Carbohydrate Research, 109 (1982) 161–166
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESIS OF D-[6-^{13}C]GLUCOSE, 2-AMINO-2-DEOXY-D-[1-^{13}C]GLUCOSE HYDROCHLORIDE, AND 2-[^{15}N]AMINO-2-DEOXY-D-GLUCOSE HYDROCHLORIDE*

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(Received January 20th, 1982; accepted for publication, February 16th, 1982)

ABSTRACT

D-[6-^{13}C]Glucose, 2-amino-2-deoxy-D-[1-^{13}C]glucose hydrochloride, and 2-[^{15}N]amino-2-deoxy-D-glucose hydrochloride have been synthesized for use in biosynthetic studies. The two amine hydrochlorides were prepared by a series of reactions involving D-arabinose and 2-(benzylamino)-2-deoxy-D-gluconononitrile. The intermediates employed in preparing D-[6-^{13}C]glucose were D-glucose, 1,2-*O*-isopropylidene-α-D-glucofuranose, 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose dimer, barium 1,2-*O*-isopropylidene-α-D-[6-^{13}C]glucofuranuronate, and 1,2-*O*-isopropylidene-α-D-[6-^{13}C]glucofuranose.

INTRODUCTION

Recent biosynthetic studies of several antibiotic substances conducted in our laboratory have made extensive use of precursors labeled with carbon-13, or some other stable isotope. An especially important and generally useful substrate has proved to be D-[6-^{13}C]glucose (**9**), which we have employed in biosynthetic studies of neomycin[1], streptomycin[2], spectinomycin[3], pactamycin[4], chloromycetin (chloramphenicol)[5], geldanamycin[6], and nybomycin[7]. In studies of the biosynthesis of the aminocyclitol antibiotic neomycin, 2-amino-2-deoxy-D-[1-^{13}C]glucose[1] (**3**) and 2-[^{15}N]amino-2-deoxy-D-glucose[3] (**5**) proved equally useful. The details of our routes to these labeled monosaccharides, not previously given, are now recorded.

DISCUSSION

The routes, involving modifications of previously described intermediates and procedures, were designed to make optimum use of the expensive carbon-13, by adding this label at a late stage in the synthesis and, in some cases, employing vacuum-

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.
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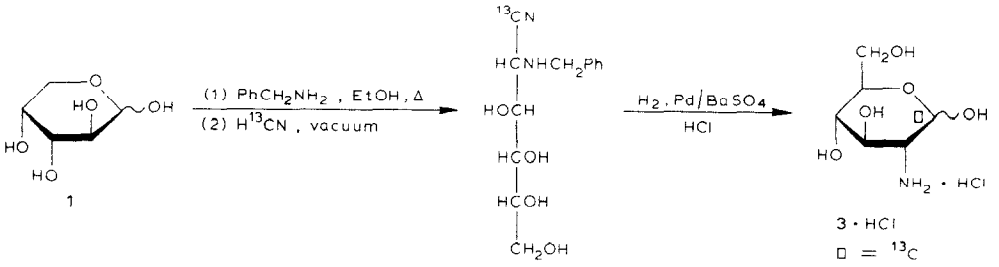
line techniques to transfer and recover hydrogen [^{13}C]cyanide. The route to compound **3** was also used for the preparation of compound **5**.

Compound **3** was synthesized by the procedure of Kuhn and Kirschenlohr[8], by converting D-arabinose (**1**) into *N*-benzyl-D-arabinosylamine, followed by addition of hydrogen [^{13}C]cyanide, to yield 2-(benzylamino)-2-deoxy-D-[1-^{13}C]glucononitrile (**2**). Compound **2** was then hydrogenated, and the amine was acidified with hydrochloric acid, to give the hydrochloride of the desired product (**3**).



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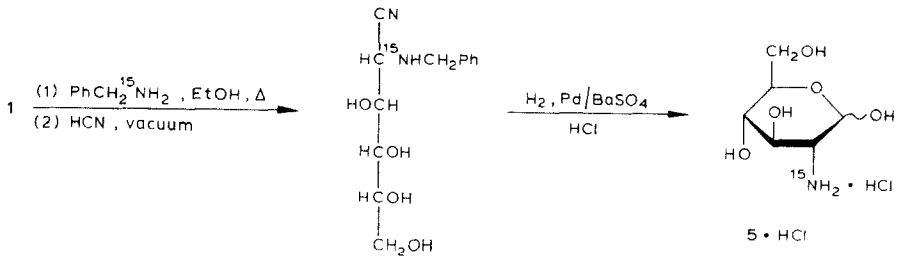
Compound **5** was prepared by the same route, except that [^{15}N]benzylamine and unlabeled hydrogen cyanide were employed. The overall route is the same as that employed by Hornemann[9] for the preparation of compound **5**, but the experimental details differ.



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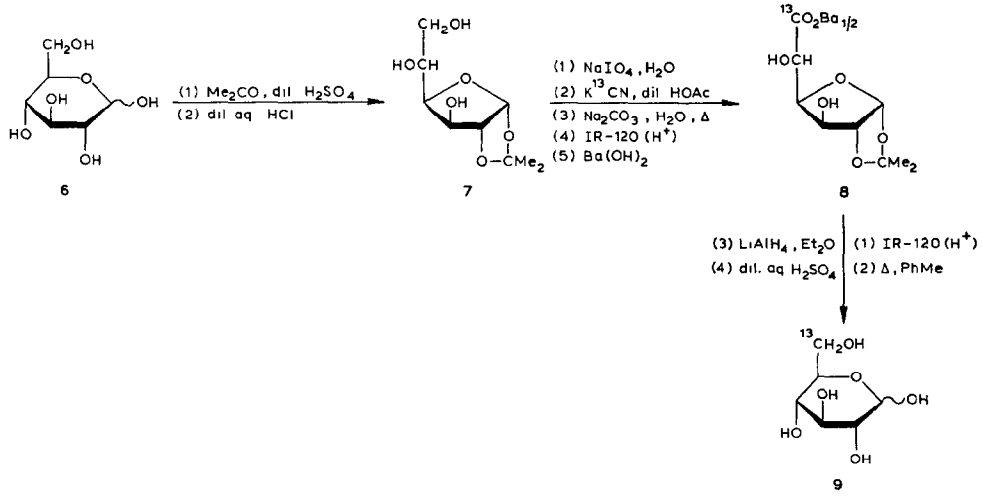
D-[6-^{13}C]Glucose (**9**) was prepared by minor modifications of the procedure of Schaffer and Isbell[10,11]. Unlabeled D-glucose (**6**) was converted, *via* 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose[12,13] and 1,2-*O*-isopropylidene-α-D-glucofuranose[12,13] (**7**) into the 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose dimer[10]; this was treated with potassium [^{13}C]cyanide, and the product was converted into barium 1,2-*O*-isopropylidene-α-D-[6-^{13}C]glucofuranuronate monohydrate (**8**), a known compound in the 6-^{14}C-labeled form[10,11], and **8** was transformed, by acidification, lactonization of the acid, reduction of the lactone, and hydrolysis of the acetal, into D-[6-^{13}C]glucose (**9**).



The overall yield of **9** was 17%, based on the potassium [^{13}C]cyanide consumed, whereas that of **3** was 51% on the same basis. The yield of **5** was 62%, based on [^{15}N]benzylamine (or 48%, based on sodium [^{15}N]nitrate).

EXPERIMENTAL

General. — All melting points were determined on a Kofler micro hot stage and are uncorrected. Infrared spectra were recorded with a Perkin–Elmer 521 or a Beckman IR-12 spectrophotometer. ^{1}H-N.m.r. spectra were determined with either a Varian A-60 or an HA-100 spectrometer, using tetramethylsilane as the internal standard in deuteriochloroform solution. Optical rotations were determined with a Zeiss polarimeter. Paper chromatography was performed by the ascending technique on Whatman No. 3MM paper at 25°. Chromatograms were detected with ninhydrin or alkaline silver nitrate reagent.

Preparation of 2-amino-2-deoxy-D-[*1*-^{13}*C*]*glucose hydrochloride* (**3**). — The synthesis of **3** was conducted by modification of the procedure of Kuhn and Kirschenlohr[8]. *N*-Benzyl-D-arabinosylamine was prepared by boiling a mixture of D-arabinose (**1**; 2.2 g, 14.6 mmol), benzylamine (2.2 mL), and absolute ethanol (5.0 mL) under reflux for 20 min, to give a clear solution which was cooled to room temperature.

Hydrogen [^{13}C]cyanide was generated by the dropwise addition of M sulfuric acid (30 mL) to potassium [^{13}C]cyanide (57 atom%, 1.07 g, 16.5 mmol), and carried, in a slow stream of helium, through a tube containing Aquasorb (Mallinckrodt Chemical Works, St. Louis, MO), to remove water, and then through two traps (cooled by liquid nitrogen) on a high-vacuum line. Sweeping the reaction mixture for 2 h gave a 78% yield of hydrogen [^{13}C]cyanide, obtained by using high-vacuum techniques. One vacuum-line connection was equipped with a stopcock attached to a hypodermic needle. A 300-mL reaction vessel equipped with a rubber septum was

connected *via* the needle to the vacuum line; then the vessel was evacuated, and cooled by liquid nitrogen, and the hydrogen [^{13}C]cyanide was introduced by performing a vacuum distillation. Next, the vessel was disconnected from the vacuum line, and the hydrogen [^{13}C]cyanide was allowed to warm to room temperature. Finally, the ethanolic solution of the *N*-benzyl-D-arabinosylamine was injected into the vessel containing the hydrogen [^{13}C]cyanide, and the mixture was shaken overnight at 25°. The resulting precipitate was filtered off, and washed with a small volume of cold ethanol, before being recrystallized from ethanol. Colorless leaflets of **2** (2.6 g, 59%), m.p. 124–126° (lit.[8] m.p. for L isomer, 130–132°) were obtained.

The products of two such experiments were combined, and **2** (3.5 g, 13 mmol) was hydrogenated in 0.5M aqueous hydrochloric acid (61 mL) in the presence of 5% palladium-on-barium sulfate (500 mg). After 14 h, 700 mL of hydrogen had been absorbed (theoretical, 582 mL), and the catalyst was filtered off, and washed with water. The filtrate and washings were combined, evaporated *in vacuo*, the residue dissolved in water (10 mL), the acid neutralized with Amberlite IR-45 (OH^-) resin, and the mixture filtered. The filtrate was added to a column of Amberlite IR-120 (H^+) resin (100 mL), which was washed with water until the eluate was neutral, and then with M hydrochloric acid. Acidic fractions were found to give a negative Nessler (ammonium ion) test. The eluates were combined, and evaporated to dryness *in vacuo*; a small volume of methanol was added to the residue, and the resulting precipitate was filtered off, and washed with a small volume of cold methanol; yield 2.45 g (87%). Paper chromatograms using the solvent systems 10 : 7 : 3 ethyl acetate–pyridine–water (R_F 0.18) and 2 : 1 : 1 1-butanol–pyridine–water (R_F 0.22) showed only one spot after development thereof by drying the paper and spraying it with ninhydrin reagent. The infrared (i.r.) spectrum of the isotopic material was superposable on that of authentic 2-amino-2-deoxy-D-glucose hydrochloride.

Preparation of 2-[^{15}N]amino-2-deoxy-D-glucose hydrochloride (**5**). — Compound **5** was prepared, in similar yield, by the same procedure as that for compound **3**, employing [^{15}N]benzylamine and unlabeled hydrogen cyanide. [^{15}N]Benzylamine was prepared from [^{15}N]ammonium nitrate *via* [^{15}N]benzamide. Employing the procedure of Swan and Kelly[14], the yield of [^{15}N]benzamide was 96% (based on [^{15}N]ammonium nitrate); m.p. 126.5–127.5° (lit.[9] m.p. 127°), and a thin-layer chromatogram showed one spot, having an R_F value identical with that of an authentic sample of benzamide. A solution of [^{15}N]benzamide (19.7 g, 161 mmol) in oxolane (400 mL) was then added dropwise during 1.5 h to a suspension of lithium aluminum hydride (10 g, 265 mmol) in oxolane (300 mL) at room temperature. After the last addition of amide, the mixture was stirred and boiled under reflux for 3 h. Water (10 mL), 15% aqueous sodium hydroxide (10 mL), and water (100 mL) were successively added, the precipitate was filtered off, the oxolane in the filtrate was evaporated, solid sodium chloride was added, the solution was extracted with ether, and the extract was dried (anhydrous sodium sulfate), and evaporated; distillation of the residue gave 14 g (80%) of colorless [^{15}N]benzylamine, b.p. 183–186°, whose i.r. spectrum was superposable on that of authentic benzylamine.

Preparation of D-[6-^{13}]*glucose* (**9**). — The procedure of Mehltretter *et al.*[12] and of Schmidt[13] was followed for the preparation of 1,2-*O*-isopropylidene-α-D-glucofuranose from **6**. Following partial hydrolysis of the diisopropylidene acetal with hydrochloric acid, pH 2, the aqueous layer was washed with two 250-mL portions of benzene, the acid neutralized with silver carbonate, the precipitate filtered off and washed with water, and the filtrate and washings were combined, and evaporated to dryness *in vacuo*. Recrystallization from ethyl acetate gave colorless crystals of 1,2-*O*-isopropylidene-α-D-glucofuranose (**7**); 45 g (37%), m.p. 151–154° (lit.[13] m.p. 161–162.5°).

The dimer of 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose was prepared by a modification of the method of Schaffer and Isbell[10], employing 44 g (0.20 mol) of **7**. The chloroform extract was dried (anhydrous sodium sulfate), decolorized with charcoal, and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel by eluting with chloroform containing 3–5% of methanol, and the fractions containing the main component were combined, and evaporated to dryness *in vacuo*. A heavy syrup was obtained that showed no carbonyl absorption band in the i.r. spectrum, but whose ^{1}H-n.m.r. spectrum showed a very weak aldehyde peak at δ 9.5. The yield of the dimer was 29.5 g (79%); it gave a semicarbazone derivative, m.p. 206–209° (lit.[15] m.p. 209–209.5°).

1,2-*O*-Isopropylidene-α-D-*xylo*-pentodialdofuranose dimer (5.55 g; 29.5 mmol, calculated as the monomer) was converted into barium 1,2-*O*-isopropylidene-α-D-[6-^{13}C]glucofuranuronate (**8**) by reaction with potassium [^{13}C]cyanide (65.0 atom%, 1.65 g, 25 mmol), employing the procedure of Schaffer and Isbell[10,11]. After removal of the excess of the barium ion as the carbonate, the filtrate was concentrated to ~50 mL *in vacuo*, nucleated with an authentic sample of **8**, and cooled in an ice bath. The resulting precipitate was filtered off, and successively washed with small volumes of cold water and 50% aqueous methanol, to give colorless crystals (2.4 g, 30% based on K^{13}CN) of the barium salt, which was directly converted into **9** by a slight modification of the procedure of Schaffer and Isbell[10,11]. After the barium salt **8** (2.4 g) had been acidified, the resulting acid lactonized, the lactone reduced, and the isopropylidene acetal hydrolyzed with acid[10,11], the resulting solution was passed through a column containing Amberlite IR-45 (OH^-) resin (100 mL), the eluate and washings combined, and concentrated to ~100 mL *in vacuo*, and the concentrate freeze-dried, to give a colorless residue. Treatment of the residue with methanol and isopropyl alcohol gave a colorless precipitate of D-[6-^{13}C]glucose (**9**): 780 mg (58%); $[\alpha]_D^{25}$ +50.0° (*c* 10.2, H_2O). The isotopic material was analyzed by paper chromatography, with development with 2:1:1 1-butanol–pyridine–water; only one spot was found (R_F 0.30), identical with that for authentic D-glucose.

ACKNOWLEDGMENT

This work was supported by a research grant (AI 01278) from the National Institute of Allergy and Infectious Diseases. It is an honor for us to extend our best wishes to Professor Sumio Umezawa on the occasion of his 73rd birthday.

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