

Synthesis of α -D-Glucose-2-C¹⁴, α -D-Mannose-2-C¹⁴, and α -D-Galactose-2-C¹⁴¹

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α -D-Glucose-2-C¹⁴, α -D-mannose-2-C¹⁴, and α -D-galactose-2-C¹⁴ have been synthesized from the corresponding 1-C¹⁴-labeled pentoses in yields of 50, 18, and 36 percent, respectively. The methods are adaptations of those previously employed in the syntheses of the 1-labeled sugars, but include certain improvements, among them, the use of sodium acid oxalate as a buffer in the reduction of D-mannono- γ -lactone, and the elimination of the crystallization of D-lyxose-1-C¹⁴.

1. Introduction and Discussion of Synthetic Procedures

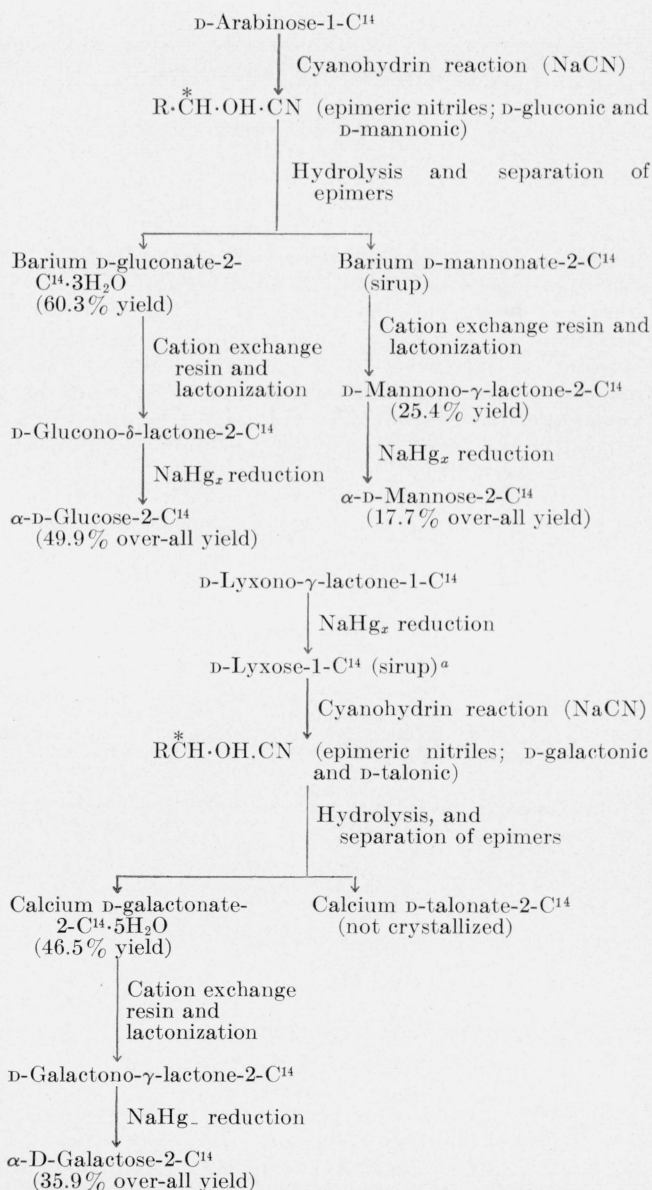
To confirm and extend fundamental knowledge obtained by research workers in many fields by use of 1-C¹⁴-labeled sugars a supply of sugars labeled at other positions is required. The present paper describes the preparation of some 2-C¹⁴-labeled sugars from D-arabinose-1-C¹⁴ and D-lyxose-1-C¹⁴ [1,2].² In each case, the labeled pentose was allowed to react with nonradioactive sodium cyanide, and the next higher sugars were prepared by methods similar to those previously developed for the production of the 1-C¹⁴-labeled sugars [1,2,3,4,5]. However, the procedure formerly employed for the reduction of D-mannono- γ -lactone [3] involved the addition and removal of benzoic acid, a step that caused considerable trouble. Consequently, the sodium amalgam reduction of D-mannono- γ -lactone was studied further, and a procedure developed for the production of D-mannose in high yield by the use of sodium acid oxalate as a buffer.

In contrast with the procedure previously reported for the preparation of D-lyxose-1-C¹⁴ [2], no attempt was made to crystallize the labeled D-lyxose used for the preparation of the nitriles of D-galactonic-2-C¹⁴ acid and D-talonic-2-C¹⁴ acid. The accompanying diagrams show the steps of the syntheses and the yields of the products based on the activities of the D-arabinose-1-C¹⁴ and D-lyxono- γ -lactone-1-C¹⁴, used as starting materials.

2. Experimental Procedures

2.1. Barium D-Gluconate-2-C¹⁴ Trihydrate and D-Mannono- γ -lactone-2-C¹⁴

A solution containing 3.49 millimoles (mM) of D-arabinose-1-C¹⁴, with 680 microcuries (μ c) of activity, and 3.6 mM of sodium cyanide in 25 ml of water was allowed to stand at room temperature for 3 days. The reaction products were then treated by the procedures described for the preparation of barium



¹ This work is part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the U. S. Atomic Energy Commission. This paper is based on the work described in an AEC report.

² Figures in brackets indicate the literature references at the end of this paper.

^a Sirup used without crystallization.

D-gluconate-1-C¹⁴ and D-mannono- γ -lactone-1-C¹⁴ [3].

The crystalline barium D-gluconate-2-C¹⁴ trihydrate had an activity of 339 μ c. By the use, in three portions, of a total of 1,030 mg of nonradioactive barium D-gluconate trihydrate as carrier, an additional quantity of 71 μ c was separated. Thus the radiochemical yield of barium D-gluconate-2-C¹⁴ from D-arabinose-1-C¹⁴ was 60.3 percent.

The mother liquor from the preparation of barium D-gluconate-2-C¹⁴, after removal of barium, and lactonization, gave 116 mg of crystalline D-mannono- γ -lactone-2-C¹⁴ having an activity of 126 μ c. By means of a total of 297 mg of carrier, used in 3 portions, the total radiochemical yield was increased to 173 μ c, corresponding to 25.4 percent of the original D-arabinose-1-C¹⁴.

2.2. Preparation of α -D-Glucose-2-C¹⁴ from Barium D-Gluconate-2-C¹⁴ Trihydrate

A 1.035-mM quantity of barium D-gluconate-2-C¹⁴ trihydrate, having an activity of 400 μ c, was converted to D-glucono- δ -lactone-2-C¹⁴ by the method previously described [3]. In the earlier work, a mixture of oxalic acid and sodium oxalate was used in the sodium amalgam reduction of the lactone to the sugar; crystalline sodium acid oxalate was later found to be more convenient [1]. Table 1 reports the results of a series of reductions of D-glucono- δ -lactone in the presence of crystalline sodium acid oxalate. The conditions of experiment 2 were followed in the reduction of D-glucono- δ -lactone-2-C¹⁴. The product, separated as crystalline α -D-glucose without carrier by the procedure given for the production of α -D-glucose-1-C¹⁴, weighed 296 mg and had an activity of 314 μ c. Additional material obtained by use of carrier amounted to 17 μ c. Thus the radiochemical yield of α -D-glucose-2-C¹⁴ from barium D-gluconate-2-C¹⁴ trihydrate was 82.8 percent, and the over-all yield from D-arabinose-1-C¹⁴ was 49.9 percent.³

TABLE 1. Reduction of D-glucono- δ -lactone^a by sodium amalgam in the presence of sodium acid oxalate

Experiment	Sodium acid oxalate monohydrate	NaHg ₂ (5 percent)	Yield of D-glucose (by analysis)
	g	g	%
1	0.6	1.65	87.2
2	1.0	2.3	90.6
3	1.5	3.45	87.9
4	2.0	4.6	83.4

^a 1.0 mM of lactone in 20 ml of water.

2.3. Preparation of α -D-Mannose-2-C¹⁴ from D-Mannono- γ -lactone-2-C¹⁴

The procedure for the preparation of α -D-mannose-1-C¹⁴ from D-mannono- γ -lactone-1-C¹⁴ was simplified by use of sodium acid oxalate as a buffer in place of benzoic acid. The experiments reported in table 2

³ Based on the percentage yield of barium D-gluconate-2-C¹⁴ from D-arabinose-1-C¹⁴.

show that a maximum yield of sugar is obtained by use of 27.6 g of 5-percent sodium amalgam per millimole of lactone in the presence of 12 g of sodium acid oxalate.

TABLE 2. Reduction of D-mannono- γ -lactone^a by sodium amalgam in the presence of sodium acid oxalate

Experiment	Sodium acid oxalate monohydrate	NaHg ₂ (5 percent)	Yield of D-mannose (by analysis)
	g	g	%
1	1.0	2.3	25.5
2	2.0	4.6	43.9
3	4.0	^b 9.2	56.5
4	6.0	^b 13.8	67.8
5	8.0	^c 18.4	88.3
6	12.0	^c 27.6	91.6
7	16.0	^c 36.8	88.6

^a 1.0 mM of lactone in 20 ml of water.

^b Added in 4.5-g quantities at 1-hr intervals. Mercury was removed before each new addition.

^c Added in 9.2-g quantities at 1-hr intervals. Mercury was removed before each new addition.

For the preparation of α -D-mannose-2-C¹⁴, a 1.4-mM composite sample of D-mannono- γ -lactone-2-C¹⁴, having an activity of 294 μ c, was reduced in the presence of 16.8 g of sodium acid oxalate, by use of 39 g of 5-percent sodium amalgam in three portions. Salts were removed by precipitation with alcohol and use of ion-exchange resins, as previously described. The crystalline sugar was prepared by concentrating the solution, dissolving the sirup in methanol, and adding 2-propanol. The crystalline α -D-mannose-2-C¹⁴, obtained without carrier, weighed 158 mg and had an activity of 185 μ c; by use of non-radioactive α -D-mannose as carrier, an additional 20 μ c of the sugar was obtained. Thus the radiochemical yield from D-mannono- γ -lactone-2-C¹⁴ was 69.7 percent and the over-all yield from D-arabinose-1-C¹⁴ was 17.7 percent.⁴ By elution of the resin used for removal of the salts, 49 μ c of crude D-mannonic-2-C¹⁴ acid was reclaimed.

2.4. Calcium D-Galactonate-2-C¹⁴ Pentahydrate

Calcium D-galactonate-2-C¹⁴ pentahydrate can be prepared readily from crystalline β -D-lyxose-1-C¹⁴ by the process developed for the production of calcium D-galactonate-1-C¹⁴ [4]. However, by starting the synthesis with a solution of D-lyxose-1-C¹⁴, without separation of the crystalline sugar, considerable saving of time and material can be made. In the present study, the D-lyxose-1-C¹⁴ was obtained by reduction [2] of 3 mM of D-lyxono- γ -lactone-1-C¹⁴ containing 1,918 μ c of carbon-14. The solution of D-lyxose was concentrated to 25 ml and cooled to 0° C. Six millimoles of sodium bicarbonate and 6 ml of aqueous 0.5 N sodium cyanide were added, and the solution was stored at room temperature. After 4 days, the reaction mixture was treated by the procedure described for the preparation of calcium D-galactonate-1-C¹⁴. The calcium D-galactonate-2-C¹⁴ pentahydrate obtained without carrier weighed

⁴ Based on the percentage yield of D-mannono- γ -lactone-2-C¹⁴ from D-arabinose-1-C¹⁴.

240 mg and had an activity of 593 μc . Additional salt, obtained by use of carrier, amounted to 299 μc . Thus the radiochemical yield of calcium D-galactonate-2- C^{14} pentahydrate was 892 μc or 46.5 percent of the original D-lyxono- γ -lactone-1- C^{14} . The residual liquor, containing approximately 1,000 μc of carbon-14 largely in the form of calcium D-galactonate-2- C^{14} , was set aside for future use.

2.5. Preparation of α -D-Galactose-2- C^{14} From Calcium D-Galactonate-2- C^{14} Pentahydrate

A solution of D-galactonic-2- C^{14} acid was prepared from 763 mg of calcium D-galactonate-2- C^{14} pentahydrate having an activity of 803 μc . The acid was lactonized, and reduced by the procedure developed for the preparation of α -D-galactose-1- C^{14} [4]; a total of 18 g of 4.2-percent sodium amalgam and 6.6 g of sodium acid oxalate was used. The sugar solution after removal of salts, and concentration, gave crystalline α -D-galactose-2- C^{14} that, once recrystallized, had an activity of 567 μc . By means of 400 mg of nonradioactive sugar, used in two portions as

carrier, additional sugar containing 54 μc was obtained. Thus the total radiochemical yield of α -D-galactose-2- C^{14} was 621 μc or 77.3 percent of the calcium D-galactonate-2- C^{14} pentahydrate; the overall yield was 35.9 percent.⁵ Extraction with aqueous acetic acid of the mixed resin column used for deionization of the sugar solution liberated 117 μc of activity. This material, largely D-galactonic-2- C^{14} acid, was stored for use in a subsequent preparation.

3. References

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⁵ Based on the percentage yield of calcium D-galactonate-2- C^{14} from D-lyxono- γ -lactone-1- C^{14} .

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