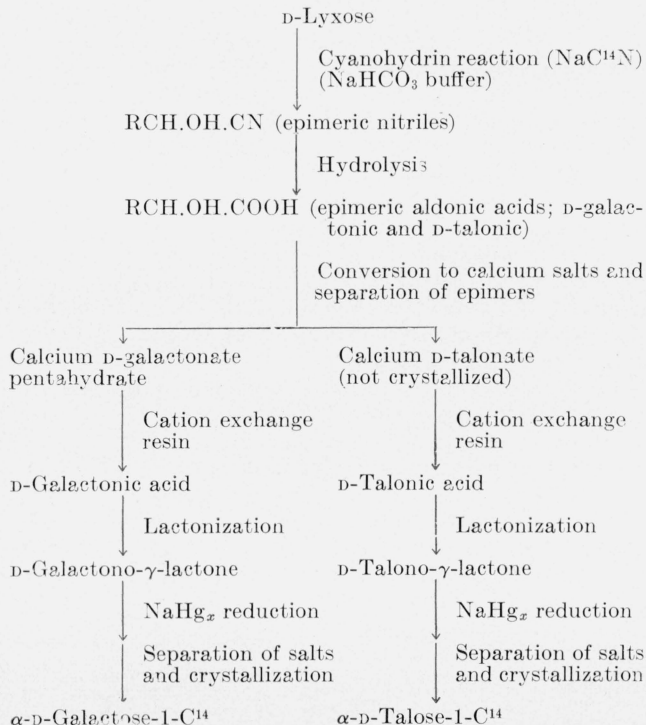# [1] Synthesis of D-Galactose-1-C$^{14}$ and D-Talose-1-C$^{14}$

## Horace S. Isbell, Harriet L. Frush, and Nancy B. Holt

An efficient procedure is presented for the preparation of α-D-galactose-1-C$^{14}$ and α-D-talose-1-C$^{14}$ from D-lyxose and C$^{14}$-labeled cyanide. The epimeric acids from the cyanohydrin synthesis were separated as calcium D-galactonate-1-C$^{14}$ pentahydrate and D-talono-γ-lactone-1-C$^{14}$ in yields of 67 and 28 percent, respectively. The calcium D-galactonate-1-C$^{14}$ was converted quantitatively to D-galactono-γ-lactone-1-C$^{14}$ and this was reduced, in 86-percent yield, to D-galactose-1-C$^{14}$. The over-all radiochemical yield of D-galactose-1-C$^{14}$ from NaC$^{14}$N was thus 57 percent. D-Talono-γ-lactone-1-C$^{14}$ was reduced to α-D-talose-1-C$^{14}$ in 56-percent yield, an over-all radiochemical yield of 16 percent.

## 1. Introduction

D-Galactose, one of the most important natural sugars, occurs in numerous polysaccharides, in certain glycosides (e. g., cerebrosides), in lactose, and in other substances of biological importance. Prior to the present study, D-galactose-1-C$^{14}$ had been prepared in 21-percent yield from D-lyxose and NaC$^{14}$N [1],[2] but the sugar was not generally available to research workers; the epimeric sugar, D-talose-1-C$^{14}$, had not been prepared. In order to make D-galactose-1-C$^{14}$ available for tracer studies in biological systems, and to facilitate its preparation by others, a study was made of its synthesis by techniques similar to those described in previous publications from this laboratory [2, 3, 4, 5, 6, 7]. α-D-Galactose-1-C$^{14}$ and α-D-talose-1-C$^{14}$ have now been synthesized in good yield by the following procedure:

D-Lyxose

    Cyanohydrin reaction (NaC$^{14}$N)
    (NaHCO$_3$ buffer)

RCH.OH.CN (epimeric nitriles)

    Hydrolysis

RCH.OH.COOH (epimeric aldonic acids; D-galactonic and D-talonic)

    Conversion to calcium salts and separation of epimers

Calcium D-galactonate     Calcium D-talonate
pentahydrate     (not crystallized)

    Cation exchange     Cation exchange
    resin     resin

D-Galactonic acid     D-Talonic acid

    Lactonization     Lactonization

D-Galactono-γ-lactone     D-Talono-γ-lactone

    NaHg$_x$ reduction     NaHg$_x$ reduction

    Separation of salts     Separation of salts
    and crystallization     and crystallization

α-D-Galactose-1-C$^{14}$     α-D-Talose-1-C$^{14}$

## 2. Discussion of the Experimental Method

It was found that in the reaction of cyanide with D-lyxose, general acid catalysts favor formation of the galactonic epimer. Accordingly, the preparation of the radioactive cyanohydrins was conducted in the presence of sodium bicarbonate. The epimeric acids were conveniently separated by means of the calcium salts, one of which, calcium D-galactonate-1-C$^{14}$ pentahydrate, crystallizes readily. The salts were converted to the corresponding acids, and these were lactonized and then reduced to the corresponding sugars. In a preparation beginning with 7.5 millicuries of NaC$^{14}$N, calcium D-galactonate-1-C$^{14}$ pentahydrate and D-talono-γ-lactone-1-C$^{14}$ were obtained in radiochemical yields of 67 and 28 percent, respectively. The calcium D-galactonate-1-C$^{14}$ was converted to D-galactono-γ-lactone-1-C$^{14}$, and this was reduced to the sugar by means of sodium amalgam in the presence of sodium acid oxalate [3]. The radiochemical yield of α-D-galactose-1-C$^{14}$ from calcium D-galactonate-1-C$^{14}$ pentahydrate was 86 percent, and the over-all yield from NaC$^{14}$N was 57 percent.[3] Similarly, the radiochemical yield of α-D-talose-1-C$^{14}$ from D-talono-γ-lactone-1-C$^{14}$ was 56 percent, and the over-all yield was 16 percent.

## 3. Experimental Details

### 3.1. Preparation of Calcium D-Galactonate-1-C$^{14}$ Pentahydrate

A solution consisting of 20 ml of water, 10 mM of sodium hydroxide and 12 mM of NaC$^{14}$N, having an activity of 7,500 microcuries, was frozen in a 100-ml round-bottomed glass-stoppered flask, and a carbon dioxide-saturated solution containing 13 mM of D-lyxose [8] and 10 mM of sodium bicarbonate in 10 ml of water was added. The flask was stoppered and kept in gentle motion until the ice had melted. After storage for 5 days at 0° C, the mixture was heated at 80° C under a stream of air with the addition of water from time to time, until ammonia was no longer detectible in the vapor (about 12 hr). The solution was diluted with 25 ml of water and passed

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

[3] If allowance is made for calcium D-galactonate-1-C$^{14}$ reclaimed by carrier technique after the reduction, the yield in the reduction step becomes 98 percent, and the over-all yield, 66 percent. The reclaimed material was of low activity, and suitable for use as a carrier in a subsequent preparation.

through 70 ml of cation exchange resin.[4] The column was washed until the activity was negligible, and the total effluent was evaporated to 100 ml under reduced pressure; an aqueous suspension of calcium hydroxide was then added in sufficient amount to give a faint, but permanent, pink color in the presence of phenolphthalein indicator. The solution, filtered and evaporated under reduced pressure, yielded a sirup that crystallized readily upon addition of methanol and seed crystals. After 3 days, a crop of calcium D-galactonate-1-C[14] pentahydrate was separated and recrystallized once by dissolving it in the minimum amount of hot water, cooling the solution, and cautiously adding methanol. The crystals, when separated and dried, weighed 1.838 g (equivalent to 7.07 mM) and had an activity of 4,435 $\mu$c. By the successive use of four 700-mg quantities of nonradioactive D-galactose as carrier an additional 583 $\mu$c was separated. Thus the total yield of calcium D-galactonate-1-C[14] pentahydrate was 5,018 $\mu$c, or 66.9 percent. The residues, containing 2,400 $\mu$c of radioactivity, were used for the preparation of D-talono-$\gamma$-lactone-1-C[14], as described in section 3.4.

## 3.2. Preparation of D-Galactono-$\gamma$-lactone-1-C[14]

A 1.42-g quantity of calcium D-galactonate-1-C[14] pentahydrate having an activity of 3,600 $\mu$c[5] was dissolved in 100 ml of water, and the solution was passed through a column containing 70 ml of cation exchange resin. The column was thoroughly washed, and the total effluent was concentrated under reduced pressure to 25 ml. The solution was then transferred in equal parts to 5 reduction tubes.[6] In order to lactonize the D-galactonic-1-C[14] acid, the tubes were placed in a bath at 80° C, and each solution was concentrated in a current of air; from time to time the residues were dissolved in ethylene glycol monomethyl ether (Methyl Cellosolve), and the evaporation was continued. During this treatment, the needle-like crystals of D-galactonic acid, first observed, were slowly converted to the typical wedge-shaped crystals of the lactone. When the lactonization seemed to be complete, as evidenced by the lack of amorphous material and needle crystals (about 3 days), the tubes were stored in a desiccator until used for production of the sugar.

## 3.3. Reduction of D-Galactono-$\gamma$-lactone-1-C[14] to $\alpha$-D-Galactose-1-C[14]

Prior to the preparation of D-galactose-1-C[14], the experiments given in table 1 were conducted with

nonradioactive D-galactono-$\gamma$-lactone in order to ascertain optimum conditions for reduction of the lactone. In each experiment, the crystalline sodium acid oxalate and the lactone were placed in a reduction tube immersed in an ice bath. Twenty milliliters of ice water was added, and vigorous stirring was begun. The 5-percent amalgam[7] was then added in one portion through the side arm. Stirring was continued until the amalgam had completely reacted (about 2 hr). The solution was filtered, and the amount of sugar formed was determined by the modified Scales method [9]. The data of table 1 show that 1 mM of D-galactono-$\gamma$-lactone was reduced most effectively by 4.6 g of 5-percent sodium amalgam in the presence of 2 g of sodium acid oxalate (approximately a 50-percent excess); hence these proportions were used in the reduction of the labeled lactone.

TABLE 1. *Reduction of D-galactono-$\gamma$-lactone* [a] *by sodium amalgam in the presence of sodium acid oxalate*

| Experiment | Sodium acid oxalate | NaHg$_x$ (5-percent) | Yield of D-galactose (by analysis) |
|---|---|---|---|
| | *g* | *g* | *%* |
| 1 | 1.0 | 2.3 | 70.2 |
| 2 | 2.0 | 4.6 | 95.3 |
| 3 | 2.4 | 5.5 | 89.2 |

[a] 1.0 mM of lactone in 20 ml of ice water.

The 5 aliquots of D-galactono-$\gamma$-lactone-1-C[14] described in section 3.2 (each containing 1.1 mM with 720 $\mu$c of activity) were reduced separately[8] by use of 5 g of 5-percent sodium amalgam and 2.3 g of sodium acid oxalate. After 2 hr, the mercury was separated, and the products from the 5 tubes were combined and diluted with 5 volumes of methanol. The resulting crystalline salts were separated by filtration and discarded after they were found to be nonradioactive. The alcoholic filtrate was concentrated under reduced pressure to 20 ml, cooled in ice water, and neutralized with aqueous sodium hydroxide until it had a faint, but permanent, pink color in the presence of phenolphthalein indicator. An equal volume of methanol was added, the mixture was refiltered, and the residue, likewise found to be nonradioactive, was discarded. After the filtrate had been concentrated to about 15 ml and diluted with 2 parts of methanol, a third crop of salts with negligible radioactivity was removed by filtration and discarded. The filtrate was concentrated to remove most of the methanol and then passed through a column containing 50 ml of a mixture of equal parts of cation[9] and anion[10] exchange resin. Five milliliters of cation exchange

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[4] Amberlite IR 120–H, analytical grade, Resinous Products Division of Rohm & Haas Co., Philadelphia, Pa.

[5] The calcium D-galactonate-1-C[14] pentahydrate employed here was part of that described in section 3.1, to which a small amount of material from another preparation was added. The level of activity (2.54 $\mu$c/mg) is slightly higher than that of section 3.1 (2.41 $\mu$c/mg).

[6] The reduction apparatus employed here has been fully described [5]; it consists of a heavy-walled glass tube, 200 by 25 mm, having a 24/40 standard-taper joint. A side arm, 50 by 15 mm, attached just below the joint, facilitates addition of the amalgam. A stainless-steel, standard taper stopper, equipped with oilless bearings, supports a stainless-steel stirrer, which just touches the bottom of the tube.

[7] The amalgam was prepared in small pellets by pouring it in molten condition through a heated alundum thimble, having a small hole in the bottom, into a 2-ft "shot tower" of mineral oil. The pellets were stored under mineral oil, and just before use, were blotted dry, weighed, and rinsed with benzene.

[8] Since this work was completed, a larger stainless-steel reduction apparatus has been employed, which makes portion-wise reduction unnecessary.

[9] Amberlite IR 100–H, analytical grade, Resinous Products Division of Rohm & Haas Co., Philadelphia, Pa.

[10] Duolite A–4, Chemical Process Co., Redwood City, Calif.

resin was also placed at the top and at the bottom of the mixed resins. The absence of free acid in the effluent showed that sufficient resin was present to remove all salts. The column was washed until the activity was negligible, and the effluent was concentrated to 20 ml under reduced pressure. The solution was then filtered through a slender column containing 0.1 g of a decolorizing carbon, and the filtrate was freeze-dried. After the residue had been dissolved in 3 ml of methanol, and 2-propanol added to the point of incipient turbidity, crystallization of D-galactose-1-$C^{14}$ began immediately. At the end of 3 days, the mother liquor was removed with a capillary pipet, and the crystals were carefully washed in place with ethanol. To recrystallize, the material was dissolved in 1 ml of water by warming, 5 ml of methanol was added, and then 2-propanol almost to the point of saturation. After 48 hr, the crystals were separated as described before and dried; they weighed 760 mg and contained 2,780 $\mu c$ of activity. By use of three 500-mg portions of nonradioactive D-galactose as carrier, an additional 300 $\mu c$ of activity was recovered from the mother liquor. Hence the radiochemical yield of D-galactose-1-$C^{14}$ was 3,080 $\mu c$, or 85.6 percent of the activity of the original calcium D-galactonate-1-$C^{14}$ pentahydrate (3,600 $\mu c$).

When the column of mixed resin used for the purification of the sugar was extracted with 250 ml of 10-percent aqueous acetic acid, 435 $\mu c$ of activity was recovered. The solution was concentrated under reduced pressure almost to dryness, and finally was freeze-dried. An aqueous solution of the residue was de-cationized, neutralized with a solution of calcium hydroxide, filtered, and used to dissolve 500 mg of nonradioactive calcium D-galactonate pentahydrate. After concentration of the solution under reduced pressure, and addition of methanol, a crop of calcium D-galactonate-1-$C^{14}$ pentahydrate was obtained, which, once recrystallized, contained 366 $\mu c$ of activity. This material was used as a carrier in a later preparation. If allowance is made for the reclaimed calcium D-galactonate-1-$C^{14}$ pentahydrate, the yield of D-galactose-1-$C^{14}$ from the calcium salt becomes 98.3 percent.

### 3.4. D-Talono-γ-lactone-1-$C^{14}$

The liquor that remained after separation of the last traces of crystalline calcium D-galactonate-1-$C^{14}$ pentahydrate in the experiment described in section 3.1 was diluted with 25 ml of water and passed through a column containing 25 ml of cation exchange resin. The effluent was concentrated under reduced pressure, and the resulting sirup was finally transferred to a test tube by means of Methyl Cellosolve. The D-talonic-1-$C^{14}$ acid in the sirup was lactonized by immersing the tube in a water bath at 60° C, and passing a current of air over the warm liquid; a few drops of Methyl Cellosolve were added at 2-hr intervals. After 8 hr the residue was taken up in about 5 ml of 2-propanol. Crystalline

D-talono-γ-lactone (200 mg) was added, the mixture was warmed to dissolve the carrier, and the solution was seeded and set aside for 24 hr. The first crop of D-talono-γ-lactone-1-$C^{14}$, after one recrystallization from 2-propanol, weighed 606 mg, and had an activity of 1,625 $\mu c$. The combined mother liquors, when treated with 2 additional 200-mg quantities of carrier, yielded 482 $\mu c$. The total radiochemical yield was thus 2,107 $\mu c$, corresponding to 28 percent of the Na$C^{14}$N used in the cyanohydrin synthesis.

### 3.5. Preparation of α-D-Talose-1-$C^{14}$

Prior to the reduction of the labeled lactone, suitable conditions for the production of the sugar were determined by means of the experiments reported in table 2.

TABLE 2. *Reduction of D-talono-γ-lactone* [a] *by sodium amalgam in the presence of sodium acid oxalate*

| Experiment | Sodium acid oxalate | NaHg$_x$ (5-percent) | Yield of D-talose (by analysis) |
|---|---|---|---|
| | *g* | *g* | *%* |
| 1 | 1.6 | 2.3 | 54.7 |
| 2 | 3.2 | 4.6 | 75.0 |
| 3 | 4.8 | 6.9 | 76.1 |
| 4 | 6.4 | 9.2 | 75.0 |
| 5 | 6.4 | [b] 18.4 | 66.7 |

[a] 0.5 mM of lactone in 20 ml of ice water.
[b] Added in two portions.

Experiments 2, 3, and 4 of table 2 gave nearly the same yield of sugar. The conditions of experiment 2 were adopted for the reduction of the labeled material, as they called for less of the reagents. In a typical preparation, 430 mg of D-talono-γ-lactone-1-$C^{14}$ having an activity of 378 $\mu c$, was reduced in two portions by use of 13 g of 4.2-percent amalgam [11] and 7.5 g of sodium acid oxalate for each portion. The product was treated in essentially the same manner as the product from the reduction of D-galactono-γ-lactone-1-$C^{14}$. The sugar solution, after de-ionization, was concentrated to 50 ml under reduced pressure and was then freeze-dried. The residue was dissolved in 0.5 ml of ethanol, *n*-butanol was added to the point of incipient turbidity, and the solution was seeded with α-D-talose previously prepared [10]. After several days, the mother liquor that remained was withdrawn with a capillary pipet, and the crystalline α-D-talose-1-$C^{14}$ was washed with a few drops of 2-propanol. The product, recrystallized once from the minimum amount of water by the addition of 2-propanol, weighed 170 mg, and had an activity of 142 $\mu c$. By use of 600 mg of carrier in 2 portions, an additional 70 $\mu c$ of α-D-talose-1-$C^{14}$ was separated from the combined mother liquors. The total yield was thus 212 $\mu c$, or 56 percent of the D-talono-γ-lactone-1-$C^{14}$ used.

## 4. References

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