[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Synthesis of L-Ascorbic-1-C^{14} Acid from D-Sorbitol[1]

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L-Ascorbic-1-C^{14} acid has been prepared from D-sorbitol *via* L-xylose, L-xylosone and the cyanohydrin reaction, giving a 70% yield based upon cyanide-C^{14}.

The synthesis of ascorbic acid on a semi-micro scale presents unusual difficulties due to the lability of the vitamin. However, the cyanohydrin method[2,3,4] was successfully adapted to an isotopic synthesis[5] which has been carried out repeatedly in these laboratories. Scarcity of the starting material, L-xylose, and the continued interest in the labeled vitamin for biochemical investigations prompted us to examine methods leading from D-sorbitol to L-ascorbic-1-C^{14} acid. The resultant modifications, as presented, permit a rapid and convenient synthesis of the compound, giving an active product in good yield from the commercially available D-sorbitol.

Experimental

L-Xylose.—L-Xylose was synthesized from D-sorbitol via the 2,4-benzylidene derivative by a modification of the method of Vargha.[6] The crude monobenzylidene derivative was washed with 5% sodium carbonate solution, with ice-water, and finally with ethyl ether. One recrystallization from an excess of ethanol by slow evaporation yielded pure monobenzylidene D-sorbitol, m.p. 172–173° (cor.). The powdered material (5 g.) was heated with 30 ml. of glacial acetic acid to 50°. Lead tetraacetate (8.2 g.) as determined by titration[7,8] in 30 ml. of glacial acetic acid at 50°, was added in one portion. After shaking for 5 minutes, the clear solution gave a negative starch–iodide paper test. The solvent was thoroughly removed *in vacuo*, at 30°. The sirup was triturated with ethyl acetate while being warmed and lead salts were filtered off. After removal of the solvent, hydrolysis with 15% acetic acid under reflux for 1 hour, concentration *in vacuo* to a glassy sirup, and two crystallizations from glacial acetic acid, colorless crystals of L-xylose were obtained. Seeding was unnecessary; m.p. 142–143.5° (cor.), $[\alpha]^{20}D$ −93.2 (initial rotation by extrapolation, −19.2 at equilibrium), (H_2O, *c*, 7.9); yield 82–85%.

Anal. Calcd. for $C_5H_{10}O_5$: C, 40.0; H, 6.71. Found: C, 40.3, 40.2; H, 6.71, 6.72.

L-Xylosone.—L-Xylosone was synthesized by modification of a method previously described.[9] The direct oxidation of L-xylose with cupric acetate afforded much higher yields of the osone than obtained by the cleavage of osazones. Optimum conditions for the synthesis of L-xylosone were found to be as follows: 1.6 g. of L-xylose was dissolved in 7 ml. of water. To this, 100 ml. of methanol and 7 g. of powdered cupric acetate monohydrate were added. The solution was slowly refluxed in a flask fitted with a condenser and a mercury-sealed stirrer for 20 minutes. After cooling, the cuprous oxide was removed by filtration. The excess cupric acetate was removed by passage of the methanolic solution through a 2.5 × 50-cm. column containing a 30-cm. bed of Amberlite IR-120.[10] The resin had been previously converted to the hydrogen form by washing with 1.5 *N* hydrochloric acid, followed by water and thorough washing by upflow with methanol. The latter step eliminated formation of bubbles upon contact of the resin with the reaction mixture. The use of the cation column insured the convenient removal of all cupric ion from the L-xylosone solution. All of the cupric ion must be removed because of the effect it would have later in catalyzing the oxidative decomposition of the ascorbic acid.

After passing through the column, the osone solution was evaporated *in vacuo* under nitrogen to a foamy semi-solid. This material was then dissolved in 15 ml. of water and the concentration of L-xylosone in the solution was determined as follows. A 0.5-ml. aliquot of the osone solution was diluted with 3 ml. of water. After passing nitrogen through the solution for 10 minutes a threefold excess of sodium cyanide (20 mg.) was added to react with the L-xylosone, forming imino-L-ascorbic acid. Nitrogen was bubbled through the solution for an additional 20 minutes. The solution was then acidified with hydrochloric acid and the excess cyanide removed by a stream of nitrogen. The concentration of imino-ascorbic acid in this solution was determined by titration of a convenient aliquot with indophenol dye which had been standardized against ascorbic acid. The results of the above method indicated that L-xylosone was obtained from L-xylose in 40 to 50% yield.

Ascorbic-1-C^{14} Acid.—L-Xylosone (600 mg., 4.0 mmoles) in 15 ml. of water was added dropwise over a 20-minute period to an equal volume of a solution containing 3 mmoles of labeled sodium cyanide[11] (3 mc. of C^{14}) and 6 mmoles of sodium hydroxide. The reaction was carried out in a 125-ml. erlenmeyer flask provided with a magnetic stirrer, dropping funnel, and inlet and outlet tubes for nitrogen which did not extend into the liquid. Provision was also made for sealing the flask completely by means of stopcocks. A slow stream of oxygen-free nitrogen was passed through the reaction vessel and a trap, into a flask containing 50 ml. of 2.5 *N* sodium hydroxide. Ten minutes after completion of the addition, sufficient concentrated hydrochloric acid was added to the reaction mixture to make the solution 2.5 *N*, followed by 600 mg. of carrier ascorbic acid. In trial runs, cyanide addition was found to be quantitative under these conditions, as determined by indophenol titration. The stopcocks were then closed and the flask was placed in a water-bath at 50° for 24 hours to hydrolyze the imino-L-ascorbic acid. At the end of this period nitrogen was again passed through the flask and the radioactive carbon dioxide (formed by decarboxylation of ascorbic acid) was collected in the trap.

The solution after hydrolysis was evaporated under nitrogen *in vacuo* at 30°. The sirupy residue was dissolved in 100 ml. of water and passed through a 2 × 50-cm. column which contained a 30-cm. bed of Amberlite IR-4B in the acetate form.[12] The ascorbic acid was eluted from the column with 1 *N* hydrochloric acid, and fractions containing no ascorbic acid, as determined by titration, were discarded. Ascorbic acid was recovered almost quantitatively through this ion-exchange purification step. The use of an anion column in the acetate form instead of in the base form, as

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(11) Purchased from Tracerlab, Inc., Boston, Massachusetts.

(12) Amberlite IR-4B resin,[10] conditioned by the method of S. S. Jackel, E. H. Mosbach and C. G. King, *Arch. Biochem.*, **31**, 442 (1951), was converted to the acetate form by upflow with 10% acetic acid. The column was then washed with water and saturated with inactive ascorbic acid. The adsorbed anions were removed with 2 *N* ammonium hydroxide and the resin reconverted to the acetate form.

previously reported,[5] made unnecessary the prior use of a cation column.[13]

The eluate from the anion column was evaporated at 30° *in vacuo* under nitrogen. The pale yellow sirup was dissolved in 30 ml. of absolute alcohol and 400 ml. of peroxide-free absolute ether was added. A small amount of flocculent precipitate was removed by filtration. The clear filtrate was evaporated under nitrogen. The resulting crystals of ascorbic acid were washed with 1:1 absolute ethanol–redistilled ligroin until the supernatant was no longer colored, followed by an additional wash with absolute ether.

The colorless crystals of ascorbic acid (667 mg.) had an absolute activity of 2.6 μc./mg. when counted as barium carbonate, which represented a yield of 58% based on the radioactive sodium cyanide. Additional amounts of less active material were recovered from the ether precipitate and supernatants by adding carrier ascorbic acid. The over-all yield of ascorbic acid based on radioactive sodium cyanide was 70%. The ascorbic acid was pure, as measured by indophenol dye titration, and melted at 187–189°. The specific activity remained constant during recrystallization and preparation of the isopropylidene derivative. Analysis of the product from a trial synthesis with non-radioactive cyanide, after one crystallization from glacial acetic acid, gave the following results.

Anal. Calcd. for $C_6H_8O_6$: C, 40.9; H, 4.58. Found: C, 40.8, 40.8; H, 4.69, 4.56.

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(13) Subsequent work has resulted in a further improvement in the isolation procedure, to be published later.

[CONTRIBUTION FROM THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

Synthesis of Ascorbic Acids by the Osone–Cyanide Method[1]

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Conversion of sugars into the corresponding osones is readily brought about by the action of alcoholic cupric acetate. Without further purification, these osones may be converted into ascorbic acids by condensation with potassium cyanide followed by hydrolysis with dilute mineral acid. If carried out at 95° rather than 45–50° as formerly prescribed the hydrolysis can be completed in 3 hours instead of 1–2 days.

Vitamin C (L-xyloascorbic acid) is essential for the prevention of scurvy[2] but little is known of its actual role in biochemical systems: In attempts to throw light on this problem, experiments devised by Dr. C. D. May and his associates required D-(levo)-ascorbic acid (the enantiomorph of vitamin C).[3] By investigating the effect of this compound which has no antiscorbutic activity[4] it seemed likely that information would be forthcoming which would indicate whether D-ascorbic acid, with the same oxidation–reduction potential as vitamin C, could be utilized by an animal in certain specific biological oxidation–reduction systems.

The work reported herein deals with a simple synthesis of D-xyloascorbic acid, a substance that is virtually unobtainable at the present time. The method finally adopted for this purpose, which involves the reaction of a sugar osone with alkali cyanide, can also be used for the preparation of vitamin C or any of its analogs, with radioactive carbon at C_1. When osones are available this reaction, first used by Haworth and his associates,[5] provides an excellent route to the corresponding ascorbic acids. Hitherto, the methods normally used for preparing osones, such as the action of fuming hydrochloric acid on hexose phenylosazones[6] or benzaldehyde on pentose phenylosazones[5,7,8] provide at best poor yields and consequently the availability of certain ascorbic acids is thereby greatly restricted. This is true in spite of alternative methods for synthesizing ascorbic acid and its analogs.[9–12]

Osones are said to be obtainable in yields of 40–60% by heating an aldose or ketose with cupric acetate in methanol.[13] This is by far the best method for making osones but it does not seem to have received the recognition that it deserves.

When D-xylose is oxidized with cupric acetate a 50–55% yield of D-xylosone is readily produced and this upon condensation with potassium cyanide in aqueous solution immediately gives the corresponding imino-D-xyloascorbic acid. Formerly, the latter was converted into the corresponding D-ascorbic acid by hydrolysis with dilute mineral acid for 40–48 hr. at 40–50°.[5] It is shown herein that when carried out at 95–100° the reaction is complete in 3 hours and good yields of D-ascorbic acid (m.p. 192° dec., $[\alpha]_D$ −23° in water) can readily be isolated. By using the cupric acetate method for preparing osones and allowing the latter to react with $NaC^{14}N$, ascorbic acid and its analogs labeled at C_1 with radioactive carbon become readily accessible.[14]

Experimental

D-Xylosone.—To a solution of D-xylose (12 g.) in water (30 ml.), methanol (750 ml.) and cupric acetate (60 g.) were added. The mixture was quickly brought to the boil, refluxed for 10 minutes, cooled and filtered to remove cuprous oxide. The copper acetate in the filtrate was precipitated by hydrogen sulfide and after adding charcoal the solution was filtered and concentrated *in vacuo* at 35–40°. The D-xylosone was obtained as a colorless sirupy substance.[13] It

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