

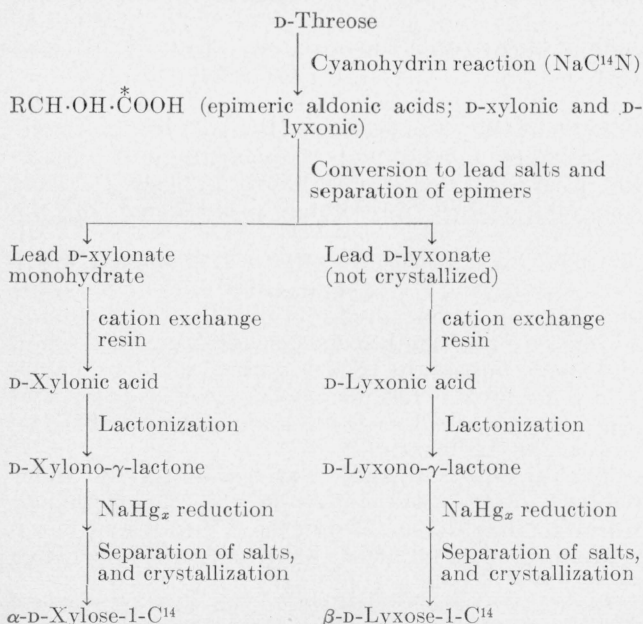
Synthesis of α -D-Xylose-1-C¹⁴ and β -D-Lyxose-1-C¹⁴ ¹

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Methods are presented for the preparation of α -D-xylose-1-C¹⁴ and β -D-lyxose-1-C¹⁴ from D-threose and C¹⁴-labeled sodium cyanide. The xylonic epimer from the cyanohydrin synthesis was separated in 34-percent yield by means of the lead salt; the lyxonic epimer, in 49-percent yield by means of the lactone. Lead D-xylonate-1-C¹⁴ was converted quantitatively to D-xylonono- γ -lactone-1-C¹⁴, and this was reduced by sodium amalgam to α -D-xylose-1-C¹⁴ in 61-percent yield. D-Lyxono-1-lactone-1-C¹⁴ on reduction, gave β -D-lyxose-1-C¹⁴ in 64-percent yield. Thus, the over-all yields of α -D-xylose-1-C¹⁴ and β -D-lyxose-1-C¹⁴, based on the C¹⁴-labeled cyanide used in the cyanohydrin synthesis, were 21 and 31 percent, respectively. The methods reported greatly facilitate the preparation of α -D-xylose-1-C¹⁴ and β -D-lyxose-1-C¹⁴.

1. Introduction and Discussion

Polysaccharides containing D-xylose, the most abundant of the pentoses, accompany cellulose in a wide variety of plant products. For numerous investigations in the fields of bacteriology, plant and animal metabolism, enzymology, and polysaccharide chemistry, a supply of 1-C¹⁴-labeled xylose was needed. Prior to the present study, this substance had been obtained from D-glucose-1-C¹⁴ through the intermediate preparation of 1,2-isopropylidene-D-glucose-1-C¹⁴, followed by periodate oxidation, and reduction of the resulting 1,2-isopropylidene-D-xylotrihydroxyglutaraldehyde [1].² It seemed probable that a less complicated synthesis of D-xylose-1-C¹⁴ could be accomplished, and both of the labeled sugars D-xylose and D-lyxose, could be isolated by methods similar to those previously employed at the Bureau for the cyanohydrin synthesis of other 1-C¹⁴-labeled sugars [2]. The method developed is shown in the accompanying outline.



2. Experimental Procedures

2.1. D-Threose

D-Threose, required as a starting material for the cyanohydrin synthesis, was prepared from calcium D-xylonate dihydrate [3] by the Ruff degradation [4, 5] with certain modifications. Three grams of crystalline calcium D-xylonate dihydrate (7.38 mM) was added to a solution prepared from 0.6 of barium acetate monohydrate, 0.15 g of ferric sulfate (approximately, a hexahydrate) and 50 ml of water. The mixture was heated to 50° C, and treated with 1.8 ml of 30-percent hydrogen peroxide. After the solution had darkened, a second 1.8-ml quantity of 30-percent hydrogen peroxide was added. When the solution had again darkened, indicating that reaction was complete, it was filtered through decolorizing carbon, and concentrated under reduced pressure to a thick sirup, which was mixed with 70 ml of methanol, and then with 140 ml of ethanol. The resulting precipitate was separated by filtration, washed with ethanol, and discarded. The filtrate was concentrated under reduced pressure almost to dryness, and the residual sirup was diluted first with 25 ml of methanol and then with 25 ml of 2-propanol. The alcoholic solution was filtered through 1 g of decolorizing carbon, the filter was washed with a 1:1 ethanol-2-propanol mixture, and the filtrate was concentrated under reduced pressure to remove the alcohol. The residue was then dissolved in 20 ml of water, and the solution was passed through a column containing 20 ml of mixed cation³ and anion⁴ exchange resins; the column was washed with 100 ml of water. The effluent was concentrated under reduced pressure, and finally was adjusted to a volume of 50 ml. A 5-ml aliquot of this solution, when treated with 20 ml of N/10 iodine and 30 ml of N/10 sodium hydroxide by the method of Kline and Acee [6], reduced iodine equivalent to 0.825 mM of aldose. Thus the yield of D-threose was 8.25 mM, or 56 percent of the theoretical. The material was used without further purification in the cyanohydrin synthesis.

2.2. Lead D-Xylonate-1-C¹⁴ Monohydrate

A portion of the solution prepared above (20 ml, containing 3.3 mM of D-threose) was treated with

¹ Part of a project on the development of methods for the synthesis of radioactive carbohydrates, sponsored by the 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.

³ Amberlite IR 120-H, Rohm & Haas Co., Philadelphia, Pa.

⁴ Duclite A-4, Chemical Process Co., Redwood City, Calif.

4.4 mM of sodium bicarbonate, and cooled to 0° C. It was then mixed with 10 ml of a solution containing 2.2 mM of NaC¹⁴N, with an activity of 8.8 mc, and 2.2 mM of sodium hydroxide.⁵ After 1 day at 0° C and 4 days at room temperature, the reaction mixture was heated at 80° C under a stream of air, with the addition of water from time to time, until the evolution of ammonia ceased. The solution was then diluted with water and passed through a column of cation exchange resin to convert the salts to the free acids. To facilitate removal of carbon dioxide, the resin (20 ml) was placed in a tube 25 mm in diameter, and agitated during the passage of the liquid so as to cause the liberated carbon dioxide to rise to the surface. The column was washed thoroughly with water, and the total effluent, to which was added 295 mg of lead carbonate (PbCO₃), was concentrated under reduced pressure to about 100 ml, and then filtered. A 500-mg quantity of carrier lead D-xylonate monohydrate was dissolved in the filtrate, the solution was concentrated under reduced pressure to about 5 ml, and methanol was added dropwise to the point of incipient turbidity. The crystalline lead D-xylonate-1-C¹⁴ monohydrate that formed in the course of 1 day was separated, and recrystallized from water by the addition of methanol. The two mother liquors were combined and concentrated under reduced pressure to remove most of the methanol. By the successive use of four additional 500-mg quantities of nonradioactive carrier, four more crops of lead D-xylonate-1-C¹⁴ monohydrate were obtained. Finally, the five crops of lead D-xylonate-1-C¹⁴ monohydrate were combined and recrystallized once from water by the addition of methanol. The recrystallized salt weighed 2.557 g and contained 3,009 μc of activity, corresponding to a radiochemical yield of 34.2 percent of the NaC¹⁴N used. The residual liquors, containing approximately 5,800 μc of carbon-14, were used for the preparation of D-lyxono-γ-lactone-1-C¹⁴, as described in section 2.5.

2.3. D-Xylonono-γ-Lactone-1-C¹⁴

A water solution of the lead D-xylonate-1-C¹⁴ described above was passed through a column containing 20 ml of cation exchange resin, which was then washed with about 1 liter of water. The total effluent was concentrated under reduced pressure, and the volume was adjusted to 50 ml. Ten-milliliter aliquots of the solution were transferred to each of five reduction tubes,⁶ and each tube was heated at 60° C under a stream of air until the solution had evaporated to dryness. The residue was dissolved in a few drops of ethylene glycol monomethyl ether (Methyl Cellosolve), seeded with crystalline D-xylonono-γ-lactone, and stored at room temperature over calcium chloride in a desiccator; it was moistened each day with a few drops of Methyl

Cellosolve. After 2 weeks, when the product appeared to be entirely crystalline, the material was used for the preparation of α-D-xylose-1-C¹⁴.

2.4. α-D-Xylose-1-C¹⁴

In order to determine suitable conditions for reduction of the C¹⁴-labeled lactone, samples of non-radioactive D-xylonono-γ-lactone were reduced with various quantities of sodium amalgam⁷ in the tubes described in footnote 6. The results, given in table 1, show that the highest yield of reducing sugar is obtained by the use of 4.6 g of 5-percent sodium amalgam per millimole of lactone. Hence this proportion was used in the preparation of D-xylose-1-C¹⁴.

TABLE 1. Reduction of 1.0 mM of D-xylonono-γ-lactone^a by sodium amalgam in the presence of sodium acid oxalate

Experiment	Sodium acid oxalate	NaHg ₂ (5-percent)	Yield of D-xylose (by analysis)
1-----	ℓ 1.6	g 2.3	% 77.6
2-----	3.2	4.6	87.0
3-----	4.8	6.9	81.3
4-----	6.4	9.2	71.8

^a In 20 ml of water.

The five aliquots of D-xylonono-γ-lactone-1-C¹⁴ described in section 2.3 (each having 600 μc in 1.84 mM) were reduced by the successive addition to each reduction tube of 5.8 g of sodium acid oxalate, 20 ml of ice water, and 8.3 g of 5-percent sodium amalgam in pellet form. The mixtures were stirred vigorously in an ice bath until the amalgam was spent, at which point the mixtures were combined and separated from the mercury. The crystalline sodium salts were removed by filtration, washed with ice water, and discarded. The filtrate, concentrated under reduced pressure to 25 ml, was treated at ice temperature with sodium hydroxide until it had a faint, but permanent, pink color in the presence of phenolphthalein indicator; it was then diluted with two volumes of methanol, and the resulting precipitate was separated, washed with methanol, and discarded after a test showed negligible radioactivity. A small third crop of salts was obtained by reconcentrating the filtrate to 10 ml, adding 50 ml of methanol, concentrating to a sirup, and finally mixing the residue with 25 ml of methanol. The insoluble residue, when separated, and thoroughly washed with methanol, likewise had no appreciable radioactivity.

The alcoholic filtrate was concentrated under reduced pressure to remove most of the methanol, then diluted with water and passed through a column containing 20 ml of mixed cation⁸ and anion exchange

⁵ A 50-percent excess of D-threose was used to insure complete utilization of the cyanide.

⁶ Heavy-walled glass tubes, each 200 by 25 mm, with a 24/40 standard-taper joint, and an oblique sidearm 50 by 15 mm, attached just below the joint, for addition of amalgam after vigorous stirring has begun. A stainless-steel standard-taper stopper, 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 aluminum thimble, having a small hole in the bottom, into a 2-ft "shot tower" of mineral oil. The pellets were stored under oil, and just before use, were blotted dry, weighed, and rinsed with benzene.

⁸ Amberlite IR 100-H, Rohm & Haas Co., Philadelphia, Pa. This resin was used in the purification of the labeled sugars in place of the Amberlite IR 120-H used elsewhere.

resins, and an additional 5 ml of cation exchange resin at the base of the column. The salt-free and neutral effluent was concentrated to approximately 25 ml under reduced pressure, filtered, and freeze-dried; the residue was dissolved in 2 ml of methanol. After 24 hr a crop of crystalline α -D-xylose-1-C¹⁴ was obtained, which, when recrystallized from a few drops of water by the addition of methanol, weighed 485 mg and had an activity of 1,052 μ c. By use of a total of 1.1 g of carrier D-xylose in four portions, an additional 630 μ c of α -D-xylose-1-C¹⁴ was recovered from the mother liquor. However, the final mother liquor contained a substantial quantity of uncrystallizable material having an activity of 1,158 μ c. This may have consisted of glycosides formed by reaction of the sugar with the alcohol used as a solvent, of oligosaccharides formed by condensation of two or more sugar molecules, or of sugar anhydrides, together with the polyhydric alcohol formed by over-reduction. In order to reclaim D-xylose-1-C¹⁴, the mother liquor was hydrolyzed by heating for 3 hr with 10 ml of 0.05 *N* aqueous hydrochloric acid in a boiling-water bath. The acid was removed by means of 10 ml of anion exchange resin, and the neutral effluent was concentrated under reduced pressure to a sirup. By use of a total of 600 mg of D-xylose in three portions as carrier, 154 μ c of D-xylose-1-C¹⁴ was obtained. As 1,682 μ c of α -D-xylose-1-C¹⁴ had been separated previously, the total yield was 1,836 μ c, or 61 percent of the activity of the lead D-xylose-1-C¹⁴ originally used.

2.5. D-Lyxono- γ -Lactone-1-C¹⁴

A solution of the residue from the preparation of lead D-xylose-1-C¹⁴ monohydrate (section 2.2) was decationized by means of a column containing 10 ml of cation exchange resin. One gram of non-radioactive D-lyxono- γ -lactone was dissolved in the effluent. The solution was concentrated under reduced pressure to a sirup and lactonized by heating it at 90° C under a jet of air for 8 hr, with the addition of 0.2-ml portions of glacial acetic acid at 2-hr intervals. The residue was dissolved in 1 ml of ethanol, and 2-propanol was added to the point of incipient turbidity. After the addition of seed crystals, and storage at room temperature for 24 hr, a crop of crystalline D-lyxono- γ -lactone-1-C¹⁴ was obtained. When recrystallized once from ethanol by the addition of 2-propanol, it weighed 574 mg and had an activity of 2,627 μ c. By use of an additional 600 mg of nonradioactive D-lyxono- γ -lactone as carrier, 1,658 μ c of lactone was obtained in three portions from the combined mother liquors. The total yield was 4,285 μ c, or 48.7 percent, of the activity of the NaC¹⁴N used in the original cyanohydrin synthesis.

2.6. α -D-Lyxose-1-C¹⁴

Prior to the preparation of α -D-lyxose-1-C¹⁴, the experiments of table 2 were conducted. The proportions of experiment 3, which gave the highest

yield of D-lyxose, were used in the preparation of the labeled sugar.

In a typical preparation, 1 mM of D-lyxono- γ -lactone-1-C¹⁴, having an activity of 657 μ c, was reduced in the manner described for D-xylo- γ -lactone-1-C¹⁴, but with the use of 22 g of 4.2-percent sodium amalgam,⁹ 8 g of sodium acid oxalate, and 20 ml of water. Stirring of the preparation was continued at 0° C for 3 hr, and the resulting β -D-lyxose-1-C¹⁴ was separated by essentially the same procedure as that described in section 2.4. A 32-mg crop of β -D-lyxose-1-C¹⁴, having an activity of 140 μ c, was obtained without carrier. By use of 600 mg of carrier D-lyxose, a total of 210 μ c of the labeled sugar was separated in three portions. Hydrolysis of the mother liquor, deionization, concentration, and use of 0.4 g of carrier in two portions gave 71 μ c of β -D-lyxose-1-C¹⁴. The total yield was 421 μ c, or 64 percent of the activity of the parent lactone.

TABLE 2. Reduction of 1.0 mM of D-lyxono- γ -lactone^a by sodium amalgam in the presence of sodium acid oxalate

Experiment	Sodium acid oxalate	NaHg ₂ (5-percent)	Yield of D-lyxose (by analysis)
1.....	<i>g</i> 6.4	<i>g</i> 9.2	% 41.0
2.....	9.6	13.8	66.3
3.....	12.8	18.4	76.2
4.....	^b 25.6	^b 36.8	50.5

^a In 20 ml. of water.

^b Added in two equal portions. Mercury was removed before the second addition.

3. References

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⁹ Equivalent to 18.4 g of 5-percent amalgam. A smaller excess of sodium acid oxalate was used than the proportions given in table 2.

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