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Synthesis of α-L-Fucose-*1*-^{14}C (6-Deoxy-L-galactose-*1*-^{14}C)

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α-L-Fucose-*1*-^{14}C was synthesized in a radiochemical yield of 30 percent. The synthesis involved degradation of nonradioactive L-fuconic acid to 5-deoxy-L-lyxose and synthesis from this of α-L-fucose-*1*-^{14}C by use of ^{14}C-labeled cyanide in the cyanohydrin reaction. The resulting epimeric, ^{14}C-labeled aldonic acids were separated as either the barium or the sodium salts. Both salts of L-fuconic acid crystallize more readily than the corresponding salts of the epimeric 6-deoxy-L-talonic acid.

The preparation of barium L-fuconate by the electrolytic oxidation of L-fucose in the presence of barium carbonate and barium bromide is described.

Key Words: Carbon-14-labeled L-fucose, L-fuconic acid-*1*-^{14}C, L-fucose-*1*-^{14}C, radioactive carbohydrates, synthesis of radioactive sugars.

1. Introduction

Polysaccharides containing L-fucose are found in the cell walls of marine algae, in a few gums, and in certain substances of animal origin, such as red blood-cells, blood serum, gastric mucin, ovarian cystofluid, and many other materials of biological importance. Because of the widespread occurrence of L-fucose, the ^{14}C-labeled sugar is a valuable research tool. We have carried out the synthesis of α-L-fucose-*1*-^{14}C, and have supplied the radioactive sugar to numerous workers engaged in biological research. The method developed for the preparation is outlined below.

The α-L-fucose used as the starting material for the separation of the labeled product was obtained from the seaweed *Ascophyllum nodosum* by a procedure developed earlier, as described in [1].[1] The sugar was oxidized electrolytically by the method of Isbell and Frush [2], and the product was separated as crystalline barium L-fuconate. The barium salt was converted into the calcium salt, and this was degraded to 5-deoxy-L-lyxose by the method of Ruff [3] as modified by Hockett and Hudson [4], the proportion of reducing sugar formed from calcium L-fuconate being determined by iodine titration in alkaline solution [5]. In order to ascertain conditions favorable for formation of the *fuco* (*galacto*) configuration in the cyanohydrin reaction, test syntheses, beginning with 5-deoxy-L-lyxose, were conducted at the tracer level, and the products were analyzed by the isotope-dilution technique [6, 7]. The results showed that formation of a higher proportion of the *fuco* configuration is favored by the presence of a general acid-catalyst.

A portion of the crude 5-deoxy-L-lyxose was then treated with sodium cyanide-^{14}C in the presence of sodium bicarbonate. The cyanohydrins were carried through the reaction steps shown in figure 1. The resulting α-L-fucose-*1*-^{14}C crystallized directly from solution (without the intermediate preparation of the phenylhydrazone, a step ordinarily found necessary in the preparation of L-fucose from seaweed). The sodium 6-deoxy-L-talonate-*1*-^{14}C obtained as a by-product was retained for future use.

2. Experimental Details

2.1. Barium L-Fuconate [2]

A mixture of 20.5 g (0.125 mole) of α-L-fucose, 4 g of barium bromide, and 12.5 g of barium carbonate in 500 ml of water was placed in a 2-liter, 3-necked flask fitted with two graphite electrodes (1 cm in diameter) and an efficient stirrer. The flask was cooled in ice water, and a direct current of 0.2 A was passed through the stirred solution. After 34 hr (6.8 A-hr), a test for reducing sugar with Benedict reagent [8] was faint, and the electrolysis was stopped. The residual bromine and the barium bromide in the solution were respectively removed by adding 5 g of a decolorizing carbon and 4.2 g of silver sulfate. The suspension was filtered, and the residue on the filter was washed with hot water.[2] The filtrate was treated with hydrogen sulfide to remove residual silver salts, refil-

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[1] Figures in brackets indicate the literature references at the end of this paper.

[2] Barium L-fuconate may occasionally crystallize before filtration is conducted. Hence, the precipitate must be thoroughly washed, in order to avoid loss of material.

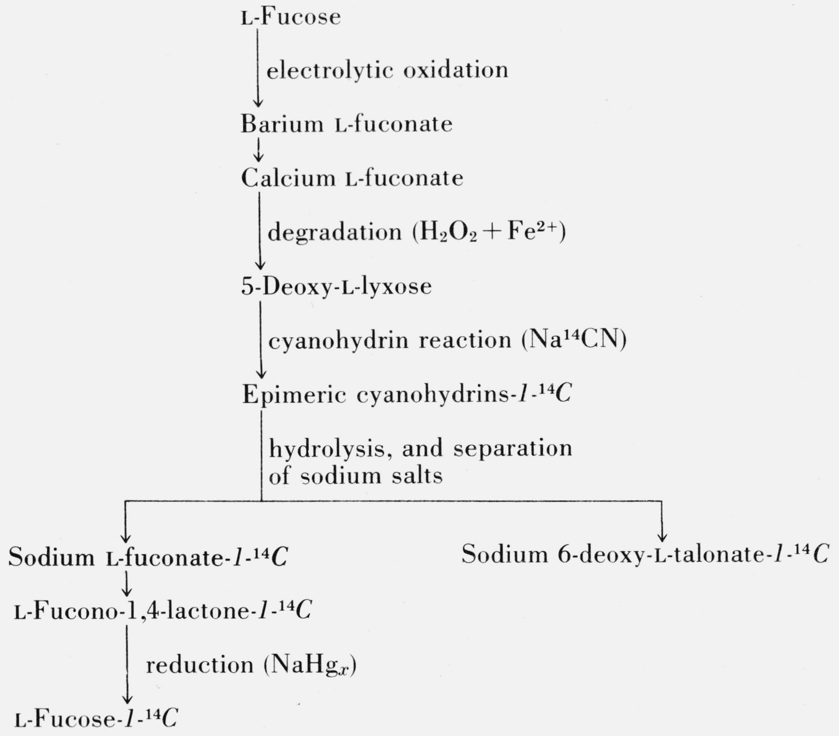

FIGURE 1. *Synthesis of α-L-Fucose-*1-^{14}C.

tered, and concentrated under reduced pressure to a thin syrup, which was diluted with ethyl alcohol to the point of incipient turbidity and nucleated with crystalline barium L-fuconate.

After several hours, a crop of crystals was separated by filtration; by reconcentrating the filtrate and diluting it with ethanol, a second crop was obtained; combined yield, 26 g (84%). The salt, $Ba(C_6H_{11}O_6)_2$, was recrystallized from hot water. It was only slightly soluble in water, and showed no optical rotation, in agreement with the reported properties of barium L-fuconate [9].[3]

2.2. 5-Deoxy-L-lyxose

Under vigorous stirring, 1.5 g (3 mmoles) of barium L-fuconate in 50 ml of hot water was successively treated with 6.2 ml of 1 *N* sulfuric acid and 350 mg of calcium carbonate. Without removal of the precipitate, the mixture, now containing a solution of calcium L-fuconate, was placed in a water bath at 50 °C; 62 mg of barium acetate, 63 mg of ferrous sulfate, and, finally, 0.75 ml of 30-percent hydrogen peroxide were added, whereupon the mixture turned brown. After 1 hr, a second quantity (0.75 ml) of hydrogen peroxide was added. After an additional hour, the suspension was diluted with 25 ml of methyl alcohol and 50 ml of ethyl alcohol, and filtered. The filtrate was concentrated under reduced pressure to about 25 ml, and passed through a column containing 25 ml of mixed (1 : 1) cation- and anion-exchange resins. The column was well washed with water, and the effluent was concentrated to about 25 ml. Titration of an aliquot with iodine in alkaline solution [5] showed the presence of 3.25 mmoles of reducing sugar (54% of the theoretical yield).

2.3. Reaction of 5-Deoxy-L-lyxose With Sodium Cyanide

In order to ascertain favorable conditions for formation of the L-fuconic cyanohydrin from 5-deoxy-L-lyxose, the following aqueous reaction mixtures were sealed in glass ampoules and kept for 4 days at about 5 °C.

1. 0.079 mmole of 5-deoxy-L-lyxose in 0.65 ml of solution,
   0.07 mmole of sodium cyanide-^{14}C (0.73 mCi/mmole), and
   0.30 mmole of sodium hydroxide in 1 ml of solution.

2. 0.079 mmole of 5-deoxy-L-lyxose in 0.65 ml of solution,
   0.05 g (approximately) of solid carbon dioxide,
   0.07 mmole of sodium cyanide-^{14}C (0.73 mCi/mmole), and
   0.30 mmole of sodium hydroxide in 1 ml of solution.

In the preparation of the second reaction mixture, the solid carbon dioxide was shaken with the sugar solution and allowed to vaporize before the alkaline

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[3] For preparing small quantities of L-fuconic acid, oxidation of L-fucose by bromine [10] or by iodine in alkaline solution [5] is more convenient than the electrolytic method described here. The resultant bromide or iodide is removed as the silver salt.

cyanide solution was added. After 4 days, each ampoule was opened, and the contents were hydrolyzed and treated with cation-exchange resin in a manner similar to that described below for the large preparation. Barium L-fuconate [4] (100 mg) was added to each solution; after several recrystallizations of the salt, the activities and the corresponding yields of barium L-fuconate-*1*-^{14}C in the cyanohydrin reaction were as follows: (1) 0.22 μCi/mg, 46.5 percent; (2) 0.28 μCi/mg, 60.5 percent. Hence, the presence of bicarbonate (a general acid-catalyst) favors the formation of the L-fuconic epimer.

2.4. Sodium L-Fuconate-*1*-^{14}C

In a 100-ml, round-bottomed flask in an ice-bath, 6 ml of 0.13 *M* 5-deoxy-L-lyxose (0.78 mmole) and 202 mg of sodium bicarbonate were shaken with a small lump (0.1 g) of solid carbon dioxide. To this mixture was added 5 ml of a cold solution containing 0.71 mmole (9.36 mCi) of sodium cyanide-^{14}C and 1.72 mmoles of sodium hydroxide. The mixture was kept in a refrigerator for one day, and at room temperature for four days. Then, 530 mg of sodium carbonate in 10 ml of water was added, and the flask, equipped with an air condenser, was heated in a boiling-water bath until evolution of ammonia had ceased. The solution was stirred with 5 ml of a cation-exchange resin [5] and passed through a column containing 20 ml of the resin. The effluent (containing 7.74 mCi of activity) was partially concentrated under reduced pressure (to remove carbon dioxide), and neutralized with sodium hydroxide.

Nonradioactive sodium L-fuconate (100 mg) was added, and the solution was concentrated to a syrup which was saturated with methyl alcohol and nucleated. The crystals of sodium L-fuconate-*1*-^{14}C that formed were separated, and washed with 50 percent aqueous methyl alcohol. The mother liquor was diluted with a solution of 500 mg of nonradioactive sodium L-fuconate and concentrated to a syrup, and the crystals that formed were combined with the first crop and recrystallized; yield, 522 mg, 4,330 μCi. A third crop, obtained by use of nonradioactive carrier, contained 40 μCi of activity. The total yield, 4,370 μCi, is 46.7 percent, based on the sodium cyanide-^{14}C used.

The mother liquors, containing approximately 3 mCi of activity, chiefly in the form of sodium 6-deoxy-L-talonate, were held for future use.

2.5. L-Fucono-1,4-lactone-*1*-^{14}C and Reduction to L-Fucose-*1*-^{14}C

A solution containing 2.58 mmoles of sodium L-fuconate-*1*-^{14}C having an activity of 4,330 μCi was passed through a column containing 10 ml of cation-exchange resin. The effluent was concentrated under reduced pressure, and then divided equally between two reduction tubes described previously [6]. The aqueous solution in each tube was concentrated to a syrup by means of a stream of air introduced through a capillary tube. The acid was lactonized by heating the syrup at 70 °C with glacial acetic acid. After 1 hr of heating, the completeness of lactonization was tested by paper chromatography and by scanning of the radioactivity in a paper-chromatogram scanner. (Unlactonized acid remains near the origin in the usual developing solvents.) Heating was repeated for an hour, and the tubes were allowed to stand over soda-lime in a desiccator for several days. Lactonization appeared to be nearly complete, as shown by test chromatograms.

The lactone (not crystalline) was reduced by the previously described method [6, 11], by use of 20 ml of water in each tube, and, per mmole of lactone, 4.6 g of 5-percent sodium amalgam in the form of pellets [12] and 3.2 g of sodium hydrogen oxalate. The reduction products in the tubes were combined, and the residual acids were neutralized with sodium hydroxide. Five volumes of methyl alcohol were added, and the mercury and precipitated salts were separated on a filter, washed with methyl alcohol and discarded. The filtrate was concentrated under reduced pressure to about 10 ml and diluted with five volumes of methyl alcohol. The salts that precipitated were removed by filtration, and the filtrate was concentrated to remove the alcohol. The syrup was dissolved in water, and the solution was passed through a column containing 25 ml of a 1:1 mixture of cation- and anion-exchange resins.

The effluent (which was shown to be salt-free by testing with a commercial conductivity-meter) was freeze-dried. The residue was dissolved in methyl alcohol, isopropyl alcohol was added to the point of incipient turbidity, and the solution was nucleated. The crystals of α-L-fucose-*1*-^{14}C that formed in 24 hr were separated and recrystallized; yield, 113 mg having an activity of 1,168 μCi. A second crop, weighing 214 mg after recrystallization, and having an activity of 655 μCi, was obtained from the mother liquors by use of 200 mg of nonradioactive L-fucose as the carrier.

When the ion-exchange column was eluted with 10-percent aqueous acetic acid, it yielded salts having an activity of 2,057 μCi. The effluent was freeze-dried, and the residue was dissolved in water and passed through a column containing 15 ml of a cation-exchange resin. The acid solution was concentrated under reduced pressure, transferred to a reduction tube, lactonized, and reduced with sodium amalgam as described above. After removal of the salts and unreacted L-fuconic acid-*1*-^{14}C, the sugar solution was combined with the mother liquor of the first preparation, yielding crystalline α-L-fucose-*1*-^{14}C which, when recrystallized, weighed 288 mg and had an activity of 777 μCi.

The elution, lactonization, and reduction procedures were again repeated, and yielded 222 mg of α-L-fucose-*1*-^{14}C having an activity of 222 μCi. Thus, the

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[4] Although barium L-fuconate was used in the tracer experiments, sodium L-fuconate was later found to have more satisfactory crystallizing properties. Accordingly, the latter salt was used for separation of the epimers in the high-activity preparation reported in the following sections.

[5] This caused the evolution of carbon dioxide, and obviated, in part, the formation of gas pockets in the resin column. The column was also back-washed several times during use. Finally, all of the resin was washed until it was substantially free from radioactivity.

total radiochemical yield of α-L-fucose-*1*-^{14}C from sodium L-fuconate-*1*-^{14}C was 2.8 mCi or 65 percent; the yield of the labeled sugar from sodium cyanide-^{14}C was 30 percent.

3. References

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