[1] Synthesis of β-Gentiobiose-1-C^{14}

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Gentiobiose-1-C^{14} was synthesized in 24.3 percent radiochemical yield. Its preparation involved the degradation of nonradioactive gentiobiose by the Ruff method to 5-*O*-β-D-glucosyl-D-arabinose, the use of the latter material with sodium cyanide-C^{14} in the Fischer-Kiliani cyanohydrin synthesis, and the crystallization of β-gentiobiose-1-C^{14}. The procedure is relatively simple and is satisfactory for the production of gentiobiose-1-C^{14} in any desired amount.

1. Introduction

Labeled disaccharides are required for chemical, biological, bacteriological, and medical research. Methods for the preparation of C^{14}-labeled lactose and C^{14}-labeled maltose have been described in prior publications of the Bureau [1,2].[2]

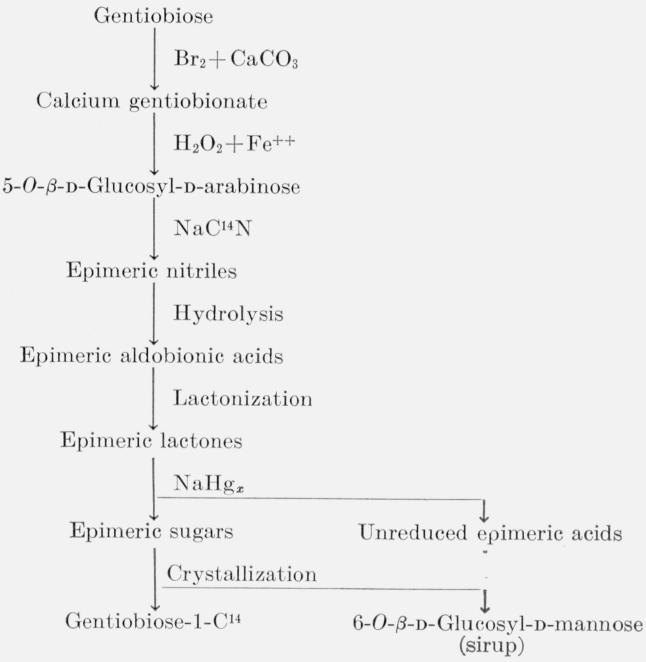
A radioactive gentiobiose with a uniformly labeled nonreducing glucose moiety was synthesized by Sowden and Spriggs [3]. The material was obtained in 11.9-percent yield by a modified Koenigs-Knorr synthesis, starting with a uniformly labeled D-glucose-C^{14}. The high cost of the starting material and the low yield make production of this material very expensive.

The present paper reports the synthesis of gentiobiose labeled in the reducing group by application of the cyanohydrin synthesis. The starting material, 5-*O*-β-D-glucosyl-D-arabinose, had been prepared previously by degradation of gentiobiose oxime by the method of Wohl [4,5]. For the present synthesis the material was prepared by the Ruff degradation [6] and was purified by the chromatographic method of Whistler and Durso [7]. The glucosyl-arabinose was allowed to react with sodium cyanide-C^{14} under conditions previously found favorable for production of the gluconic epimer. No attempt was made to separate the epimeric products of the cyanohydrin reaction prior to the sugar stage. At this point nonradioactive gentiobiose was added, and the C^{14}-labeled product was separated by cocrystallization.

The yield was 24.3 percent, based on the C^{14}-labeled cyanide used. The procedure makes gentiobiose-1-C^{14} available at reasonable cost.[3]

2. Discussion of the Method

The production of gentiobiose-1-C^{14} involved the following steps:



3. Experimental Procedure

3.1. Preparation of Calcium Gentiobionate

A solution of 26.6 g of gentiobiose in 750 ml of ice-cold water was stirred with 15 g of calcium carbonate and 5.0 ml of bromine [8]. After 24 hours in darkness the excess bromine was removed by a stream of air, and the excess calcium carbonate separated. To remove the bromide the solution was treated first with 15 g of oxalic acid and filtered, and then treated with 10 g of silver carbonate. After refiltration, the solution was cooled to 0° C, passed into a column containing 120 ml of ice-cold Amberlite IR–120H, [4] and washed through the resin with 500 ml of ice-cold water. The effluent was treated with an excess of calcium carbonate, the mixture then filtered, and the filtrate concentrated under vacuum to give amorphous calcium gentiobionate.

[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.
[2] Figures in brackets indicate the literature references at the end of this paper.
[3] Supplied to research workers by NBS at $1.00 a microcurie in quantities of from 10 to 100 μc.
[4] Product of Rohm & Haas Co., Phila., Pa.

3.2. Preparation of 5-O-β-D-Glucosyl-D-Arabinose

About 20 g of amorphous calcium gentiobionate, 1.045 g of barium acetate monohydrate, and 1.065 g of ferrous sulfate heptahydrate were combined in 300 ml of water. The mixture was heated to boiling and then filtered. After the filtrate was cooled to 40° C, 6 ml of 30-percent hydrogen peroxide was added. When the hydrogen peroxide had reacted, the solution was recooled to 40° C, and a second 6-ml portion of hydrogen peroxide was added [9]. On completion of the second reaction, the solution was cooled in ice and passed through a tube containing an ice-cold mixture of 100 ml of Amberlite IR–120H and 100 ml of Duolite A–4.[5] The mixed resin bed was washed with ice-cold water, and the combined effluent was concentrated under vacuum to about 100 ml.

The concentrated solution was introduced onto a column (75×250mm) of Darco G–60 [6] and Celite 535 [7] (1:1 by weight) [7]. The column was eluted in turn with water, 5-percent ethanol, and 10-percent ethanol. The fraction eluted with 10-percent ethanol contained the glucosyl-arabinose. It was concentrated to a 50-ml volume, and the sugar concentration was determined by titration with iodine. Portions of this solution were used in the preparation of gentiobiose–1–C^{14}.

3.3. Preparation of β-Gentiobiose-1-C^{14}

An ice-cold solution of 4.43 millimoles (mM) of sodium cyanide-C^{14} (5.6 millicuries (mc)) and 4.8 mM of sodium hydroxide in 10 ml of water was combined with 4.85 mM of 5-*O*-β-D-glucosyl-D-arabinose and 0.78 g of sodium bicarbonate dissolved in 15 ml of ice-cold water. The mixture was stoppered and stored at room temperature. After 8 days, 0. 53 g of sodium carbonate in 25 ml of water was added, and the solution was heated at 90° C under a current of air for 4 hours. The resulting product was dissolved in ice-cold water, and the solution was passed through 50 ml of ice-cold Amberlite IR–120H. The resin was washed with 200 ml of ice-cold water, and the combined effluent, which assayed 5.11 mc, was freeze dried. The residue was dissolved in Methyl Cellosolve (ethylene glycol monomethyl ether), and the solution was transferred in approximately equal portions into 4 glass reduction tubes described previously [1]. Under a gentle stream of air the solvent was evaporated at room temperature. Fresh solvent was added to the concentrate, and the evaporation repeated. After 2 weeks of repeated cycles of dilution (with Methyl Cellosolve) and concentration, the tubes were stored for 1 week in a desiccator over calcium chloride. Then in each of the tubes, which were fitted with an efficient stirrer and immersed in an ice bath, the lactonized material was treated through the side arm of the tube with 2.0 g of sodium acid oxalate and 20 ml of ice-cold water; vigorous stirring was begun, and 3.5 g of pellets of 5.1-percent sodium amalgam was quickly added. After 2 hours of vigorous stirring the mixture was neutralized with sodium hydroxide, treated with 3 volumes of methanol, and filtered. The filtrate was concentrated under vacuum to about 15 ml, treated with 6 volumes of methanol, and refiltered. It was again concentrated under vacuum, diluted to 30 ml with water, and passed, ice cold, through a column containing 60 ml of a mixture of Amberlite IR–120H and Duolite A–4 at ice temperature. The resin was washed with 300 ml of ice-cold water, and the total effluent was freeze dried. When the residue was treated with a few milliliters of methanol, it yielded a small amount of insoluble material, which was removed by filtration. The solutions from the four reduction mixtures, each of which had been treated as described above, were combined into two parts, and these were concentrated. The first part was treated with 1 g of nonradioactive β-gentiobiose as carrier. A little water was added to dissolve the mixture, which was then warmed to 55° C, treated with absolute ethanol to incipient turbidity, and seeded with β-gentiobiose [10]. As the crystallization proceeded, additional ethanol was added. The radioactive sugar thus crystallized was added as a first carrier to the second part of the product obtained from the reduction. After a similar crystallization process, and recrystallization, 508 mg of β-gentiobiose-1-C^{14} assaying 1.16 μc/mg was obtained. In all, 4 carrier crystallizations were run, and the total radioactive gentiobiose obtained amounted to 1.36 mc. Based on 5.6 mc of radioactive cyanide, the radiochemical yield of β-gentiobiose-1-C^{14} was 24.3 percent.

The unreduced acids, which had been separated from the sugars by the mixed resins, were recovered in the amount of 1.8 mc by treatment of the mixed resin with 10-percent acetic acid.

[5] Product of Chemical Process Co., Redwood City, Calif.
[6] Product of Atlas Powder Co., New York, N. Y.
[7] Product of Johns-Manville Co., New York, N. Y.

4. References

[1] H. L. Frush and H. S. Isbell, J. Research NBS **50,** 133 (1953) RP2400.
[2] H. S. Isbell and R. Schaffer, J. Am. Chem. Soc. **78.** 1887 (1956).
[3] J. C. Sowden and A. S. Spriggs, J. Am. Chem. Soc. **76.** 3539 (1954).
[4] A. Wohl, Ber. deut. chem. Ges. **26,** 730 (1893).
[5] N. S. MacDonald and Wm. L. Evans, J. Am. Chem. Soc. **64,** 2731 (1942).
[6] O. Ruff and G. Ollendorf, Ber. deut. chem. Ges. **33,** 1798 (1900).
[7] R. L. Whistler and D. F. Durso, J. Am. Chem. Soc. **72,** 677 (1950).
[8] C. S. Hudson and H. S. Isbell, J. Am. Chem. Soc. **51,** 2225 (1929).
[9] R. C. Hockett and C. S. Hudson, J. Am. Chem. Soc. **56,** 1632 (1934).
[10] A. Thompson and M. L. Wolfrom, J. Am. Chem. Soc. **75,** 3605 (1953).

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