JOURNAL OF RESEARCH of the National Bureau of Standards—A. Physics and Chemistry
Vol. 66A, No. 1, January–February 1962

Tritium-Labeled Compounds VIII. Confirmation of the Position of the Tritium in D-Glucose-6-*t* and D-Glucitol-5-*t*[1]

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(August 29, 1961)

The radiochemical purity of D-glucose-6-*t* and D-glucitol-5-*t* was established by oxidation of the labeled compounds and determination of the radioactivity of the products. The results show that, in each instance, the tritium is present solely in the position cited.

1. Introduction and Discussion

Carbohydrates position-labeled with tritium have proved useful for studying reaction mechanisms and for investigating complex biological problems. In order to interpret the results of studies conducted with tritium-labeled carbohydrates, it is essential that the position of the tritium label be accurately known, and that the carbohydrate be labeled exclusively in the position cited.

As has previously been demonstrated, D-glucose-1-*t*, prepared by reduction of D-glucono-1,5-lactone either with sodium amalgam in tritiated water or with lithium borohydride-*t* in pyridine solution, is labeled exclusively at C–1 [1].[2] In the present study, it has been shown that the D-glucose-6-*t* and D-glucitol-5-*t* distributed under the NBS labeled-sugar project [2] are labeled exclusively at C–6 and C–5, respectively.

The compounds in question were synthesized by reduction of suitable substances with lithium borohydride-*t* [3]. D-Glucose-6-*t* was obtained by hydrolysis of 1,2-*O*-isopropylidene-D-glucose-6-*t* prepared by reduction of 1,2-*O*-isopropylidene-D-glucurono-6,3-lactone [4]. D-Glucitol-5-*t* was prepared by reduction of L-sorbose, and was separated by crystallization as the D-glucitol–pyridine complex [5].

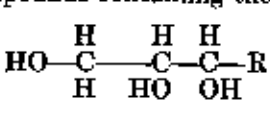
It seemed possible that, in the reduction of the carbonyl group with lithium borohydride-*t*, enolization might occur between the carbonyl group and the adjacent group, leading to unsaturation and subsequent introduction of tritium at a position other than the desired one. This study was undertaken to determine whether each of the materials was labeled only in the position cited.

In order to prove the position of tritium, the labeled sugar was so oxidized as to remove all of the hydrogen or tritium at one position or more in the carbon chain, and the molar activity of the derivative was compared with that of the original compound.

Thus, in the analysis of D-glucose-6-*t*, a sample of the sugar was oxidized with dilute nitric acid to potassium hydrogen D-glucarate [6, 7]. This compound contains no hydrogen or tritium attached to C–1 or C–6. Radioassay of the purified potassium hydrogen D-glucarate showed that it contained no tritium. Consequently, all of the tritium in D-glucose-6-*t* is at C–1 or C–6.

Next, a sample of the D-glucose-6-*t* was oxidized with iodine by the Kline–Acree method [8] to potassium D-gluconate-6-*t*, which contains no hydrogen or tritium attached to C–1. The potassium D-gluconate-6-*t* was purified by recrystallization to constant activity, and was found to have the same molar activity as the D-glucose-6-*t*. Since all of the tritium in D-glucose-6-*t* must be at C–1 or C–6, and there is no tritium at C–1, all of the tritium in D-glucose-6-*t* must be at C–6.

In the determination of the position of the label in D-glucitol-5-*t*, a bacterial oxidation with *Acetobacter suboxydans* was conducted. *A. suboxydans* attacks only those compounds containing the



group in the D-configuration, oxidizing the penultimate group to a carbonyl group [9]. In the oxidation of D-glucitol, the hydrogen (or tritium) atom at C–5, the site of oxidation, is removed, to yield L-sorbose [10, 11]. When D-glucitol-5-*t* was oxidized, the resulting L-sorbose contained no radioactivity; hence there could have been no tritium at positions other than C–5 in the D-glucitol-5-*t*.

Radioactivity assays were made with a 2π, windowless, gas-flow, proportional counter. Materials of very low activity were counted in films of sodium *O*-(carboxymethyl)cellulose (CMC) on 2-in. planchets [12, 13]. Materials of higher activity were counted in solutions of formamide [14]. Table 1 gives a summary of the results.

TABLE 1. *Analysis of* D-*glucose*-6-t *and* D-*glucitol*-5-t

Compound analyzed	Positions of non-labile hydrogen or tritium [a]	Radioactivity	Percent of original radioactivity
		mc/mmole	
D-Glucose-6-*t*	1,2,3,4,5,6*	1.10	100
↓ Potassium hydrogen D-glucarate	2,3,4,5	0.0	0
Potassium D-gluconate-6-*t*	2,3,4,5,6*	1.10	100
D-Glucitol-5-*t*	1,2,3,4,5*,6	0.075	100
↓ L-Sorbose	1,3,4,5,6	.0	0

[a] The asterisk indicates the tritium-bearing carbon atom.

[1] Part of a project on the production of radioactive carbohydrates sponsored by the Division of Research of the Atomic Energy Commission.

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

2. Experimental Procedures

2.1. Preparation of Derivatives of D-Glucose-*6-t*

a. Potassium Hydrogen D-Glucarate

A 5.669-mg sample of D-glucose-*6-t* (0.031 mmole, 1.10 mc/mmole) was combined with 101.9 mg (0.56 mmole) of nonradioactive D-glucose in a 50-ml porcelain casserole. Thirty milliliters of 1-*M* nitric acid was added, and the solution was concentrated to dryness on a steam bath. The residue was dissolved in distilled water, and the solution was transferred to a 100-ml standard-tapered flask, neutralized with 5-percent potassium carbonate solution to the phenolphthalein end-point, reacidified with glacial acetic acid, and concentrated under reduced pressure to a sirup. After the sirup had been transferred to a test tube with glacial acetic acid, potassium hydrogen D-glucarate crystallized. After about two hours, the mother liquor was removed from the potassium salt with a capillary pipet, and the solid was washed once with water. It was then recrystallized by dissolving it in the minimal amount of hot water, adding decolorizing carbon, and filtering through infusorial earth and decolorizing carbon. The yield after one recrystallization was 43 mg (29 percent). After three recrystallizations, the potassium hydrogen D-glucarate was radioassayed by counting in films of CMC. Both the sample and the background planchets (5 each) were counted to a total of 20,000 counts: they gave the same average number of counts per second.

b. Potassium D-Gluconate-*6-t*

A 36.7-mg sample of D-glucose-*6-t* (0.204 mmole, 1.10 mc/mmole) was placed in a 50-ml flask and dissolved in 1 ml of water. An excess of iodine, 4.79 ml of 0.0511-*M* solution (0.245 mmole), was added, followed by 6.16 ml of 0.0993-*M* sodium hydroxide (0.612 mmole), added dropwise while the contents of the flask were mixed. The solution was allowed to stand for about 10 min and was then passed through a column containing 5 ml of cation-exchange resin (Amberlite IR-120). To the effluent was added 0.5 mmole of freshly prepared silver carbonate, and the mixture was stirred until all iodide had been converted to silver iodide. The solid was removed by filtration, and the filtrate, which gave a negative test for iodide ion, was passed through 7 ml of cation-exchange resin. The effluent was concentrated under reduced pressure and then neutralized to the phenolphthalein end-point with dilute potassium hydroxide. The solution of potassium D-gluconate-*6-t* was filtered into a standard-tapered test tube, concentrated, and brought to crystallization by dropwise addition of methanol. After 1 day, 37 mg (77 percent) of potassium D-gluconate-*6-t* had separated. Radioassays of the compound in formamide solution after two and three recrystallizations gave activities of 1.10 and 1.09 mc/mmole, respectively.

2.2. Preparation of Derivative of D-Glucitol-*5-t*

a. L-Sorbose

A 181-mg sample of D-glucitol-*5-t* (1.0 mmole, 0.075 mc/mmole) was oxidized to L-sorbose with *A. suboxydans*, according to the procedure previously described for the oxidation of D-mannitol to D-fructose [15]. The broth and inoculum were prepared as before, but with D-glucitol (instead of D-mannitol) as the substrate. The agar slants used for maintenance of the culture were prepared from 10-ml aliquots of an aqueous suspension containing, by weight, 1.5 percent of agar, 1 percent of yeast extract, 0.3 percent of D-glucose, and 1 percent of calcium carbonate.

The L-sorbose, produced in a yield of 89 percent, was purified by several recrystallizations from methanol–2-propanol, and was assayed in films of CMC; it contained no radioactivity.

3. References

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(Paper 66A1–138)