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Note

Synthesis of 2-amino-2-deoxy-D-[1-^{13}C]glucose and 2-amino-2-deoxy-D-[1-^{13}C]mannose

THOMAS E. WALKER* AND ROBERT BARKER
Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824 (U.S.A.)
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The synthesis of [1-^{13}C]-enriched sugars cannot be accomplished in high yields by exactly the same procedures used for the [^{14}C] analogs. In the case of 2-amino-2-deoxy-D-glucose, synthesis of the [1-^{13}C] analog has been reported[1]; however, no experimental details were given. Although two methods for preparing 2-amino-2-deoxy-D-[1-^{14}C]glucose have been reported[2], both utilized ^{12}C carrier. In the first method, a small amount of labeled cyanide was added to *N*-phenyl-D-arabinosylamine, followed by a much larger quantity of unlabeled cyanide. The second method involved the addition of labeled cyanide to a sample of the unlabeled nitrile under exchange-reaction conditions. Neither method is applicable for the synthesis of highly enriched ^{13}C amino sugars.

In the present study, [^{13}C]cyanide is mixed in approximately equimolar amount with *N*-benzyl-D-arabinosylamine and acetic acid to generate H^{13}CN *in situ*. This procedure obviates the handling of the small amounts of hydrogen cyanide involved in the literature[3] synthesis of 2-amino-2-deoxyglucopyranose, and provides relatively high yields without using an excess of cyanide. The major drawback to the method is the need to separate the products from the salt formed during generation of the cyanide. Although the enriched gluconononitrile derivative readily crystallizes from the mixture, the mother liquor contains sodium acetate, unreacted starting-material, and a mixture of the glucono- and mannono-nitrile derivatives, which may be recovered after conversion into the reducing sugars.

After crystallization of the gluconononitrile derivative, the mother liquor is processed quickly to prevent hydrolysis or epimerization. The solution is evaporated to an oil at 30° and treated with sufficient hydrochloric acid to make the solution neutral, and then 0.5M with respect to the acid. After reduction and concentration, the mixture contains 2-amino-2-deoxy-D-glucose and -mannose hydrochlorides, plus sodium chloride and, perhaps, some arabinose. The amino sugars are isolated by

*Present address: Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87545, U.S.A.

TABLE I

^{13}C CHEMICAL SHIFTS OF THE AMINO [1-^{13}C]SUGARS

Compound	*Chemical shift (p.p.m.)*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
2-Amino-2-deoxy-α-D-[1-^{13}C]glucopyranose	90.7	56.0 (d 44.3)[b]	71.2	71.2	73.1 (d 1.5)	62.0 (d)[c]
2-Amino-2-deoxy-β-D-[1-^{13}C]glucopyranose	94.3	58.5 (d 43.8)	73.6 (d 2.4)	71.2	77.6	62.0 (d)
2-Amino-2-deoxy-α-D-[1-^{13}C]mannopyranose	91.8	56.0 (d 44.7)	68.3	67.6	73.1 (d 1.7)	61.7 (d)
2-Amino-2-deoxy-β-D-[1-^{13}C]mannopyranose	92.4	57.1 (d 41.3)	70.9 (d 1.8)	67.4	77.4	61.8 (d)

[a]All resonances are given in p.p.m. downfield from external tetramethylsilane. [b]The d designates a resonance observed as a doublet; couplings are given in Hz. [c]Coupling observed, but not measurable because of overlapping resonances.

chromatography on Dowex-50 (H^+) resin. Separation of the *manno* and *gluco* epimers was accomplished partially by this chromatographic separation, and completed by fractional recrystallization. The overall yield of amino sugars was 67%, based on sodium [^{13}C]cyanide.

According to Kuhn[3], *N*-benzylarabinosylamine may be produced *in situ* and treated directly with $Na^{13}CN$; however, this procedure gives lower yields of purified products.

^{13}N.m.r.-spectral assignments of the ^{13}C-enriched amino sugars were in general agreement with published data[4,5]. The intense ^{13}C-1 signals for the *gluco* epimer were in 2:1 ratio for the α and β anomers respectively, whereas the *manno* epimer gave a 1:2 ratio for the α and β anomers. C-2 for both epimers gave peaks for both α and β anomers, having splittings consistent with 90% enrichment at C-1. The chemical shifts of the enriched compounds are listed in Table I. The coupling constants between C-1 and other atoms have been published earlier[5].

As expected, the i.r. spectra of the enriched compounds display several peaks that are shifted with respect to the natural-abundance compounds[1]. Most noteworthy is the CN stretching frequency in the glucononitrile derivative, which shifted from 2238 cm^{-1} in the natural-abundance compound to 2185 cm^{-1} in the ^{13}C-enriched compound.

EXPERIMENTAL

Materials and methods. — Benzylamine, D-arabinose, and palladium chloride were obtained from Sigma Chemical Company. Sodium [1-^{13}C]cyanide (90% enriched) was a gift from Max Goldblatt of the Stable Isotope Resource at the Los Alamos Scientific Laboratory; it contained approximately 8% of sodium hydroxide.

Palladium oxide hydrate on barium sulfate was prepared as described by Kuhn and Haas[6]. Pulse ^{13}C n.m.r. spectra (15.08 MHz) were obtained for aqueous solutions with a Bruker WP-60 spectrometer. All spectra were obtained at ambient temperature (about 40°) with the spectrometer locked to the resonance of D_2O contained in a capillary tube. Spectra were recorded with a spectral width of 3623 Hz and 4K spectral points with a 13-μsec (90°) pulse, with proton-noise decoupling. The acquisition time was 1.13 sec, with no pulse delay. All chemical shifts are given relative to external tetramethylsilane. Melting points were measured with a Thomas–Hoover oil-bath apparatus, and are uncorrected. I.r. spectra for KBr pellets were obtained with a Perkin–Elmer Model 215 spectrophotometer. Sugars in solution were determined by the ferricyanide-reduction method as described by Park and Johnson[7], or by the liberation of formaldehyde[8] following treatment with sodium periodate.

N-Benzyl-D-arabinosylamine. — D-Arabinose (20 g, 133 mmol) was added to 60 ml of ethanol containing benzylamine (16 ml, 146 mmol). The suspension was stirred and heated until all of the sugar had dissolved (10–15 min); on cooling to 40°, the product crystallized to a solid mass. The mixture was filtered and the crystals were washed with ethanol and ether to give 23.5 g (98.7 mmol, 74%) of product in four crops; m.p. 115–116°; lit.[1] m.p. 113–115°.

2-Benzylamino-2-deoxy-D-[1-^{13}C]glucononitrile. — *N*-Benzyl-D-arabinosylamine (19.1 g, 80 mmol) was dissolved with heating and stirring in 150 ml of ethanol in a 500-ml, round-bottomed flask. While the solution was still warm, sodium [1-^{13}C]-cyanide (3.75 g, 75 mmol) was added and allowed to dissolve with continued stirring and heating. The solution was cooled in an ice bath to room temperature and then acetic acid (4.68 ml, 81.5 mmol) in ethanol (50 ml) was added through a dropping funnel. After several min, crystallization began and the flask was stoppered and kept for 1 h at room temperature and then for 1 h at −10°. The crystals were filtered off, washed with ethanol and ether, and dried over phosphorus pentaoxide *in vacuo* to give 8.6 g (32.3 mmol, 43%), m.p. 124–125°; lit.[1] m.p. 130–132°.

A second crop of crystals (2.4 g) was obtained from the mother liquor by the addition of ethyl acetate; however, this material was determined to be a salt (melting point >260°) that lacked contiguous hydroxyl groups[8]. The mother liquor was evaporated to an oil at 30° *in vacuo*, acidified, and reduced without further purification to produce both 2-amino-2-deoxy-D-glucose and -mannose.

2-Amino-2-deoxy-D-[1-^{13}C]glucose. — 2-Benzylamino-2-deoxy-D-[1-^{13}C]gluco-nonitrile was reduced with palladium oxide on barium sulfate in a Parr pressure-reaction apparatus. The catalyst (2 g) was first reduced at 60 lb. in.$^{-2}$ in 40 ml of water for about 10 min. The nitrile (8.6 g, 32.3 mmol) was then added, together with 75 ml of hydrochloric acid and 30 ml of water. The reduction was started at 60 lb. in.$^{-2}$ and allowed to proceed for 11 h to a pressure of 40 lb. in.$^{-2}$. The catalyst was centrifuged off, and washed, and the combined supernatants were filtered. The filtrate was evaporated and the product (2-amino-2-deoxy-D-glucose hydrochloride) was crystallized from water–ethanol; yield: 4.56 g (21.1 mmol, 65%), m.p. 190° (decomp.). The mother

liquors were saved, and additional product was obtained by chromatography and crystallization as described in the following section.

2-Amino-2-deoxy-D-[*1*-^{13}C]*mannose.* — The oily residue from crystallization of the gluconononitrile (see foregoing) was dissolved in 150 ml of water containing sufficient hydrochloric acid to titrate the sodium acetate present and to produce a final concentration of 0.5M hydrochloric acid. The mixture was filtered to remove 130 mg of brown crystals melting at 156–158° (dec.), which were possibly a nitrile hydrochloride [an authentic sample of D-gluconononitrile recrystallized from ethanol-M hydrochloric acid had m.p. 149–150° (dec.)]. The filtrate was hydrogenated at 60 lb. in.$^{-2}$ for 6 h with 4 g of pre-reduced palladium oxide on barium sulfate. The catalyst was centrifuged off, and washed, and the supernatant filtered. The filtrate was evaporated to about 20 ml, 30 ml of methanol was added, and the solution was stored overnight at 4°. The crystals that formed (1.0 g) were identified as sodium chloride.

The filtrate was evaporated to an oil that was fractionated by chromatography and crystallization to yield 2.20 g of 2-amino-2-deoxy-D-glucose · HCl, 1.22 g of 2-amino-2-deoxy-D-mannose · HCl, and 1.6 g of a mixture of the epimers. The total recovery of ^{13}C as the desired products was 67%.

The most satisfactory separation of the epimers was obtained by using a column (2 × 50 cm) of Dowex-50 (H^+), 200–400 mesh, for the fractionation of 3–4 mmol of the mixture of amino sugars. The column was developed with a gradient produced by mixing 1 liter of 0.5M hydrochloric acid into 1 liter of water. Fractions (10 ml) were taken, and analyzed by the periodate-chromotropic acid method[8], and the ferricyanide-reduction method[7]. A small proportion of a ^{13}C-enriched compound containing a carbonyl group emerged first, followed by the *gluco* isomer, and then the *manno* isomer. Peaks were broad, and some mutual contamination was apparent by n.m.r. analysis. The presence of an excess of sodium chloride interfered with the separation. Pure substances could be obtained by concentration of pooled fractions to low volume and crystallization by the addition of methanol.

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