THE SYNTHESIS OF PER-C-DEUTERATED D-GLUCOSE*

HANS J. KOCH AND RONALD S. STUART

Merck Frosst Laboratories, P.O. Box 1005, Pointe Claire-Dorval, Quebec H9R 4P8 (Canada)

ABSTRACT

A new, isotopic hydrogen-exchange technique that allows the introduction of deuterium by catalytic exchange with carbon-bound hydrogen was employed for the preparation of per-C-deuterated D-glucose.

INTRODUCTION

Fully C-deuterated D-glucose is available from the hydrolyzate of the carbohydrate fraction of algae grown in a deuterated medium. In addition to the difficulties encountered in growing deuterated algae, the procedure requires large quantities of deuterium oxide. The isolation of D-glucose-$^2H_7$ from the complex carbohydrate mixture is tedious.

In a recent communication[1], we described a method for the direct, catalytic C-deuteration of carbohydrate derivatives with deuterium oxide in the presence of deuterated Raney nickel at those carbon atoms that are bound to free hydroxyl groups. This method simply involves the boiling under reflux of the carbohydrate derivative, in deuterium oxide, with deuterated Raney nickel. The exchange appears to involve a dehydrogenation–deuterogenation of the alcohol groups. In the case of cyclic polyhydric alcohols, the exchange occurs with little loss of configuration, because the rate of isomerization is much lower than that of deuterium exchange. This seems to suggest that the molecule is attached to the nickel surface at several positions, and does not leave, or alter its relative position to, the catalytic surface during the exchange steps. However, for such polyhydric alcohols as alditols or aldonic acids, which have less restricted conformations, isomerizations are quite rapid. Thus, D-gluconic acid is not a suitable material for exchange prior to preparation of C-deuterated D-glucose through reduction of the deuterated 1,5-lactone. Similarly, D-glucose cannot be exchanged directly, because of its rapid reduction to D-glucitol and the deactivation of the catalyst.

DISCUSSION

The preparation of deuterated Raney nickel from the nickel–aluminum alloy and sodium deuteroxide in deuterium oxide is well known[2]. This procedure is both costly and time consuming. As the hydrogen adsorbed on Raney nickel exchanges rapidly with the deuterium of deuterium oxide, the deuterated Raney nickel was prepared by washing light* Raney nickel prepared in the usual[3] way, or the commercial product (W. R. Grace No. 28 Raney active nickel catalyst), with deuterium oxide in a chromatography column.

As was pointed out previously[1,4], the proton-decoupled ^{13}C-n.m.r. spectra of partially deuterated carbohydrates allow a very facile determination of the sites of deuteration, because the signals of carbon atoms having directly bonded deuterium atoms become very weak and broad, and seemingly disappear from the spectrum; this requires that the assignments of the signals be known. The spectra of the methyl glycopyranosides have been assigned[5,6].

The deuterium atom at C-1 of the fully C-deuterated D-glucose was introduced by reduction of D-glucono-1,5-lactone with deuterated borane in tetrahydrofuran. All of the other deuterium atoms were introduced by catalytic exchange of suitable derivatives. 1,2-O-Isopropylidene-α-D-glucofuranose-1-^{2}H permitted the exchange at C-5 and C-6; methyl α-D-glucopyranoside-1,5,6,6-^{2}H$_4$ permitted the exchange at carbon atoms 2, 3, and 4. This reaction sequence was also reversed, namely, exchanging at carbon atoms 2, 3, 4, and 6 of methyl α-D-glucopyranoside-1-^{2}H and then exchanging at C-5 of 1,2-O-isopropylidene-α-D-glucofuranose-1,2,3,4,6,6-^{2}H$_6$; however, the former sequence was the more convenient.

D-Glucono-1,5-lactone (1) was reduced with deuterated borane in tetrahydrofuran (THF) to give D-glucose-1-^{2}H (2). The reduction of aldonolactones with borane in THF has been reported[7]; this reaction is particularly useful for the reduction of 1,5-lactones of the *gluco* configuration[7,8], and is more convenient than reduction with sodium amalgam in deuterium oxide[9]. Compound 2 was converted into 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose-1-^{2}H (3) (ref. 10) which, in turn, was partially hydrolyzed to 1,2-O-isopropylidene-α-D-glucofuranose-1-^{2}H (4) (ref. 11) by using well established but slightly modified methods. Both 3 and 4 also serve as derivatives for purposes of identification. Compound 4 was heated under reflux in deuterium oxide containing deuterated Raney nickel, to give, primarily, 1,2-O-isopropylidene-α-D-glucofuranose-1,5,6,6-^{2}H$_4$ (5) ($\sim$60%), 1,2-O-isopropylidene-β-L-idofuranose-1,5,6,6-^{2}H$_4$ ($\sim$20%), and small amounts of the correspondingly deuterated 6-deoxy compounds. The approximate composition was determined from the relative intensities of the ^{13}C-n.m.r. signals of the mixture, and by g.l.c. of the peracetylated mixture. As C-5 is exocyclic, isomerization occurs quite readily at that carbon atom, as reported previously for alditols[1]. Deuterium exchange does not occur at C-3, because of the

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*The term "light" refers to undeuterated material.

proximity of the 1,2-*O*-isopropylidene group[1]. The reduction at C-6 to the 6-deoxy compounds is not surprising, as it also occurs with alditols[8]. The isomerization and reduction will be reported separately.

One recrystallization of the mixture from ethyl acetate afforded pure 1,2-*O*-isopropylidene-α-D-glucofuranose-1,5,6,6-$^2H_4$ (5) which is less soluble than the corresponding L-idose derivative. Compound 5 was converted into methyl α-D-glucopyranoside-1,5,6,6-$^2H_4$ (6) by reaction with methanol containing anhydrous hydrogen chloride. (Compound 7 could also be prepared directly from the mixture of deuterated 1,2-*O*-isopropylidene derivatives, although in lower yield.) Compound 6 was then deuterated at carbon atoms 2, 3, and 4 in refluxing deuterium oxide containing deuterated Raney nickel, to give methyl α-D-glucopyranoside-1,2,3,4,5,6,6-$^2H_7$ (7), the methyl α-pyranoside of fully *C*-deuterated D-glucose. The methyl signal of 7 has the same chemical shifts, in both the p.m.r. and proton-decoupled, ^{13}C-n.m.r. spectra, as methyl α-D-glucopyranoside; however, all of the other signals are absent. Similarly, the tetraacetate of 7 and of "light" methyl α-D-glucopyranoside were compared. The p.m.r. and proton-decoupled, ^{13}C-n.m.r. signals due to the glycosidic methyl groups and the four acetoxyl groups were identical; in the deuterated compound, no other signals were present. Methyl α-D-glucopyranoside-1,2,3,4,5,6,6-$^2H_7$ was hydrolyzed to D-glucose-1,2,3,4,5,6,6-$^2H_7$ (8, D-glucose-$^2H_7$) with 3M hydrochloric acid.

The R_F or R_G (glucose) values of fully *C*-deuterated carbohydrates on paper chromatograms are generally slightly lower than those of the corresponding "light" compounds. D-Glucose-$^2H_7$ (8) and D-glucose-$^2H_7$ obtained from deuterated algae
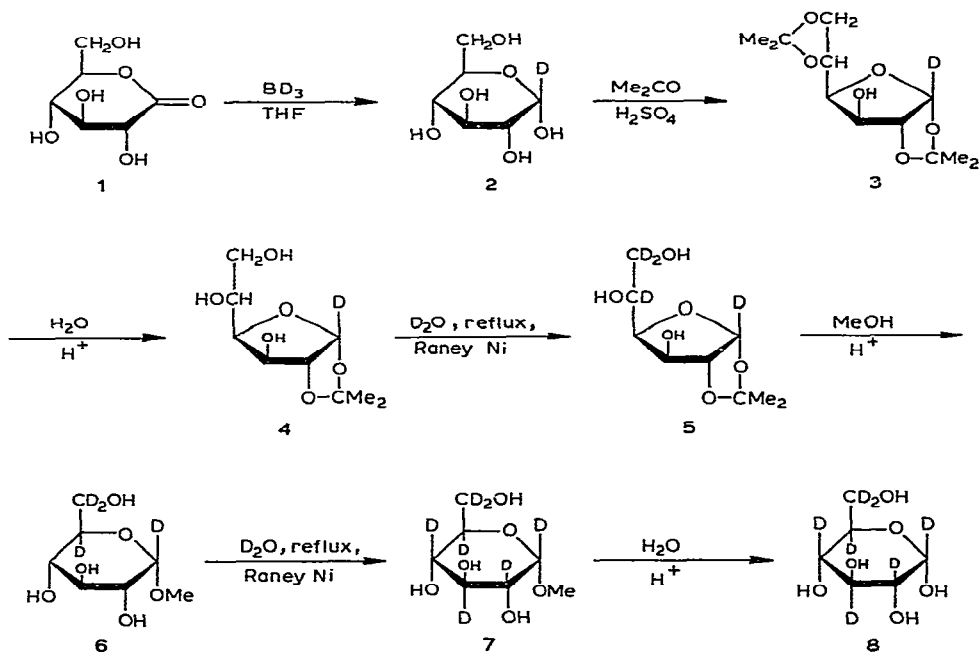
have an R_G value of 0.95 on Whatman No. 1 paper, using water-saturated 1-butanol containing 5% of ethanol as the eluant. In the same solvent system, D-galactose-^{2}H$_7$ (R_G 0.78) also moves slightly more slowly than "light" D-galactose (R_G 0.82). Similarly, the gas-chromatographic retention-times differ slightly, the deuterated compound generally having the lower retention-time. The separation of β-D-glucose from β-D-glucose-^{2}H$_7$ as the per(trimethylsilyl) derivatives has been reported[12].

EXPERIMENTAL

General methods. — Melting points are uncorrected, Mixture melting points were performed with "light" material; reported constants are those of "light" compounds. Solutions were evaporated under vacuum. N.m.r. spectra were recorded with Varian EM 360 and CFT 20 spectrometers. Deuterated Raney nickel was measured as settled volume in deuterium oxide. Paper chromatography was conducted on Whatman No. 1 paper, using water-saturated 1-butanol containing 5% (v/v) of ethanol as the eluant.

Deuterated Raney nickel*. — Wet Raney nickel (under water) prepared in the usual way[3], or commercial Raney nickel (W. R. Grace, Raney active nickel catalyst No. 28), was placed in a chromatography column having a coarse, porous-glass disc, the water was allowed to run off, and then deuterium oxide was allowed to flow through the nickel until the effluent water had a deuterium content of at least 98% (n.m.r.). After ~30 min, a little more deuterium oxide was passed through the nickel, to ensure complete exchange of the adsorbed hydrogen by deuterium. The deuterated Raney nickel was kept under deuterium oxide in a refrigerator, and was ready for deuterium exchanges.

Borane-^{2}H$_3$ in tetrahydrofuran. — Borane-^{2}H$_3$ in tetrahydrofuran (~1M) was prepared from sodium borodeuteride and boron trifluoride-etherate by the method used for the synthesis of "light" borane in tetrahydrofuran by Zweifel and Brown[13].

α-D-*Glucopyranose-1-^{2}H* (2). — To D-glucono-1,5-lactone (1; 5 g) in a 250-ml flask was added a solution of borane-^{2}H$_3$ in tetrahydrofuran (~1M, 100 mL), the flask was closed with a drying tube (not a stopper!), and kept overnight at room temperature; after several hours, a clear gel had formed. Then, water was added slowly until the vigorous evolution of gas had stopped, and the solution was evaporated to dryness. Methanol (20–30 mL) was added to, and evaporated from, the residue five times, to remove methyl borate. The resulting, syrupy D-glucose-1-^{2}H was crystallized slowly from water–2-propanol; yield 4 g (80%), m.p. 145–146°, $[\alpha]_D^{25}$ +49° (after 24 h; c 10, water); lit.[14] m.p. 146°, $[\alpha]_D$ +52.7° (c 10, water); R_G 1.0, paper chromatogram.

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose-1-^{2}H (3). — Compound 2 (3.8 g) was suspended, by stirring, in anhydrous acetone (500 mL) containing concentrated sulfuric acid (3 mL) for 15 h. Then, an excess of anhydrous sodium

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*Caution: It should be remembered that dry Raney nickel is pyrophoric.

carbonate was added, and the mixture was stirred until the yellow color of the solution disappeared and the solution was neutral to wet, universal-indicator paper. The solids were filtered off, and washed with a little acetone, and the combined solutions were evaporated to a crystalline residue, which was recrystallized from petroleum ether (b.p. 65–110°), to give pure 3; yield 4.0 g (74%), m.p. 108–110°, mixture m.p. 110°, $[\alpha]_D^{25}$ −17° (c 2, water); lit.[15] m.p. 110–111°, $[\alpha]_D$ −18.5° (c 5, water).

1,2-O-Isopropylidene-α-D-glucofuranose-1-^2H (4). — Compound 3 (3.7 g), water (100 mL), and washed Amberlite IR-120 (H$^+$) cation-exchange resin (10 mL) were stirred for 5 h at room temperature. Then, the resin was filtered off, and the filtrate was evaporated to a crystalline residue which, upon recrystallization from ethyl acetate, gave pure 4; yield 2.7 g (85%), m.p. 160°, mixture m.p. 160°, $[\alpha]_D^{25}$ −10° (c 5, water); lit.[15] m.p. 161°, $[\alpha]_D$ −11.8° (c 8, water); ^{13}C-n.m.r. signals in ^2H_2O, with DSS* as the internal standard, as follows.

Compound 4		Light compound
C-1	missing	106.6
C-2	86.3	86.3
C-3	75.7	75.7
C-4	81.8	81.7
C-5	70.4	70.4
C-6	65.4	65.4
Isopropylidene group		
C-1'	27.5	27.5
C-2'	114.5	114.5
C-3'	27.1	27.1

1,2-O-Isopropylidene-α-D-glucofuranose-1,5,6,6-$^2H_4$ (5). — Compound 4 (3 g) was dissolved in deuterium oxide (20 mL), and the solution was evaporated to dryness in order to exchange with the hydrogen of the hydroxyl groups. Then, deuterium oxide (100 mL) and deuterated Raney nickel (20 mL) were added, and the mixture was boiled under reflux for 15 h. The nickel was removed by filtration through several layers of filter paper, and the filtrate was evaporated to afford a semi-crystalline mass. T.l.c. on silica gel (Bakerflex), using ethyl acetate as developer and iodine vapor as detector, showed the presence of 5 (R_F 0.29), the L-*ido* derivative (R_F 0.46), and two poorly resolved, fast spots (R_F 0.87). Recrystallization of the mixture from ethyl acetate gave pure 5; yield 1.2 g (40%), m.p. 158–160°, $[\alpha]_D^{25}$ −10.5° (c 5, water), mixture m.p. 158–160°; lit.[15] m.p. 161°, $[\alpha]_D$ −11.8° (c 8, water); ^{13}C-n.m.r. signals in ^2H_2O, with DSS as the internal reference, as follows.

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*Sodium 2,2-dimethyl-2-sila-5-pentanesulfonate.

Compound 5		Light compound
C-1	missing	106.6
C-2	86.3	86.3
C-3	75.7	75.7
C-4	81.8	81.7
C-5	missing	70.4
C-6	missing	65.4

Isopropylidene group		
C-1'	27.5	27.5
C-2'	114.5	114.5
C-3'	27.1	27.1

Methyl α-D-*glucopyranoside-1,5,6,6-*$^2H_4$ **(6)**. — Compound **5** (1.0 g) was boiled for 15 h under reflux in methanol (30 mL) containing 1% of anhydrous hydrogen chloride. The solution was cooled, stirred with an excess of silver carbonate until it was neutral, and the silver salts were filtered off. The filtrate was treated with a little activated carbon, and the suspension filtered through a bed of Celite. Evaporation of the filtrate, and recrystallization of the residue from anhydrous ethanol, gave **6**; yield 0.6 g (68%), m.p. 165–167°, $[\alpha]_D^{20}$ +153° (*c* 4, water), mixture m.p. 166–167°; lit.[16] m.p. 167–169°, $[\alpha]_D$ +157° (*c* 2, water); ^{13}C-n.m.r. signals in ^2H_2O, with DSS as the internal reference, as follows.

Compound 6		Light compound
C-1	missing	101.2
C-2	73.2	73.2
C-3	75.1	75.1
C-4	71.7	71.6
C-5	missing	73.6
C-6	missing	62.6
CH$_3$	57.0	57.0

Compound **6** was also prepared directly from the mixture of 1,2-*O*-isopropylidene derivatives, but the yield was smaller (25%).

Methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside-1,5,6,6-$^2H_4$ **(6a)**. — Compound **6** was acetylated with acetic anhydride in pyridine in the usual way[17]. The product was recrystallized from ethanol; m.p. 100–102°, $[\alpha]_D^{22}$ +128° (*c* 4, chloroform), mixture m.p. 100–102°; lit.[16] m.p. 101–103°, $[\alpha]_D^{25}$ +130° (*c* 2, chloroform).

Methyl α-D-glucopyranoside-1,2,3,4,5,6,6-$^2H_7$ **(7)**. — Compound **6** (1 g) was dissolved in deuterium oxide (~10 mL), and the solution was evaporated to dryness in order to exchange with the hydrogen of the hydroxyl groups. Then, deuterium oxide (50 mL) and deuterated Raney nickel (10 mL) were added, and the mixture was boiled under reflux for 15 h. While the exchange mixture was still hot, the nickel

was removed by filtration through several layers of filter paper, and the filtrate was evaporated to dryness. The solid residue was recrystallized from ethanol; yield 0.6 g (60%), m.p. 164–166°, $[\alpha]_D^{25}$ +150° (*c* 0.5, water), mixture m.p. 164–166°; lit.[16] m.p. 167–168°, $[\alpha]_D$ +157° (*c* 2, water); paper chromatogram, 7 was very slightly slower than the light material; ^{13}C-n.m.r. signals in ^2H_2O, with DSS as the internal reference, as follows.

Compound 7		*Light compound*
C-1	missing	101.2
C-2	missing	73.2
C-3	missing	75.1
C-4	missing	71.6
C-5	missing	73.6
C-6	missing	62.6
CH$_3$	57.0	57.0

Methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside-1,2,3,4,5,6,6-$^2H_7$ (*7a*). — Compound 7 was acetylated with acetic anhydride in pyridine in the usual way[17]. The product was recrystallized from ethanol; m.p. 99–101°, $[\alpha]_D^{22}$ +127° (*c* 1, chloroform), mixture m.p. 99–101°; lit.[16] m.p. 101–103°, $[\alpha]_D^{25}$ +130° (*c* 2, chloroform); p.m.r. data (60 MHz, Me$_4$Si as the internal reference) in chloroform-^2H: δ 3.45 (s, 3 H, OMe), 2.12 (s), 2.10 (s), 2.05 (s), and 2.03 (s) (total 12 H, 4 OAc), no other signals. The corresponding signals of authentic "light" material have identical chemical shifts (mixed sample).

D-Glucose-$^2H_7$ (*8*). — Compound 7 (0.5 g) was hydrolyzed in 3M hydrochloric acid for 10 h under reflux. Then, the solution was made neutral with Dowex-1 X8 anion-exchange resin in the hydrogencarbonate form. The resin was filtered off, the filtrate was evaporated to dryness, and the residue was crystallized from water–2-propanol; yield 0.3 g (65%); m.p. 142–144°, $[\alpha]_D^{22}$ +47° (*c* 1, water), mixture m.p. 142–145°; lit.[14] m.p. 146°, $[\alpha]_D$ +52.7° (*c* 10, water); paper chromatogram, R_G 0.95; D-glucose-$^2H_7$ from algae, 0.95.

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