The Synthesis of L-Ascorbic-C_6^{14} and L-Ascorbic-6-C^{14} Acid[1]

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The synthesis of L-ascorbic acid, labeled with C^{14} in the carboxyl carbon,[2,3] which was accomplished by addition of sodium cyanide-C^{14} to L-xylosone, has made possible experiments leading to the elucidation of some aspects of the fate of Vitamin C in laboratory animals.[4] The procedure reported in this note for preparing uniformly C^{14} labeled L-ascorbic acid from uniformly C^{14} labeled D-glucose, has made possible experiments in which the metabolism of the entire carbon chain of the vitamin was compared to the metabolism of the carboxyl carbon.[5]

To prepare L-ascorbic-C_6^{14} acid and L-ascorbic-6-C^{14} acid (prepared from D-glucose-1-C^{14}), the method of Bothner-By, Gibbs, and Anderson[6] was modified for greater ease and yields were tripled. For the acetonization of L-sorbose-C^{14} the method of Reichstein and Grüssner[7] was modified as suggested by others[8,9,10] in order to take advantage of higher yields obtainable by allowing the reaction to proceed at a low temperature. The resulting crude diacetone L-sorbose-C^{14} was oxidized with potassium permanganate, while impurities were preferentially oxidized to non-interfering substances. The resulting crude potassium diacetone 2-keto-L-gulonate-C^{14} was directly transformed to L-ascorbic-C^{14} acid by a modification of the method of Elger[11] and others.[12,13] Purification was achieved by an anion exchange technique previously reported.[2,3] The uniformly labeled 2-keto-L-gulonic acid which was readily obtained from the corresponding diacetone derivative was used in studies of the biosynthesis of the vitamin.[14]

EXPERIMENTAL

D-Sorbitol-C^{14}. D-Glucose-C^{14} was reduced with Raney nickel[6] and D-sorbitol-C^{14} purified *via* the pyridine derivative.[15] The latter tracer was also obtained by the method of Karabinos and Ballun.[16, 17] Nickel, which interferes with the subsequent bacterial step, was removed by a cation exchange column (Amberlite IR 120).

L-Sorbose-C^{14}. The D-sorbitol-C^{14} was oxidized using *Acetobacter suboxydans.*[6] The bacteria[18,19] were grown in an aqueous broth consisting of 10% D-sorbitol and 1% yeast extract. They were collected by centrifugation, washed with five times their volume of water, then added to a 10% solution of D-sorbitol-C^{14} and incubated overnight at 30°. The organisms were removed by centrifugation and L-sorbose-C^{14} was measured spectrophotometrically,[20] and an equal amount of carrier was added. Following evaporation *in vacuo*, the diluted L-sorbose-C^{14} was recrystallized from water-ethanol whereupon white crystalline material was obtained, m.p. 162° (yield 60–80%).

Diacetone L-sorbose-C^{14}. In a 65-ml. flask 500 mg. of L-sorbose-C^{14} was finely pulverized with a glass rod and a cooled mixture of 25 ml. of dry, freshly distilled acetone and 1 ml. of concentrated sulfuric acid was added. The stoppered flask was cooled at 5–10° with occasional gentle shaking. Every 30 minutes the flask was removed from the cooling bath and allowed to stand at room temperature for 5 minutes. After 2–3 hours of such treatment most of the starting material had dissolved. The mixture then was placed in the icebox for 18–20 hours. The resulting yellow, acid solution was added in small portions to a solution of 4.00 g. of potassium carbonate in 30 ml. of water, while stirring, and cooling the flask in an ice-bath. Then 100 ml. of acetone was used to rinse the original flask which was added to the mixture. The precipitated salts were removed by suction filtration and washed with additional acetone. The washings and the filtrate were combined and the acetone was removed *in vacuo*. The remaining aqueous solution was extracted seven times with 50-ml. portions of ether and the ethereal solution was dried over potassium carbonate. The solution was filtered and was evaporated to a yellow, viscous oil by a stream of nitrogen. Two ml. of ligroin (b.p. 66–75°) were added and the solvent was removed *in vacuo;* this last step was repeated. Crystallization was induced by seeding and placing under a vacuum, affording 602–649 mg. light yellow, crude diacetone L-sorbose-C^{14} m.p. 75–76° (yield 81–89%).

Potassium diacetone 2-keto-L-gulonate-C^{14}. In 7 ml. of cold $4^1/_2$% potassium hydroxide diacetone-L-sorbose-C^{14} was dissolved and, while stirring and cooling at 5–10°, 0.95 times its weight of potassium permanganate in 20–25 ml. of water was added dropwise during $1–1^1/_2$ hours. After stirring 4 additional hours at room temperature, the solution was left standing overnight in the icebox. The temperature

(1) This investigation was supported in part by the Josiah Macy, Jr. Foundation.

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(15) Stetten and Stetten, Jr., *J. Biol. Chem.*, **193**, 157 (1951).

(16) Karabinos and Ballun, *J. Am. Chem. Soc.*, **75**, 4501 (1953).

(17) Obtained from Tracerlab Inc., 130 High Street, Cambridge, Mass.

(18) (a) Burns, Mosbach, Schulenberg, and Reichenthal, *J. Biol. Chem.*, **214**, 207 (1955); (b) Porter, *Bacterial Chemistry and Physiology*, Wiley & Sons, Inc., New York N. Y., 1955, pp. 980–982.

(19) The culture of *Acetobacter suboxydans* #621 was obtained from the American Type Culture Association, 2029 M. Street, N. W., Washington 6, D. C.

(20) Higashi and Peters, *J. Lab. and Clin. Med.*, **35**, 475 (1950).

then was raised to 50° for 15 minutes and 25 ml. of ethanol was added. The precipitated manganese dioxide was removed by suction filtration and washed first with hot water, then with ethanol. The filtrate and washings were combined and flushed with carbon dioxide to bring the solution to *pH* 8 and then evaporated to dryness *in vacuo*. The organic material in the residue was extracted with hot absolute ethanol and the solution was filtered. The solvent was removed with a stream of nitrogen while gently warming the flask. There remained about 650 mg. of a colorless material. From this residue unreacted material was removed by washing three times with 3-ml. portions of anhydrous ether, leaving behind 424–486 mg. crude potassium diacetone 2-keto-L-gulonate-C^{14}. Crystallization and reoxidation of the recovered material and combination of both batches of diacetone 2-keto-L-gulonate-C^{14} gave a total yield of 651–723 mg. (about 90% yield based on diacetone-L-sorbose-C^{14} oxidized).

L-Ascorbic acid-C^{14}. In a 65-ml. flask equipped with a ground glass joint, 651 mg. of potassium diacetone 2-keto-L-gulonate-C^{14} (prepared from 500 mg. of L-sorbose-C^{14} having a specific activity of 0.18 μcuries/mg.) were covered with 30 ml. of chloroform and while cooling the flask, 1.5 ml. of concentrated hydrochloric acid was added dropwise. The flask was stoppered and the mixture was allowed to stand in the dark at room temperature for 9–10 days.[21] The resulting brown mixture was extracted three times with 30-ml. portions of 0.01 *N* hydrochloric acid and the dissolved chloroform was removed from the aqueous phase by flushing with nitrogen. The volume was adjusted to 100 ml. and an aliquot was titrated with indophenol dye,[22] which indicated the presence of 170 mg. of L-ascorbic-C^{14} acid. Then 50 mg. of carrier was added and the material was purified by an anion exchange column technique, described previously,[3, 23] except that the Amberlite IR-4B resin was used in the acetate form and 2 *N* formic acid was employed to elute the L-ascorbic-C^{14} acid from the column (resin bed 15 cm. long and 2.5 cm. in diameter). The resulting material was washed twice with 0.5-ml. portions of cold acetone. Recrystallization afforded 146 mg. of pure L-ascorbic-C^{14} acid, m.p. 190–191°, having a specific activity of 0.13 uc/mg. Correcting for unreacted diacetone L-sorbose-C^{14}, this represents a yield of C^{14} of 22% based on L-sorbose-C^{14}. Addition of carrier to the acetone washings and recrystallization of the resulting material gave less active L-ascorbic-C^{14} acid which brought the total yield to 27%.[24] Radioactive purity was established by the following criteria:[25] the specific activity remained constant on recrystallization with carrier and L-ascorbic-C^{14} acid, its 2,4-dinitrophenylosazone and dimedone[25] derivatives had the same molar specific activity.

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(21) If instead of allowing the mixture to stand, it was refluxed for 8 hours the yield was slightly lower.

(22) The Association of Vitamin Chemists, Inc., *Methods of Vitamin Assay*, Interscience Publishers, Inc., New York, N. Y., 1951, pp. 77–80.

(23) Jackel, Mosbach, and King, *Arch. Biochem.*, **31**, 442 (1951).

(24) This method has also been carried out starting with 150 mg. of L-sorbose-C^{14}.

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2-Alkynyl Formals Formed During the Synthesis of 2-Alkynols[1a]

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Many times we have prepared large quantities of 1-alkynols in yields as high as 85% by reaction of alkynylmagnesium bromide with formaldehyde. In certain instances however, products boiling higher than the expected alkynol have been formed with a corresponding decrease in the yield of alkynol.

This paper deals with the elucidation of structure and the origin of the high-boiling product formed during the synthesis of 2-heptynol. Sufficient quantity of this higher-boiling material was available from a previous synthesis. The major component of this material was identified as di-2-heptynoxymethane, $(C_4H_9C{\equiv}C-CH_2O)_2CH_2$, **I**, on the basis of elemental analysis, molecular weight, infrared spectrum, hydrogenation equivalent, and identification of the hydrogenated product. An independent synthesis of **I** was also achieved by heating 2-heptynol with formaldehyde in the presence of magnesium bromide or ferric chloride.

Attention was also directed to determining the origin of **I** during a conventional synthesis of 2-heptynol. Conant, *et al.*[2] noted that formals are by-products of the synthesis of saturated primary alcohols when a considerable excess of gaseous formaldehyde is passed into the Grignard reagent. Isolation of the product in the usual way (which in their case was hydrolysis with sulfuric acid followed by steam-distillation) converted as much as two-thirds of the alcohol to the formal.[2]

Addition of excess formaldehyde to hexynylmagnesium bromide followed by conventional (rapid) hydrolysis did not give **I** however. No higher-boiling products were formed if the reaction mixture was kept cool as excess formaldehyde was being added but if the reaction mixture was allowed to become warm, a higher-boiling product having a double bond rather than an acetylenic

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