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A NEW SYNTHESIS OF L-*threo*-HEX-2-ENARO-1,4-LACTONE ("SACCHAROASCORBIC" ACID): A METHOD FOR THE PROTECTION OF THE ENEDIOL OF ASCORBIC ACID

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ABSTRACT

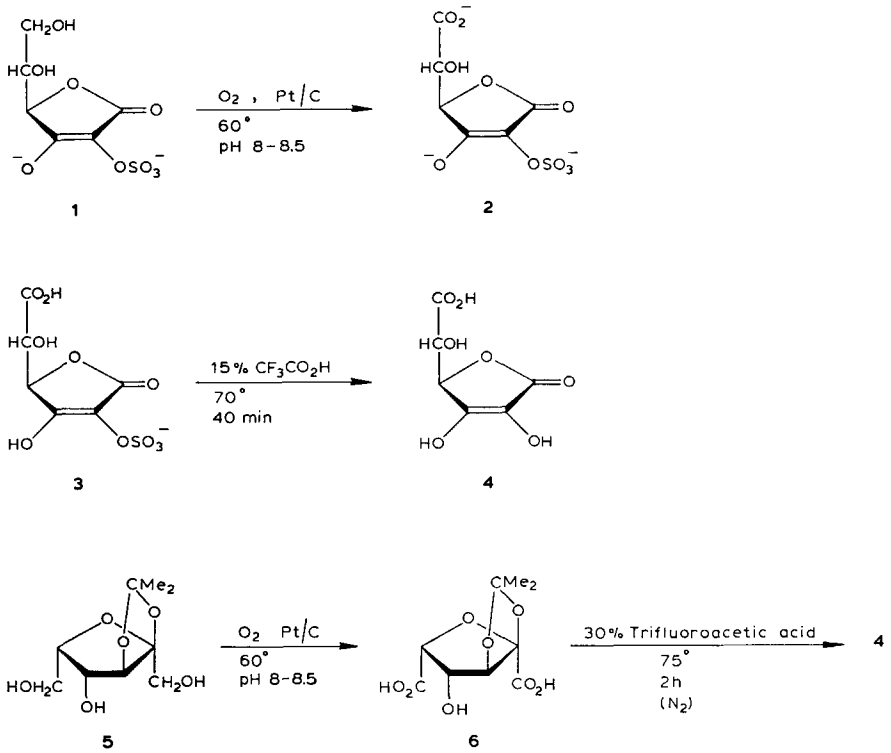
A new synthesis of L-*threo*-hex-2-enaro-1,4-lactone (**4**) ("saccharoascorbic acid") is presented, whose unique feature involves oxidation of the side chain of ascorbic acid. Ascorbate 2-sulfate (**1**) was selectively oxidized in water at pH 8–8.5 with platinum-on-carbon catalyst to yield the 2-sulfate (**3**) of **4**. Hydrolysis of **3** in 15% trifluoroacetic acid for 90 min at 70° yielded **4**. The procedure affords a useful preparation of **4**, and demonstrates the excellence of sulfation for protection of the enediol of ascorbic acid during synthetic manipulations of the side chain. The sulfated ring is stable to oxidizing agents and to base, yet sulfate is readily removed by acid hydrolysis. The properties of a new compound (**3**) of biological significance, and those of the previously uncharacterized **4**, are reported.

INTRODUCTION

The C-6 oxidized derivative of ascorbic acid, L-*threo*-hex-2-enaro-1,4-lactone[1] (**4**), was described in a 1947 patent by N. R. Trenner[2], who named the compound L-gulosaccharoascorbic acid. The patented synthesis involved oxidation of an *O*-isopropylidenesorbose derivative, with subsequent isomerization and lactonization. The product was identified only by its m.p. No further chemical or biological characterization of **4** has since been reported, although it is unique in being the only side-chain oxidized derivative of ascorbic acid to have been described.

Recent studies imply that C-6 oxidation of ascorbic acid is an important metabolic process[3] and compound **4** has been postulated as a metabolite of ascorbic acid[4], although **4** was not then available to verify this proposal. Hornig[5] has proposed that the major, polar metabolite of ascorbate 2-sulfate (**1**) observed in the urine of rats and guinea pigs is the 2-sulfate (**3**) of **4**. This compound has not been previously synthesized, nor has it been identified as a product from natural sources.

This paper presents a new synthesis of **4** in which **2** is prepared as an intermediate. Purification and characterization of both compounds is described. The synthesis introduces a procedure of potential general value for the protection and subsequent liberation of the labile ring-hydroxyl groups of ascorbic acid. We were



not able to obtain **4** by Trenner's patented procedure as written, and modifications of his synthesis are also outlined here.

EXPERIMENTAL

Preparation of potassium ascorbate 2-sulfate (K_2-**1**). — The salt K_2-**1** was prepared by a published procedure[6] developed in our laboratory.

Catalytic oxidation of **1**: *preparation of* **2**. — The potassium salt of **1** (K_2-**1**, (2.0 g, 6.0 mmol) was dissolved in 150 ml of doubly-distilled water and the pH adjusted to 8.5 with 0.5M potassium hydroxide. The solution was placed in a 250-ml, three-necked flask equipped with a paddle-type stirrer capable of 1700 r.p.m. (no load). A pH probe was placed in one neck of the flask and a buret filled with 0.5M potassium hydroxide was placed so that the base could be added dropwise during the reaction. The temperature was maintained at 60° by means of a water bath. Platinum-on-carbon catalyst (5%, 0.5 g, Matheson Coleman and Bell) was added to the solution and sweeping with oxygen (~0.5 l/min) was begun.

The pH decreased immediately, and was maintained between 8.0 and 8.5 by addition of 0.5M potassium hydroxide. The volume of alkali consumed in this manner was recorded. After 9 h, the reaction was stopped and the mixture was filtered through fine paper to remove the catalyst, which was recovered. The solution was

concentrated to 10 ml by rotary evaporation and centrifuged to remove the last traces of carbon. The pH was adjusted to 10 with potassium hydroxide, and the product was precipitated at 50° by addition of methanol. Filtration yielded 1.9 g of a non-hygroscopic powder. This crude product was dissolved in 10 ml of double-distilled water and warmed to 50°. Methanol was added dropwise until the stirred solution remained cloudy. Crystallization occurred on continued stirring, with cooling to room temperature. Filtration yielded colorless, non-hygroscopic crystals of the tripotassium salt of **2**; yield 1.75 g (70%), m.p. 240–245° dec., λ_{max} 254 nm (ε 16,800) at pH 6.86.

Anal. Calc. for $C_6H_3K_3O_{10}S \cdot 2H_2O$: (420.1) C, 17.13; H, 1.67; S, 7.64. Found (Huffman Laboratories): C, 17.21; H, 1.86; S, 7.31.

This oxidation was also performed as before but with 10.0 g of potassium **1** in 200 ml of water, to yield 7.5 g (60%) of tripotassium **2**, m.p. 240–245° dec.

Analytical methods for and properties of **2**. — Column chromatography on Whatman DE-32 *O*-diethylaminoethyl) cellulose (sulfate form) was used as the primary assay for the purity of **2**. Elution was achieved by a pumped gradient of sulfuric acid, and the u.v. absorbance of the eluate was monitored in a flow cell. The recrystallized products showed no impurities registering >1% of the product peak on the u.v. monitor printout. The column separated **1** and **2**. Systems were developed for the separation of **1** and **2** by t.l.c.

Solvent	*T.l.c. media*	R_f**1**	R_f**2**
150:75:25 Propanol–ammonia–water (A)	Bakerflex F cellulose	0.60	0.45
150:75:25 Propanol–ammonia–water (A)	Eastman 6060 silica gel	0.59	0.47
150:75:50 Ethyl acetate–acetic acid–water (B)	Eastman 6060 silica gel	0.22	0.17

Solvent *A* on cellulose plates was the most convenient of these systems, as the same solvent developed very slowly on silica gel plates.

The K_3-**2** and K_2-**1** salts were shown to be quite stable in aqueous solution at room temperature. Solutions of 10 mg of K_3-**2** and K_2-**1** in 4 ml of water were stored in stoppered flasks for 48 days. T.l.c. of the stored solutions showed no sign of decomposition. The solutions were not discolored, and the chromatograms showed no other spots or streaking, nor any sign of material at the origin or solvent front.

The CO_2H infrared absorption in K_3-**1** did not appear as a separate band. The group is present as a carboxylate anion, and its primary absorption caused by asymmetric stretching occurs at 1610 cm^{-1}, and thus is a part of the large C-2–C-3 double-bond stretching absorption shown by both compounds at 1620 cm^{-1}. The less-intense, symmetric, stretching absorption of the carboxylate anion does occur at 1400 cm^{-1}. Assignments for K_3-**2** and K_2-**1** are the same: 3520 (O-H), 3200–3400

broad (O-H), 1720 (lactone C=O), 1620 (C=C), and 1240 cm^{-1} (sulfate S-O stretching).

The salt K_3-**2** was quite soluble in water: 7.5 g dissolved in 10 ml of water at room temperature, but it was virtually insoluble in methanol, ethanol, chloroform, acetonitrile and acetone. Aqueous solutions of **2** gave a brick-red color in the presence of ferric ions indistinguishable from the color given by **1**. The intensity of the color makes this a useful spot test for the detection of the compounds. The salt K_3-**2** does not reduce 2,6-dichloroindophenol and gives a negative Tollens' test.

Acid-catalyzed hydrolysis of **2**: *preparation of* **4**. — The K_3 salt of **2** (2.40 g, 5.71 mmol) was dissolved in 10 ml of doubly-distilled water and passed through a 2.5 × 50 cm column of Dowex-50 resin (H^+ form, 20–40 mesh), with elution by distilled water. The fractions containing **2** were collected (volume = 40 ml). This solution was placed in a tube equipped with a nitrogen inlet, consisting of a Teflon tube inserted to the bottom of the solution, and the tube was then placed in a water bath at 75°. Sweeping with nitrogen was begun and 7 ml of trifluoroacetic acid was added. Sweeping with nitrogen was maintained throughout the reaction. Aliquots of 25 μl were removed at intervals and added to 10 ml of pH 6.86 buffer, and then titrated rapidly with standardized mM 2,6-dichloroindophenol.

A 1.0 mM solution of 2,6-dichloroindophenol was prepared from 0.145 g of its sodium salt in 500 ml of doubly-distilled water. Standardization was achieved by titration with three weighed samples of reagent-grade L-ascorbic acid. The endpoint in standardization and in the assay was judged to be the appearance of the first permanent blue color.

After 90 min the reaction was complete as determined by titration with 2,6-dichloroindophenol. The solution was chilled in ice and 1.12 g (5.71 mmol) of barium carbonate were added carefully (CO_2 evolved). After stirring for 10 min, the suspension was filtered through a medium-pore glass filter to remove precipitated barium sulfate. Evaporation to a syrup, addition of abs. ethanol (25 ml) and further evaporation to a syrup was followed by drying under vacuum. Crystallization from acetonitrile–chloroform gave a non-hygroscopic solid. Recrystallization from the same solvents yielded **4** as a white, microcrystalline solid; yield 0.70 g (64%), m.p. 212–215 dec., (lit.[2] 206–210°); λ_{max}^{KBr} 3530, 3300 broad, 1760, 1710, 1660 cm^{-1}; λ_{max} 266.5 nm (ε 10,200) at pH 6.86.

Anal. Calc. for $C_6H_6O_7$: C, 37.88; H, 3.15. Found (Huffman Laboratories): C, 38.24; H, 3.10.

Analytical methods for and properties of **4**. — Column chromatography on *O*-(diethylaminoethyl)cellulose (sulfate form) was used as the primary assay for the purity of **4**. Elution was achieved by pumped gradients of sulfuric acid, and the u.v. absorption of the eluant was monitored with a flow cell. The recrystallized products showed no impurities registering above 1% of the product peak area on the u.v. monitor printout. A small amount of an impurity in the crude product appeared as a peak at the void volume. The column separated **4** from ascorbic acid.

The identity of **4** as just obtained with that from Trenner's synthesis was demonstrated by chromatographic identity, their identical i.r. spectra, and an un-

changed mixed m.p. The carbonyl-stretching vibration in the i.r. spectrum of the carboxylic acid group in **4** appears at 1710 cm^{-1}, well distinguished from the 1760 cm^{-1} (lactone C=O) and 1660 cm^{-1} (C=C) absorptions seen for ascorbic and **4**. The acid **4** is quite soluble in water and shows appreciable solubility in acetonitrile. It is less soluble in methanol and ethanol, and is insoluble in chloroform. It reduces 2,6-dichloroindophenol solutions.

Preparation of 2,3-O-isopropylidene-α-L-sorbofuranose (**5**). — Finely ground L-sorbose (30 g, 0.17 mol) was added to 500 ml of reagent-grade acetone in a 1-liter round bottom flask. Concentrated sulfuric acid (30 ml) was added during 0.5 h to the stirred suspension. The temperature remained at 25 ± 3° and the sorbose did not completely dissolve in this time. After an additional h of stirring the solution was cooled in ice and neutralized with 50% sodium hydroxide, keeping the temperature below 20°. The precipitated sodium sulfate was filtered off and the solution concentrated (rotary evaporator) to remove acetone, the final volume being 80 ml.

The solution was poured into a solution of 40 g of concentrated sulfuric acid in 220 ml distilled water. This solution was stirred for 1 h at room temperature and then cooled with ice and neutralized with 50% sodium hydroxide at no warmer that 25°. Rotary evaporation to a syrup (volume ~25 ml) and extraction with four 30-ml portions of ethyl acetate was followed by concentration of the ethyl acetate extract to 60 ml. Compound **5** crystallized readily from this solution on standing, forming large white to transparent crystals, yield 7.6 g (20%); m.p. 92–93° (Fisher–Johns); λ_{max}^{KBr} 3450 broad (O-H), 2900 (C-H), 1390 and 1380 cm^{-1} (*gem*-dimethyl, C-H bending).

Preparation of 2,3-O-isopropylidene-α-L-xylo-hexulosaric acid (**6**). — Compound **5** (4 g, 18 mmol) was dissolved in 150 ml of double-distilled water. The pH was adjusted to 8.5 with potassium hydroxide and 0.5 g of platinum-on-carbon (Matheson Coleman and Bell) was added. During the reaction, the pH was maintained at 7.5 to 8.5 with 0.5M potassium hydroxide, and base consumed in this manner was recorded. The reaction temperature was maintained at 60°.

When consumption of base stopped, the hot suspension was filtered through fine filter-paper, yielding a colorless solution. No discoloration nor decrease in yields were observed in reactions conducted for up to 60 h. The solution was evaporated to 25 ml, chilled to 0°, and acidified to pH with cold, M hydrochloric acid. Extraction with four 30 ml portions of ethyl acetate and evaporation of the ethyl acetate fraction to 30 ml gave a solution from which the product **6** crystallized on being kept; yield 2.0 g (44%), m.p. 202–204° dec.; λ_{max}^{KBr} 3480, 1735 (C=O), 1380 and 1390 cm^{-1} (*gem*-dimethyl C-H bending).

Acid-catalyzed conversion of **6** into **4**. — A solution of 1.0 ml trifluoroacetic acid in 2.0 ml of double-distilled water in a test tube was placed in a water bath at 75°. Nitrogen was bubbled through the solution for 10 min prior to and during the course of the reaction by means of a Teflon tube extending to the bottom of the solution. Compound **6** (100 mg) was added, and it dissolved immediately. During

the reaction, 50 μl aliquots were removed and assayed for **4** by titration with 2,6-dichloroindophenol.

The 50-μl aliquots were added to 15 ml of pH 6.86 buffer and rapidly titrated with 2,6-dichloroindophenol. After 2.3 h, the reaction was complete, as determined by titration with 2,6-dichloroindophenol and the darkened solution was chilled with ice. Evaporation of the solution to ~0.5 ml, followed by addition of 1 ml of water and evaporation, gave a syrup that was vacuum-dried overnight to complete removal of trifluoroacetic acid. This material was dissolved in 2 ml of hot acetonitrile. Chilling precipitated **4** as a solid that was filtered off and dried; yield 21 mg (25%), m.p. 190–195° dec.; λ_{KBr}^{max} 3530, 3300, 1760, 1710, 1660 cm^{-1}. The solid from the dark acetonitrile solution was slightly tan in color.

Crude products thus obtained were recrystallized from acetonitrile to yield **4** as a white, microcrystalline solid, m.p. 210–214° dec.; average yield of recrystallized product was 28%.

DISCUSSION

The synthesis described in this paper gives L-*threo*-hex-2-enaro-1,4-lactone (**4**) in 45% yield from potassium ascorbate 2-sulfate (K_2-**1**). The preparation of K_2-**1** is a direct procedure, giving the product in 90% yield from ascorbic acid; this salt is also commercially available[7]. The synthesis of **4** requires only three steps from ascorbic acid, and it is not necessary to purify the K_3-**2** intermediate, as it is the sole product detected from the catalytic oxidation. The higher yield and ease of product isolation of the method are largely a result of the absence of an enediol-lactone, ring-closure step during the synthesis.

This procedure is the first reported synthetic oxidation of the side chain of ascorbic acid. The difficulty of performing any synthetic manipulation on the side chain of ascorbic acid arises from the presence of four reactive hydroxyl groups which must be distinguished chemically, and from the lability of the enediol to quite mild oxidizing agents. No method has previously been reported for the protection of the enediol during such a synthesis. The sulfation of ascorbic acid is an excellent method for the protection of the enediol ring as: (*a*) sulfate may be selectively introduced at the 2-position, (*b*) introduction proceeds in very high yield (90%), (*c*) compound **1** is stable to mild oxidation and to basic hydrolysis, and (*d*) sulfate is readily removed in excellent yield to give aqueous solutions of the modified ascorbate and readily precipitated sulfate ions.

The stability to atmosphere oxidation imparted to the enediol ring by protection as **1** is clearly demonstrated by this work. In contrast, ascorbic acid itself is rapidly oxidized, probably to the unstable dehydroascorbic acid, by the conditions of the catalytic oxidation. Compound **1** is stable to hydrolysis from pH 4–13, although at pH 4 it is slowly hydrolyzed and at pH 3 and below it is rapidly hydrolyzed.

There are limits to the stability of **1** to oxidizing agents. The oxidative desulfation of **1** in water by bromine was first observed by Ford and Ruoff[8]. The reaction

also occurs in *N,N*-dimethylformamide[9]. Treatment of the 5,6-isopropylidene acetal of **1** with *m*-chloroperoxybenzoic acid and 2,3-dichloro-5,6-dicyanobenzoquinone was reported to result in desulfation[10].

It is possible that methods can be developed for the use of other protecting groups. The total insolubility of K_2-**1** in nonpolar and most polar solvents is a limitation that led us to seek an uncharged, nonpolar protecting group. Attempts were made to prepare the benzyl, methoxymethyl, and tetrahydropyranyl ethers of the ascorbate enols, but pure products were not isolated. The methoxymethyl and tetrahydropyranyl ethers should show acid-labile, base-stable properties, similar to that of ascorbate sulfate, whereas the benzyl ether might be stable to both mild acids and bases. Previously reported derivatives of ascorbic acid are not promising as protected forms of the molecule. The 3-methyl and 2,3-dimethyl ethers are hydrolyzed under rather severe conditions, and the 3-esters are rapidly hydrolyzed by neutral aqueous solutions[11]. 2-*O*-Acyl esters should be more stable, and might be useful as mild-acid stable, base-labile, protected forms of ascorbic acid.

The major modification made in repeating Trenner's synthesis was the use of 30% trifluoroacetic acid in the final isomerization in place of the concentrated hydrochloric acid reported in the patent. Attempts to carry out this conversion with concentrated hydrochloric acid resulted in decomposition of the intermediate. New conditions, using trifluoroacetic acid, were developed and optimized by monitoring the reaction through titrations with 2,6-dichloroindophenol. The product was obtained and recrystallized from acetonitrile instead of from 20% hydrochloric acid.

The catalytic oxidations were monitored by recording the volume of alkali required to maintain the pH at 8.5. One equivalent of acid is formed in the oxidation. The rates were initially very rapid, and then were followed by a slow approach to completion. Heyns[12] reported that such complex kinetics are normal for these oxidations. The reason for this behavior is not known, but a reversible inhibition, not poisoning, of the catalyst seems probable. Re-use of the catalysts in our reactions resulted again in rapid, initial rates, followed by a slow approach to completion. The catalysts were re-used in up to eight reactions.

Heyns and Paulsen have reviewed the selective oxidation of organic compounds[13] and specifically carbohydrates[12,14] up through 1962. The selectivity of the oxidation for the primary hydroxyl group of **1** is in accordance with other results in the literature. It was of interest to know whether the hydroxyl group at C-5 could be slowly oxidized to a ketone under the conditions of the catalytic oxidation. Extended exposure of K_3-**2** to the conditions of the catalytic oxidation resulted in formation of small proportions (5%) of a new, u.v.-absorbing product detected by column chromatography. The identity of the product is not known. We have found no report of the catalytic oxidation of an α-hydroxy acid to an α-keto acid, the most nearly analogous system being the conversion of an α-hydroxylactone to an α-ketolactone[15].

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