[6] for erythroascorbic acid isolated from yeast and L-erythroascorbic acid prepared by Ashwell and coworkers.[11]

D-Erythroascorbic (4) and L-ascorbic acids (5) had similar stabilities in 3% metaphosphoric acid and in dilute phosphate buffer at pH 7.0. The first-order rate constants for losses of 4 and 5 were 1.2 and 1.4 h^{-1}, respectively. When the phosphate buffer was made 1 M in cupric ion, the rate constants for loss of 4 and 5 were 6.9 h^{-1} and 8.3 h^{-1}, respectively. Ferric ion (1 μM) did not accelerate the oxidation reaction. Less than 5% of 4 and 5 were lost

in 2 days at 25 °C in 3% metaphosphoric acid. Our data disagree with the observation of Reichstein[10] that L-erythroascorbic acid was much less stable in aqueous solution than L-ascorbic acid.

In one of our first attempts to purify **4**, the reaction mixture was chromatographed on a column of silica gel with chloroform/methanol (90/10, v/v) as developing solvent. The only compound eluted from the column was D-glyceric acid, which was identified by ^{13}C-NMR (data not given). This result suggests that **4** was oxidized to a mixture of oxalic acid and D-glyceric acid, in agreement[19] with the major oxidative pathway for L-ascorbic acid.

EXPERIMENTAL

General Methods. - Solutions were concentrated under reduced pressure below 50 °C. Thin-layer chromatography was done on silica gel plates, and carbohydrates were detected by spraying with 50% sulfuric acid and visualized by heating on a hot plate. Column chromatography was done using silica gel, 100-200 mesh, Grade 923 from Fisher Scientific, Fair Lawn, New Jersey. NMR spectra were recorded with a Bruker WM-400 instrument, and optical rotations on a Perkin-Elmer polarimeter, Model 241. Chemical shifts are expressed in ppm relative to internal reference of 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) in water or tetramethylsilane (TMS) in organic solvents.

D-Arabinono-1,4-lactone(2) and Methyl D-Arabinonate. The title lactone was prepared from D-glucose essentially as described by Humphlett.[14] Briefly, two gas dispersion

tubes were used to bubble oxygen through a solution of potassium hydroxide (112 g) in a mixture of water (240 mL) and methanol (1 L). The methanolic alkali was rigorously agitated at 30 °C while a solution of D-glucose (120 g) in water (240 mL) was added dropwise over a period of 2-3 h. After stirring for an additional 2 h, the oxygen supply was replaced by an air line, and stirring was continued 48 h. During that time potassium D-arabinonate crystallized from the mixture, and methanol lost by evaporation was replenished. The crystalline solid was recovered by filtration; yield 98 g (78%), mp 220-5 °C (dec).

The potassium salt (30 g) was suspended in methanol (75 mL), and the mixture saturated with hydrogen chloride using a dispersion tube for 30 min. After solid potassium chloride was removed from the hot solution, and after cooling, methyl D-arabinonate crystallized and was recovered by filtration. Yield 23 g (84%), mp 132-135 °C. Recrystallization from methanol gave pure material with mp 145 °C, Lit[14]: mp 143 °C.

To prepare D-arabinono-1,4-lactone (2), a solution of the potassium salt (20 g) in water (50 mL) was passed through a strongly acidic ion-exchange resin in the hydrogen form. The column effluent was collected, concentrated hydrochloric acid (1 mL) added, and the mixture concentrated to a syrup. Ethyl acetate (50 mL) was added three times, and after each addition the mixture was concentrated using a vacuum pump. The solid residue was recrystallized from a mixture of acetone and ethyl acetate to give 14 g (94 %) of 2 with mp 96-98 °C. Lit[20]: mp 96-98 °C.

Methyl D-Erythro-2-pentulosonate (3). The 2-keto ester was prepared using the method described by Regna and Caldwell.[21] To a solution of D-ribono- or D-arabinono-1,4-lactone (8.9 g) in a mixture of methanol (50 mL) and 85% phosphoric acid (0.25 mL) at 25 °C, sodium chlorate (3.7 g) and vanadium pentoxide (0.6 g) were added. The mixture was stirred at 18-25 °C for approximately 4 days, until the color changed from yellow to blue-green. The solid was removed, and the filtrate concentrated to a syrup (9.5 g). Thin-layer chromatography (chloroform/methanol, 4:1 v/v) showed, besides starting material (R_f 0.3), three new components with R_f 0.8, 0.62, and 0.50. The syrupy mixture was subjected to column (5 x 50 cm) chromatography on silica gel. Development of the column with 96/4 (v/v) (300 mL) and 95/5 (v/v) (250 mL) mixtures of chloroform/methanol eluted the two faster moving components (1.4 g). The desired product (3) with R_f 0.5 was eluted with chloroform/methanol (93/7, v/v). The yield of methyl D-erythro-2-pentulosonate (3) was 27% (2.9 g) from either D-arabinono- or D-ribono-1,4-lactone, but 11% from methyl D-arabinonate. $[\alpha]_D^{25} = -8.3^{\circ}$ (*c* 6.0, MeOH). ^{13}C-NMR (CD_3OD) δ 172.0 (C-1, α), 171.4 (C-1, β), 106.1 (C-2, β), 101.1 (C-2, α), 78.3 (C-3, β), 75.4 (C-3, α), 73.9 (C-5, β), 73.5 (C-5,) 71.4 (C-4, α and β), 53.8 (CH_3, α), 53.5 (CH_3, β).

D-Erythroascorbic Acid (4). To the 2-keto ester (3) (1 g) dissolved in dry methanol (100 mL) was added anhydrous sodium acetate (3 g), and the mixture was refluxed for 5 min. An aliquot (0.5 mL) of the reaction

mixture was made to volume (500 mL) with 6% aqueous metaphosphoric acid, and UV absorbance at 245 nm indicated a 78% conversion of 3 to 4. Strongly acidic ion-exchange resin (H-form), which had been solvent exchanged over several days with dry methanol, was added until the mixture was acidic (pH 2-3) to wet pH-paper. The resin was removed, and the filtrate concentrated to dryness using a vacuum pump. The solid residue was recrystallized from hot acetonitrile to give needles with mp 160-162 °C, $[\alpha]_D^{25}$ = -11 ± 1° (c = 5, H_2O). UV (water: pH ~ 1, 3% metaphosphoric acid), λ_{max} 243 nm, ϵ 8.4 X 10^3L mol^{-1} cm^{-1}; pH 7, λ_{max} 265 nm, = 14.6 x 10^3L mol^{-1} cm^{-1}. The change in λ_{max} with pH indicated[22] compound 3 had pK = 4.0. ^{13}C-NMR (water, pH 2) δ 178.8 (C-1), 160.5 (C-3), 123.5 (C-2), 82.8 (C-4), 65.0 (C-5); ^{13}C NMR (water, pH 8) 178.6 (C-1), 176.5 (C-3), 114.2 (C-2), 81.0 (C-4), 62.1 (C-5). ^{1}H NMR (D_2O, pH 8) δ 4.50 (q, H-4, $J_{4,5}$ = 2.6 Hz, $J_{4,5'}$ = 4.1 Hz), 3.97 (q, H-5, $J_{5,5'}$ = 12.7 Hz), 3.72 (q, H-5').

Anal. Calcd for $C_5H_6O_5$: C, 41.1; H; 4.11. Found: C, 41.0; H, 4.29.

Stability of D-Erythroascorbic Acid (4) in Aqueous Solution. A solution containing both 4 and 5 (each 0.05 mM) was prepared in 3% aqueous metaphosphoric acid. The mixture was held at 25 °C, for several days, and the losses of 4 and 5 were determined by high-performance liquid chromatography with electrochemical detection.[5,6,8] The retention times of 4 and 5 were 6.6 min and 6.1 min, respectively, using a mobile phase of 1:19 (v/v) methanol:

0.08M acetate buffer (pH 4.0) with 1.0 mM tetrabutylammonium phosphate and 0.1 mM EDTA together with a C-18 reverse-phase column at 35 °C.

The rates of losses of **4** and **5** (0.05 mM) also were followed in 0.05 M phosphate buffer (pH 7.0) at 25 °C in the presence and absence of 0.001 mM Cu(II) or Fe (II) over a period of several hours.

ACKNOWLEDGEMENTS

The authors thank Frank A. Loewus, Washington State University, for GC/MS measurements and helpful discussions, and Xiao Ying Wang for stability measurements. We also thank the Office of Energy Research, U.S. Department of Energy, for financial support.

Contribution No. 89-529-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS. 66506

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