

Synthesis of D-Glucose-1-C¹⁴ and D-Mannose-1-C¹⁴*

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Heretofore, radiochemical yields of sugars position-labeled with carbon-14 have been only 5 to 10 percent. This paper reports an improved cyanohydrin synthesis, and the preparation of D-glucose-1-C¹⁴ and D-mannose-1-C¹⁴ from D-arabinose in yields of about 50 percent. Low yields in prior syntheses appear to have been due to several inefficient steps, and to the fact that extension of the carbon chain yields a pair of epimeric sugar derivatives, the proportion of which may not favor the desired epimer. In the classical cyanohydrin synthesis of glucose from D-arabinose only 30 percent of the product goes to the gluconic epimer. To increase the yield of glucose, a systematic study was made of the proportions of the epimers formed from D-arabinose under a variety of conditions. It was found that the ratio of the epimers depends upon the conditions under which the reaction of the sugar with cyanide takes place. Thus, D-arabinose and cyanide, in the presence of sodium bicarbonate and carbon dioxide, yield about three parts of mannonic nitrile to one part of gluconic nitrile, but, in the presence of sodium carbonate the proportion is approximately reversed.

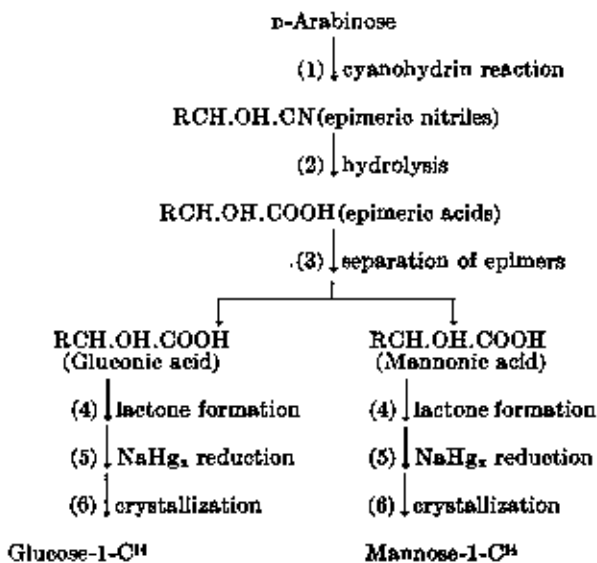
1. Introduction

Recognition by chemists, bacteriologists, and biologists that radioactive carbohydrates provide a tool for attacking many unsolved problems has led to a demand for sugars position-labeled with carbon-14. To meet the need, the Bureau, under the sponsorship of the Atomic Energy Commission, has undertaken a program for the production of C¹⁴-labeled sugars, including the development of methods of synthesis.

Previously, Sowden [3],¹ using the nitromethane method of Sowden and Fischer [4], prepared high-activity 1-C¹⁴-labeled glucose and mannose in yields of 7 and 10 percent, respectively, based on the nitromethane used.² By the same method Mahler [5] obtained 1-C¹⁴-labeled glucose and mannose in over-all yields of 5 and 7 percent, respectively, but he could not crystallize the products without the use of carriers. Koshland and Westheimer [6] used the Fischer-Kilian cyanohydrin synthesis [7] to prepare a low-activity D-glucose-1-C¹⁴ in radiochemical yield of 10 percent. They substituted the catalytic reduction of Glattfeld and Schimpff [8] for the classical sodium amalgam reduction, and used carrier techniques in the separation of both the delta lactone and the free sugar.

Thus, the history of the application of either the nitromethane or the cyanohydrin synthesis to the production of high-activity sugars appeared discouraging. From experience in the preparation of various rare sugars [9], it seemed probable that the low yields in the cyanohydrin synthesis were due, in part, to the use on a semimicro scale of techniques developed for macro preparations. A careful study was therefore made of the cyanohydrin synthesis, beginning with D-arabinose, and each step was

modified for efficient operation at the millimole level. The various steps follow and are discussed in section 2:



2. Discussion of Steps in the Cyanohydrin Synthesis

2.1. Condensation of D-Arabinose With Cyanide

Ordinarily the reaction of the aldose with the cyanide (step 1) is carried out on a macro scale with an excess of cyanide, in order that equilibrium conditions may favor the cyanohydrin. Militzer [10] has shown that combination of an aldose with cyanide under these conditions is essentially complete, but it remained to be demonstrated whether such combination could be accomplished in high degree with stoichiometric proportions. In a series of condensations with various quantities of cyanide, it was shown by titration of the excess cyanide that the condensation is nearly quantitative under a

* Presented before the Biological Section of the International Congress of Pure and Applied Chemistry, New York, N. Y., Sept. 18, 1951 [1, 2].¹

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

² A recent AEC report by Rappoport and Haasid (AECU-1853, 1961) describes the preparation by the nitromethane method of 1-C¹⁴-L-arabinose in 3-percent yield.

variety of conditions. Studies included the reaction of sodium cyanide in the presence of calcium chloride [11], ammonium chloride [12] or various quantities of sodium carbonate and bicarbonate.³

2.2. Hydrolysis of Cyanohydrins

The low yields reported in the literature for the products isolated from the cyanohydrin synthesis might be due to a partial reversal of the condensation reaction during hydrolysis. As the cyanohydrin on heating in alkaline solution yields ammonia, and the inorganic cyanide is not appreciably altered, completeness of the reaction could be judged by the ammonia evolved. It was found that the cyanohydrin from D-arabinose gave a nearly quantitative yield of ammonia, and hence there must have been no appreciable reversal.

The conditions of hydrolysis used by prior workers have been sufficiently drastic to cause deterioration of the product. Hydrolysis of the cyanohydrins from D-arabinose was found to take place satisfactorily under much milder conditions than were formerly used. It is conveniently effected by heating the cyanohydrins in sodium carbonate solution at 60° C for several hours with aeration and concentration of the solution. This procedure gives a nearly colorless product and avoids the decomposition that occurs at higher temperatures.

2.3. Partial Control of Epimeric Proportion and Separation of D-Gluconic and D-Mannonic Acids

Certain evidence has indicated that the proportion of the epimers formed in the cyanohydrin synthesis depends, in part, on the conditions under which the reaction takes place. Thus, in the condensation of mannose with HCN in the presence of ammonia, Fischer obtained 87 percent of α -mannoheptonic acid [13]⁴ but none of the beta isomer. However, the use of sodium cyanide in the presence of either calcium chloride [14] or barium chloride [15] led to the separation of substantial quantities of β -mannoheptonic acid. Partial control of the epimeric proportions is highly desirable in the synthesis of the labeled sugars. Consequently, the condensation of D-arabinose with cyanide was studied under a variety of conditions, and the proportions of gluconic and mannonic acids formed were estimated. The results, reported in section 3, showed that the ratio of gluconic acid to mannonic acid may be varied from 72:28 to 30:70 by alteration of experimental conditions. In fact, radiochemical yields as high as 70 percent of barium gluconate or 67 percent of mannonic lactone have been obtained. The ability to control the proportions of the epimeric acids over a wide range makes possible the production of the desired acid in predominating yield and greatly facilitates the separation of the epimers. For the separation of the acids, advantage was taken of the fact that mannono- γ -

lactone crystallizes more readily than either of the lactones of gluconic acid, and the fact that gluconic acid forms a crystalline barium salt⁵ whereas mannonic acid does not. In preparations designed primarily for the production of mannose, mannonic lactone was crystallized directly from the mixture of crude epimeric acids; in preparations designed for the production of glucose, gluconic acid was separated first in the form of the barium salt.

2.4. Lactonization of D-Gluconic and D-Mannonic Acids

Reduction of aldonic acids to the corresponding aldoses takes place through the lactone and not through the free acid. Consequently, the sugar acid must be converted as completely as possible to the lactone before reduction. The gluconic acid equilibrium involves complex condensation products, as well as the delta and gamma lactones. Both of the lactones as well as the free acid crystallize readily [17]; and the product obtained depends upon temperature, solvent, concentration, and the presence of seed crystals. Experiments showed that the delta lactone, on reduction with sodium amalgam, gives the sugar more readily than the gamma lactone, and that crystallization of the delta lactone can be effected satisfactorily by slow concentration at room temperature of a methyl cellosolve solution of the gluconic acid-lactone mixture. By this procedure, glucono- δ -lactone is obtained from barium gluconate in substantially quantitative yield. In some experiments the crude crystalline lactone was reduced with sodium amalgam without previous separation, and D-glucose was obtained in yields as high as 83 percent. For the preparation of pure glucono- δ -lactone-1-C¹⁴, the crude lactone was recrystallized from methyl cellosolve by the addition of ether. The conditions for the formation of mannono- γ -lactone are not critical, and satisfactory crystallization can be obtained by concentration of a solution of the epimeric acids with methanol, methyl cellosolve, acetic acid, or other organic solvent in the presence of seed crystals. In experiments designed for the production of mannose, mannonic lactone crystallized in satisfactory yields, nearly free from gluconic lactone. Recrystallization from hot isopropanol gave a substantially pure product.

2.5. Sodium Amalgam Reduction of D-Gluconic and D-Mannonic Lactones

The lactones of aldonic acids are reduced satisfactorily to sugars by sodium amalgam only in mildly acid solution. To maintain this condition, it is customary to test the solution frequently and to add acid gradually as the reaction proceeds [7b, 9, 18]. Reduction on a millimole scale in a

³ The use of sodium carbonate-bicarbonate and ammonium carbonate-bicarbonate buffers in the cyanohydrin synthesis has been under investigation at the Bureau for some time. The results obtained with various sugars will be considered in detail in a forthcoming publication.

⁴ D-Glucono- β -lactone-1-C¹⁴.

⁵ In a recent communication, Hudson [16] reported the preparation of barium L-gluconate in 50-percent yield from L-arabinose, and noted that the method of cyanide addition affects the proportion of the epimers formed. The present paper, including somewhat similar work on the D-modification, was completed and reported in abstract, (1, 2), prior to the appearance of Hudson's communication.

closed system requires an entirely different technique. Experiments at the Bureau over the course of several years have shown the practicability of maintaining a slightly acid medium for the reduction by the use of an excess of a difficultly soluble, solid organic acid or acid salt. As the sodium amalgam reacts, the acid dissolves and maintains an approximately constant pH. The buffering agent and conditions giving maximum yield of sugar vary with the lactone employed. Sodium acid oxalate with a small quantity of amalgam has been found to be most satisfactory for the reduction of glucono- δ -lactone, and benzoic acid with a large quantity of amalgam for the reduction of mannono- γ -lactone.⁶ With the improved procedure, glucono- Δ -lactone was reduced to glucose in yields of 85 to 90 percent by analysis, even when the quantities of lactone were as small as 18 mg; mannono- γ -lactone was reduced to mannose in yields of 75 percent. The efficiency of the reaction was due in part to the development of an amalgam of uniform composition that could be conveniently handled in small quantities without undue contamination from the atmosphere.

2.6. Crystallization of α -D-Glucose and α -D-Mannose

The procedure for separating the sugar formed by sodium amalgam reduction depended in part on the acid used as a buffer. In preparations in which benzoic acid was used, sufficient oxalic acid was added to convert all sodium present to sodium acid oxalate. The benzoic acid was removed by extraction with chloroform. The aqueous solution, which contained the sugar, oxalate, and small amounts of aldonic acid and polyhydric alcohol, was concentrated, and most of the oxalate was precipitated by addition of methanol. After removal of the solid oxalate by filtration, and of methanol by evaporation, the aqueous solution was ionized by means of ion-exchange resins, and lyophilized (freeze-dried). The sugar was crystallized by the addition of methanol-isopropanol mixtures in the presence of seed crystals. In preparations of radioactive sugars, one crop was separated directly from the sirup; the residual labeled sugar was removed from the mother liquor by several successive crystallizations in which either inactive or weakly radioactive sugar was used as a carrier.

Various solvents have been used successfully in the crystallization of α -D-glucose and α -D-mannose. Crude sugars crystallize more readily from acetic acid than from the methanol-isopropanol mixtures recommended here. However, acetic acid is somewhat objectionable, since unremoved traces of acid may cause gradual discoloration of the final product. Care must be used in conditioning the ion-exchange resins, because extraneous material removed from the resins sometimes inhibits crystallization of the product.

It will be seen from the yields given on page 170 that, for the production of D-mannose, the cyano-

⁶ These conditions were determined in an investigation of the sodium amalgam reduction under a variety of conditions. In the course of the work numerous lactones and methods were studied. The results will be reported in a forthcoming paper.

hydrin reaction employing the sodium bicarbonate-carbon dioxide buffer should be used; if glucose is desired, either the calcium chloride or the sodium carbonate method is preferable. With commercial C¹⁴-labeled sodium cyanide containing excess sodium hydroxide, the sodium carbonate method appears to give a slightly higher over-all yield of glucose and mannose than the calcium chloride method and does not require removal of chloride ion.⁷

3. Experimental Details

3.1. Study of the Condensation of D-Arabinose with Cyanide under Diverse Conditions

a. Establishment of the Completeness of Reaction Between Stoichiometric Quantities

To ascertain the completeness of the reaction of D-arabinose with cyanide under conditions that might prove useful for the preparation of labeled sugars, mixtures such as those given in table 1 were prepared and allowed to stand in sealed tubes at 20° C for 48 hours. The tubes were then opened, and the residual cyanide was titrated with silver nitrate by the method of Dénigès [19]. The results, expressed as percentage of cyanide reacted, show that combination of D-arabinose with cyanide is substantially complete under the conditions used.

TABLE 1. Completeness of reaction of stoichiometric quantities of D-arabinose and cyanide

Content of reaction mixture in 5 ml		Cyanide combined *	
	Millimoles	Percent	
D-Arabinose.....	2	98.0	}
NaCN.....	2		
CaCl ₂	1		
D-Arabinose.....	2	99.3	}
NaCN.....	2		
NaHCO ₃	6		

* Based on the determination of unreacted cyanide.

b. Determination by Means of Optical Rotation of the Proportions of D-Gluconic and D-Mannonic Acids Formed

As the cyanohydrin reaction is substantially quantitative under the conditions used, and the optical rotations of sodium gluconate [20] and sodium mannonate [21] differ widely, the optical rotations of the mixtures of sodium salts prepared from the cyanohydrins may be used to estimate the proportions of gluconic and mannonic acids present. In order to study the epimeric proportion under various conditions, the cyanohydrin reaction mixtures given in column 1 of table 2 were prepared. After 40 hours at 20° C, the inorganic salts listed in column 2 were added to give each mixture the same inorganic salt content. Hydrolysis was effected by digesting the solutions on the steam bath for 4 hours with reduction in volume by use of an air current. After filtration, the solutions were diluted to 25 ml and

⁷ Limited supplies of 1-C¹⁴-labeled glucose, mannose, barium gluconate, glucono- δ -lactone, and mannono- γ -lactone are now available to qualified investigators and may be purchased from the National Bureau of Standards upon authorization by the Atomic Energy Commission.

measurements of optical rotation were made. The specific rotations of sodium gluconate and of sodium mannonate were determined in solutions containing the same concentrations of inorganic salts as those present in the above experiments. These values were used to calculate the compositions of the mixtures from their optical rotations.

TABLE 2. Proportions of gluconic and mannonic acids formed in the cyanohydrin synthesis under various conditions

Reaction mixture (millimoles in 46 ml)	Salts added (millimoles)	$[\alpha]_D^{25}$ ^a	Ratio gluconate:mannonate
I D-Arabinose..... 4.0 NaCN..... 4.4	CaCl ₂ 2.2	+8.2	73 : 27
	NH ₄ Cl..... 4.4		
	NaHCO ₃ 4.4		
	Na ₂ CO ₃ 6.6		
II D-Arabinose..... 4.0 NaCN..... 4.4 CaCl ₂ 2.2	NH ₄ Cl..... 4.4	+6.1	72 : 28
	NaHCO ₃ 4.4		
	Na ₂ CO ₃ 6.6		
III D-Arabinose..... 4.0 NaCN..... 4.4 NaHCO ₃ 4.4	CaCl ₂ 2.2	+2.7	55 : 45
	NH ₄ Cl..... 4.4		
	Na ₂ CO ₃ 6.6		
IV D-Arabinose..... 4.0 NaCN..... 4.4 NH ₄ Cl..... 4.4	CaCl ₂ 2.2	+3.2	53 : 47
	NaHCO ₃ 4.4		
	Na ₂ CO ₃ 6.6		
V D-Arabinose..... 4.0 NaCN..... 4.4 NaHCO ₃ 4.4 CO ₂	CaCl ₂ 2.2	-2.2	90 : 70
	NH ₄ Cl..... 4.4		
	Na ₂ CO ₃ 6.6		
Control experiments			
Sodium gluconate, 4.0 CaCl ₂ 2.2 NH ₄ Cl..... 4.4 Na ₂ CO ₃ 9.0		+11.48	
Sodium mannonate 4.0 CaCl ₂ 2.2 NH ₄ Cl..... 4.4 Na ₂ CO ₃ 9.0		-9.15	

^a Based on the weight of the sodium salts of the aldonic acids and assuming 100-percent conversion. Volume of solution, 25 ml.
^b For manner of addition, see p. 168.

3.2. Isolation of Aldonic Acids from the Cyanohydrin Reaction

a. Manno- γ -Lactone

In order to determine the yields of barium gluconate and mannono- γ -lactone obtainable with various procedures, reaction mixtures similar to those of experiments I to IV of table 2 were prepared on an 8-millimole basis. Each solution was diluted to 100 ml and, after standing for 48 hours, was hydrolyzed by heating for several hours under reduced pressure at 50° C while the volume was kept approximately constant, and then by heating for 1 hour on the steam bath. The resulting solution was passed through a column (1.4×42 cm) of cation exchange resin.⁸ The column was washed, and the effluent was concentrated under reduced pressure to a thick sirup, which was seeded with D-manno- γ -lactone and allowed to stand at room temperature. Crystalline mannonic lactone separated overnight from the sirups of experiments III and IV; it separated more slowly from the sirups of experiments I and II, in which the proportions of the mannonic epimer were

⁸ Amberlite IR 100, analytical grade, Resinous Products Division of Rohm and Haas Co., Philadelphia, Pa.

lower. After several days, the partially crystalline mixture from each experiment was dissolved in the minimum amount of hot methyl cellosolve (about 2 ml), transferred to a small test tube, diluted with ethyl ether to incipient turbidity, and seeded with D-manno- γ -lactone. The mixture was allowed to stand for 24 to 48 hours at room temperature, during which time mannonic lactone crystallized as clusters of needles. The mother liquor was removed with a capillary pipette, and the crystals were washed twice in the test tube with 2 ml of a cold mixture of equal parts of ethanol and ether, and finally with ether alone. The crystalline mannonic lactone from each experiment was dried at 60° C in a vacuum oven and weighed. The weights of products and percentage yields for each condensation are given in table 3. In each case the product was identified by melting point, mixed melting point, and rotation.

TABLE 3. Yields of crystalline barium gluconate and mannono- γ -lactone from D-arabinose^a

Experiment	Method of addition	D-Manno- γ -lactone		Barium D-gluconate		Total yield
		Weight	Yield	Weight	Yield	
I.....	NaCN.....	0.329	23.1	0.900	41.3	64.4
II.....	CaCl ₂ +NaCN.....	.251	17.6	1.100	50.5	68.1
III.....	NaHCO ₃ +NaCN.....	.560	39.8	0.760	34.9	74.2
IV.....	NH ₄ Cl+NaCN.....	.580	40.7	.690	31.6	72.3

^a These yields were obtained with nonradioactive material. By use of carrier techniques and the methods described in section 3.5, considerably higher radiochemical yields were obtained. See p. 170.

b. Barium Gluconate

Barium D-gluconate was isolated in the following manner from the mother liquor of each of the above crystallizations of D-manno- γ -lactone: The mother liquor was concentrated under reduced pressure to a sirup, which was then dissolved in 10 ml of water. The solution was treated with 4 millimoles of aqueous barium hydroxide, digested on the steam bath for an hour, and finally acidified to phenolphthalein by means of a stream of carbon dioxide gas. The mixture was then warmed and filtered through a funnel pre-coated with diatomaceous earth and decolorizing carbon. The filtrate and washings were concentrated under reduced pressure to about 2 ml, and the solution was treated, dropwise, with methanol to incipient turbidity, and seeded with barium D-gluconate trihydrate [20].⁹ Although the typical plates of this compound appeared within a few days, crystallization was allowed to proceed for several days. The mother liquor was then removed with a fine pipette, and the crystals were washed three times with cold 25-percent methanol. The products, after drying in a vacuum oven at 80° C, gave optical rotations in substantial agreement with the value (+9°) reported for the monohydrate [20]. The weights of barium gluconate and the percentage yields from the four condensations are given in table 3.

⁹ A supply of barium gluconate was prepared at the Bureau by the electrolytic oxidation of glucose [23] in the presence of barium bromide and barium carbonate. The original preparation required a period of several months for crystallization. However, when seed crystals are available, the salt crystallizes quickly from solutions of high purity.

Table 3 shows that mannonic acid can be separated satisfactorily as the gamma lactone and gluconic acid as the barium salt. Other experiments showed that the lead salt [20] is also satisfactory for the separation of gluconic acid. In an experiment comparable to III of table 3, but on a 4-millimole level, *D*-mannono- γ -lactone was removed as described previously. The mother liquor was evaporated under reduced pressure to a sirup, and after the addition of 10 ml of water and 400 mg of neutral lead carbonate, the mixture was digested for 2 hours on the steam bath, and filtered. The filtrate and washings were concentrated under reduced pressure to about 1 ml. The sirup was treated with sufficient methanol to produce slight turbidity and seeded with crystalline lead gluconate. The crystals that formed in the course of several days were separated, washed with cold 25-percent methanol, and dried by heating under reduced pressure. The product weighed 470 mg, representing a yield of 39.4 percent.

3.3. Conversion of Barium Gluconate to *D*-Glucono- δ -Lactone

The completeness of conversion of barium gluconate to the delta lactone is closely dependent upon experimental conditions. If the barium ion is removed as barium sulfate, a trace of excess acid may hinder crystallization of the delta lactone by favoring formation of esters, condensation products, and the gamma lactone. The following was found to be the most consistently successful technique for removal of the barium ion and crystallization of glucono- δ -lactone. A solution containing 2 milliequivalents (0.581 g) of barium gluconate was passed over a column (1 \times 30 cm) of conditioned¹⁰ cation exchange resin (see footnote 8), and the column was washed immediately with water. The effluent was lyophilized at once. When lyophilization was complete, the residue was taken up in methyl cellosolve, and the solvent was evaporated in a stream of air and in the presence of seed crystals of *D*-glucono- δ -lactone. In the course of about 1 day, the solvent had evaporated, leaving a partially crystalline residue. The residue was moistened with methyl cellosolve, and evaporation was continued until the material appeared to be completely converted to the characteristic chunky crystals of the delta lactone. This material was ordinarily used directly for the preparation of *D*-glucose by reduction with sodium amalgam.

For the preparation of pure *D*-glucono- δ -lactone, the crude lactone prepared as described above was dissolved in about 10 parts of hot methyl cellosolve. The hot solution was filtered with the aid of decolorizing carbon and allowed to cool. Ether was added to the point of incipient turbidity, and the solution was seeded with crystalline glucono- δ -lactone. In the course of a day, *D*-glucono- δ -lactone separated in amount corresponding to about 90 percent of the theoretical.

¹⁰ The resin was regenerated, thoroughly washed with water, and immediately before use was washed successively with methanol and water, in order to inhibit microbiological action.

3.4. Sodium Amalgam Reduction of *D*-Glucono- δ -Lactone and Separation of Crystalline α -*D*-Glucose

Prior to the preparation of the labeled sugars, an extensive study was made of the sodium amalgam reduction of a variety of lactones on a millimole basis (see footnote 6). The procedures given here were found to be most satisfactory for the preparation of *D*-glucose and *D*-mannose. The reduction of both lactones was conducted in a heavy-walled glass tube fitted with a stainless-steel stopper having a greaseless bearing that accommodated a stirrer made by flattening one end of a stainless steel rod (fig. 1). The sodium amalgam used was in the form of pellets prepared by pouring molten 5-percent sodium amalgam into mineral oil in a 70-cm "shot tower."

For the reduction of *D*-glucono- δ -lactone, 0.7 g of oxalic acid, 0.8 g of sodium oxalate, and 20 ml of ice water were placed in the reaction flask containing 1 millimole (178 mg) of *D*-glucono- δ -lactone.¹¹ Immediately afterward, 2.3 g of 5-percent sodium amalgam pellets were added; the mixture, cooled in an ice bath, was stirred vigorously until the amalgam was spent, after which the mercury was separated. Three volumes of methanol was added, and the

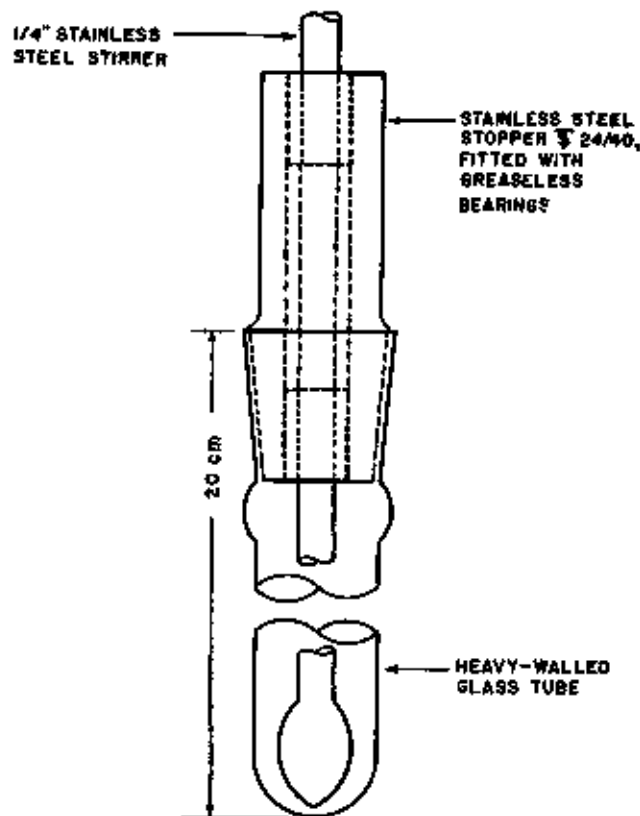


FIGURE 1. Apparatus for the sodium amalgam reduction of sugar lactones on a millimole basis.

¹¹ To avoid transferring the lactone, the methyl cellosolve solution of gluconic acid was usually evaporated and lactonized in the tube to be used for reduction.

crystalline salts that precipitated were removed by filtration, washed with methanol, and set aside for the recovery of unreacted gluconic acid. The alcoholic liquors were neutralized with aqueous sodium hydroxide and evaporated under reduced pressure almost to dryness, and the residue was extracted with several small portions of methanol (total volume was about 10 ml). The extract was filtered, diluted with water, and passed through a column (1.4×10 cm) containing equal parts of carefully conditioned cation (see footnote 8) and anion¹² exchange resins. The combined solution and washings were evaporated under reduced pressure to remove methanol, and finally lyophilized to a sirup that weighed 184 mg. The sirup was dissolved in a small amount of methanol, and the solution was transferred to a tared tube (total volume was about 4 ml). Isopropyl alcohol was added to incipient turbidity and the solution was seeded with α -D-glucose. Crystallization, which began immediately, was allowed to take place for several days. The crystals weighed 144 mg and had a specific rotation at equilibrium of +51° and a melting point of 147° to 149° C. The yield in this particular experiment was 80 percent. Analyses by copper reducing methods showed that the yield of D-glucose in the reduction process just described is 85 to 90 percent of the theoretical.

3.5. Sodium Amalgam Reduction of D-Mannono- γ -Lactone and Separation of Crystalline α -D-Mannose

¹⁰ For the reduction of D-mannono- γ -lactone, 1 millimole (178 mg) of the lactone was dissolved in 20 ml of ice water contained in the apparatus in figure 1. Benzoic acid (1.5 g) was added and stirred into the solution only long enough to effect dispersion; 4.6 g of 5-percent sodium amalgam pellets was added, and stirring was continued at 0° C for 1½ hours. At this time the mercury was removed with a pipette, additional benzoic acid (1.2 g) and sodium amalgam (4.6 g) were added, and stirring was continued until the amalgam was spent (2 hours). The mercury was removed again, and 1.26 g of oxalic acid dihydrate was added. The resulting benzoic acid was removed by extraction with chloroform. The aqueous solution was then mixed with 2 volumes of methanol and 2 of ethanol. The sodium salts that separated were collected on a filter, thoroughly washed with methanol and set aside for the recovery of any unreacted mannonic acid. The aqueous alcoholic liquor was concentrated under reduced pressure to about 10 ml, after which it was diluted again with 2 volumes of methanol and 2 of ethanol. The salts were separated, and the solution was concentrated to about 5 ml and passed through a column (1.4×10 cm) containing a mixture of equal parts of cation (see footnote 8) and anion (see footnote 12) exchange resins. The aqueous solution and washings were lyophilized. The lyophilized residue was dissolved in 1 ml of methanol, and the solution was transferred to a tared tube with 3 ml of methanol. Isopropyl alcohol was then added to the point of incipient turbidity.

¹² Duolite A-4, Chemical Process Co., Redwood City, Calif.

After the addition of seed crystals, the solution was allowed to stand for several days. It yielded 100 mg of crystalline D-mannose (55%), having an equilibrium specific rotation, $[\alpha]_D^{20}$, of +15° and a melting point of 130° to 132° C. Analyses of numerous solutions resulting from similar reductions showed that the production of D-mannose in this step varies from 75 to 80 percent of the theoretical.

3.6. Procedures Used in the Syntheses of D-Glucose-1-C¹⁴ and D-Mannose-1-C¹⁴

a. Sodium Bicarbonate-Carbon Dioxide Method for the Production of Mannono- γ -Lactone and Barium Gluconate

A flask containing 2 millimoles of radioactive sodium cyanide (20 microcuries (μ c)) and 2.24 millimoles of sodium hydroxide in 5 ml of solution was cooled in a mixture of dry ice and acetone until the solution froze. One gram of solid carbon dioxide and 20 ml of a solution containing 2 millimoles of D-arabinose were then added.

The mixture was allowed to thaw. The flask was stoppered and stored in a refrigerator overnight, and then at room temperature for 2 days. The solution was hydrolyzed by heating for 4 hours at 50° C under reduced pressure, and for 1 hour on the steam bath. Cations were removed by use of a column (1.4×24 cm) of cation exchange resin (see footnote 8), and the solution and washings were lyophilized. The residue was moistened with methanol, seeded with D-mannono- γ -lactone and allowed to stand for 3 days. During this time considerable lactone crystallized. The entire residue was then dissolved in 0.5 ml of methyl cellosolve, and the solution was treated with ether to incipient turbidity and again seeded. The D-mannono- γ -lactone-1-C¹⁴ that crystallized in the course of several days was separated, washed with ethanol and ether, and dried. It weighed 192 mg and contained 10.8 μ c of carbon-14 (54% yield¹³) $[\alpha]_D^{20}$ of the product was +53°, and the melting point was 151° to 152° C. The mother liquor and washings were combined and concentrated to a sirup to which was added 180 mg of carrier D-mannono- γ -lactone. Sirup and crystals were dissolved in 1 ml of methyl cellosolve, and ether was added to incipient turbidity. The crystallization gave 187 mg of D-mannono- γ -lactone containing 2.6 μ c of carbon-14 (13% radiochemical yield). The total recovery of radioactivity as D-mannono- γ -lactone-1-C¹⁴ was thus 13.4 μ c, or 67 percent.

To the mother liquor and washings from the crystallization of the carrier mannonic lactone, after evaporation to a sirup, were added 1 millimole of barium hydroxide octahydrate (315 mg) and 3 ml of water, and the mixture was digested on the steam bath for 1 hour. After acidification to phenolphthalein with carbon dioxide gas, the mixture was filtered by suction through a funnel precoated with diatomaceous earth and decolorizing carbon, and the combined filtrate and washings were lyophilized. The residue was dissolved in 0.5 ml of warm water, methanol was added to turbidity, and the solution was

¹³ The carbon-14 analyses reported in the paper were made by direct count in formamide solution [23].

seeded with barium gluconate trihydrate. After several days the crystalline barium 1-C¹⁴-D-gluconate trihydrate was separated and dried. It weighed 42 mg and gave carbon-14 analyses corresponding to 1.43 μ c (7.2%). To the mother liquor and washings from the above crystallization was added 100 mg of carrier barium D-gluconate trihydrate, and after crystallization, 149 mg of barium 1-C¹⁴-D-gluconate trihydrate was obtained with an activity of 2.45 μ c (12.2%). The radiochemical recovery as barium 1-C¹⁴-D-gluconate was 19.4 percent, and the total radiochemical recovery from 20 μ c of radioactive cyanide amounted to 17.3 μ c, or 86.4 percent.

A similar run made with approximately 3.5 millimoles of sodium cyanide containing 3.5 mc of carbon-14 gave the following products:

Crop	Carrier	Weight	Activity
Mannono- γ -lactone			
	<i>g</i>	<i>g</i>	μ c
1.....	None	0.2619	1,200
2.....	0.500	.4486	592
3.....	.500	.4344	245
4.....	.500	.4225	86
5.....	.500	.6002	66
		Total.....	2,189
Barium gluconate trihydrate			
1.....	1.000	0.7495	499
2.....	0.500	.6848	212
3.....	.500	.5870	69
		Total.....	780
Radiochemical recovery			
Mannono- γ -lactone.....			62.5%
Barium gluconate trihydrate..			22.3
Total.....			84.8

b. Calcium Chloride Method for the Production of Barium Gluconate and Mannono- γ -Lactone

An aqueous solution containing 3.0 g of D-arabinose (20 millimoles), 1.0 g of sodium cyanide (200 μ c), and 1.47 g of calcium chloride dihydrate was diluted to 250 ml and allowed to stand for 3 days at room temperature. It was heated at 60° to 70° C under vacuum for 4 hours and then for 1 hour on the steam bath. Cations were removed by a column (2 \times 35 cm) of cation exchange resin (see footnote 8), and the resulting solution was lyophilized to a residue. To this residue was added 10 millimoles of barium hydroxide octahydrate and 30 ml of water, and the mixture was digested on the steam bath for 1 hour. After carbonation to remove excess barium hydroxide and filtration with the aid of a decolorizing carbon, the solution was lyophilized. The solid fluffy product was dissolved in 5 ml of warm water, methanol was added to turbidity, and the solution was seeded

with barium gluconate trihydrate. The crystalline product that formed in the course of several days was separated from the mother liquor and washed with aqueous methanol and finally with methanol. The product, when dried in a vacuum desiccator, weighed 2.98 g (51%) and gave a carbon-14 analysis corresponding to 102 μ c. In a 2-percent aqueous solution, $[\alpha]_D^{25} = +10^\circ$.

Dissolution of 5 g of carrier barium gluconate trihydrate in the mother liquor and crystallization in the usual manner gave 5.52 g of the salt containing 38 μ c of carbon-14. Thus, a total of 140 μ c (70%) of the original 200 μ c of cyanide was obtained in the form of barium gluconate.

The mother liquor from the carrier barium gluconate was passed through a column (1.4 \times 30 cm) of cation exchange resin (see footnote 8), and the resulting solution was lyophilized to a residue. Carrier D-mannono- γ -lactone (2.0 g) was added with sufficient methyl cellosolve to effect solution, and ether was then added to turbidity. The mannonic lactone that crystallized weighed 2.2 g and contained 31 μ c of carbon-14, corresponding to a 15-percent yield. The total recovery of radioactive carbon from the original 200 μ c of cyanide amounted to 171 μ c, or 85 percent.

c. Sodium Carbonate Method for the Production of Barium Gluconate and Mannono- γ -Lactone

Ten milliliters of a solution containing 2 millimoles of C¹⁴-labeled sodium cyanide (4.74 μ c) and 2 millimoles of sodium hydroxide was placed in a glass-stoppered tube and frozen by use of dry ice. Then 2 millimoles of D-arabinose dissolved in 10 ml of 0.2 M sodium bicarbonate was added. The tube was loosely stoppered and shaken gently until the ice melted, allowed to stand at room temperature for 48 hours, and finally heated overnight at 60° C in a current of air. The residue was taken up in water and the solution was passed over a column (1.2 \times 20 cm) of cation exchange resin (see footnote 8).

The resulting solution was evaporated to a convenient volume, and barium hydroxide solution was added in sufficient amount to give a permanent pink color with phenolphthalein. Excess barium hydroxide was removed by carbonation and filtration. The solution was concentrated to about 1 ml, treated with methanol to the point of incipient turbidity, and seeded with barium gluconate trihydrate. After several days the crystalline barium salt was separated from the mother liquor and washed with aqueous methanol. The first crop of barium gluconate trihydrate (0.2676 g) contained 2.26 μ c, or 47.7 percent, of the 4.74 μ c in the original cyanide. Methanol was removed from the mother liquor of the first crop by evaporation in an air current, 0.5 g of carrier barium gluconate trihydrate was dissolved in the solution, and methanol was again added. A crop of barium gluconate weighing 0.5491 g and containing 0.78 μ c of carbon-14 was later separated. Thus the total radiochemical yield of barium gluconate trihydrate was 3.04 μ c, or 64 percent.

The mother liquor from the preparation of barium gluconate was passed over a column (1.4×30 cm) of cation exchange resin (see footnote 8), and the effluent and washings were evaporated substantially to dryness under reduced pressure. The residual sirup was diluted with 2 drops of methanol followed by 0.5 ml of isopropanol. Crystallization of mannono- γ -lactone, induced by seeding, was allowed to proceed for about 1 week in a glass-stoppered flask. The resulting crystalline mannono- γ -lactone was separated, washed with isopropanol, and dried. The material weighed 0.1104 g and contained 1.33 μ c of carbon-14. Addition to the mother liquor of 0.2 g of carrier mannono- γ -lactone, and recrystallization from isopropanol yielded 0.18 g of mannono- γ -lactone containing 0.11 μ c of carbon-14. The total mannono- γ -lactone-1-C¹⁴ (1.44 μ c) corresponds to a 30-percent radiochemical yield. The over-all radiochemical yield of barium gluconate and mannono- γ -lactone obtained in this particular experiment was exceptionally high, 94 percent.

A similar run made with 0.9 millimole of sodium cyanide containing 3.5 mc of carbon-14 gave the following products:

Crop	Carrier	Weight	Activity
Barium gluconate trihydrate			
1.....	None	0.0885	1,211
2.....	0.790	.8142	1,094
3.....	.500	.5173	119
4.....	.500	.4813	62
5.....	.500	.4442	21
Total			2,507
Mannono- γ -lactone			
1.....	0.500	0.4236	407
2.....	.500	.4840	76
3.....	.500	.5595	29
Total			512
Radiochemical recovery			
Barium gluconate trihydrate.....			71.5%
Mannono- γ -lactone.....			14.6
Total			86.1%

d. Sodium Amalgam Reduction of D-Glucono- δ -lactone-1-C¹⁴ and Separation of D-Glucose-1-C¹⁴

D-Glucose-1-C¹⁴ was prepared from the crops of barium gluconate obtained from the various cyanohydrin syntheses. In each case, the salt was converted to the D-glucono- δ -lactone by the method already given. The crude lactone was reduced, and the sugar was separated by the method given on page 167. Small modifications included washing the crude sugar with methanol saturated with nonradio-

active glucose. This prevents dissolution of the crystals but dilutes the mother liquor with inactive sugar. In each case additional labeled glucose was obtained from the mother liquor by use of carriers and, in some instances, the carriers were D-glucose-1-C¹⁴ of low activity obtained in previous runs. By this latter procedure the activity of the sugar was raised to a minimum of 1 μ c/mg. The radiochemical yields based on the barium gluconate were generally 80 percent. To obtain consistently good results, it is necessary to condition and wash the ion-exchange resin carefully and to avoid mold growth.

e. Sodium Amalgam Reduction of D-Mannono- γ -lactone-1-C¹⁴ and Separation of D-Mannose-1-C¹⁴

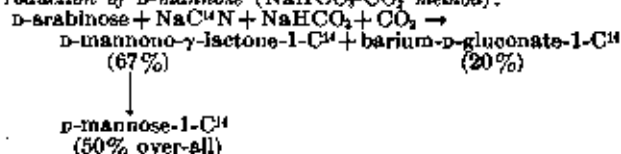
One millimole (178 mg) of D-mannono- γ -lactone-1-C¹⁴ (10 μ c) was treated as described on page 168 for the production of D-mannose. The procedure gave 99 mg of crystalline D-mannose-1-C¹⁴ with a total activity of 5.5 μ c. To the mother liquor from this material was added 200 mg of carrier D-mannose; after crystallization from a mixture of methanol and isopropanol 180 mg of D-mannose-1-C¹⁴ with a carbon-14 content of 1.96 μ c was obtained. The total radiochemical recovery from the lactone was 7.46 μ c, or 74.6 percent. As the radiochemical yield of the lactone from the cyanide was 67 percent, the over-all yield of the sugar from the cyanide was 50 percent.

Equally satisfactory results were obtained with D-mannono- γ -lactone-1-C¹⁴ having activities as high as 1 millicurie per millimole.

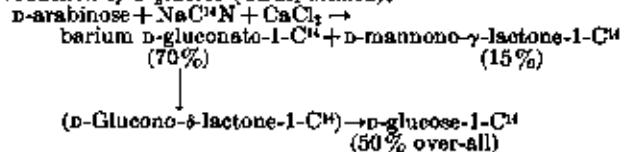
4. Summary

Procedures for the production of the several products from D-arabinose are given in the following diagrams, in which the figures in parentheses represent over-all radiochemical yields based on the amount of C¹⁴-labeled cyanide used in the initial step. The yields given here are typical. Individual preparations have given higher or lower yields, as discussed in the experimental section.

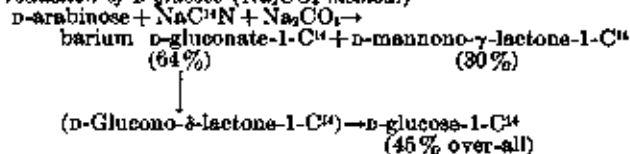
Production of D-mannose (NaHCO₃-CO₂ method):



Production of D-glucose (CaCl₂ method):



Production of D-glucose (Na₂CO₃ method):



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