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Multiply ^{13}C-substituted monosaccharides: synthesis of D-(1,5,6-$^{13}C_3$)glucose and D-(2,5,6-$^{13}C_3$)glucose

Jian Wu, Anthony S. Serianni*,
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556 (U.S.A.)
and Paul B. Bondo
Omicron Biochemicals, Inc., 19882 Alou Lane, South Bend, IN 46637 (U.S.A.)

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ABSTRACT

D-(1,5,6-$^{13}C_3$)Glucose (**7**) has been synthesized by a six-step chemical method. D-(1,2-$^{13}C_2$)Mannose (**1**) was converted to methyl D-(1,2-$^{13}C_2$)mannopyranosides (**2**), and **2** was oxidized with Pt–C and O_2 to give methyl D-(1,2-$^{13}C_2$)mannopyranuronides (**3**). After purification by anion-exchange chromatography, **3** was hydrolyzed to give D-(1,2-$^{13}C_2$)mannuronic acid (**4**), and **4** was converted to D-(5,6-$^{13}C_2$)mannonic acid (**5**) with $NaBH_4$. Ruff degradation of **5** gave D-(4,5-$^{13}C_2$)arabinose (**6**), and **6** was converted to D-(1,5,6-$^{13}C_3$)glucose (**7**) and D-(1,5,6-$^{13}C_3$)mannose (**8**) by cyanohydrin reduction. D-(2,5,6-$^{13}C_3$)Glucose (**9**) was prepared from **8** by molybdate-catalyzed epimerization.

INTRODUCTION

Isotopically-enriched D-glucoses are commonly used in studies of biological metabolism[1,2], often in conjunction with analytical methods such as nuclear magnetic resonance spectroscopy and mass spectrometry. Singly-substituted derivatives (*e.g.*, D-(1-^{13}C)glucose and D-(1-^{2}H)glucose[3]) are employed most often because of their ease of synthesis, commercial availability, and/or relatively low cost. Uniformly (^{13}C)- and (^{2}H)-substituted D-glucoses have also found applications in various chemical and biological studies[4a–e], and in the preparation of isotopically substituted proteins[4f–g]. In contrast to singly substituted D-glucoses, uniformly labeled derivatives are prepared biosynthetically[5,6].

In some cases, however, singly or uniformly isotopically substituted D-glucoses may provide only partial solutions to structural or metabolic problems, and access to multiply labeled D-glucoses containing similar or different isotopes (*e.g.*, D-(1,6,6'-$^{2}H_3$;6-^{13}C)glucose[7]) may be desirable. Enzymic routes are frequently employed for their preparation. For example, the glycolytic enzyme, aldolase (EC 4.1.2.13), has been used to prepare D-(4,5-$^{13}C_2$)fructose 1-phosphate from 1,3-dihydroxy-2-propanone phosphate (dihydroxyacetone phosphate, DHAP) and DL-(1,2-$^{13}C_2$)glyceraldehyde[8,9]. D-(4,5-$^{13}C_2$)Fructose 1-phosphate can be dephosphorylated with acid phosphatase, and

* Author for correspondence.

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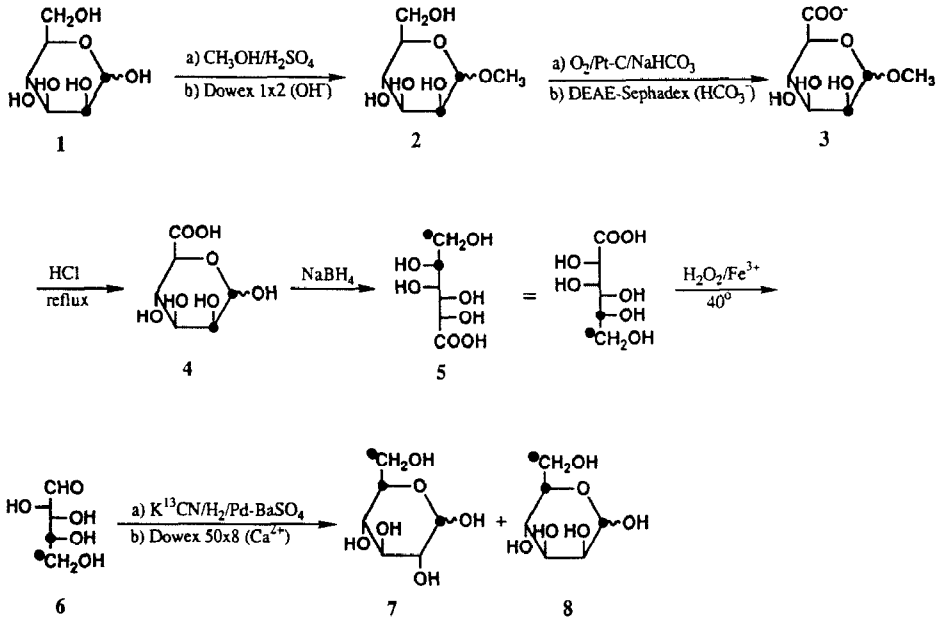

● Indicates site of ^{13}C-substitution.

Scheme 1

the resulting D-(4,5-$^{13}C_2$)fructose is converted to D-(4,5-$^{13}C_2$)glucose with immobilized D-xylose (D-glucose) isomerase[3,9].

Recent interest in D-(1,5,6-$^{13}C_3$)glucose (**7**) as a metabolic tracer in studies of malignant tissue metabolism[7] stimulated the present investigation. Although enzymic routes to **7** may be envisioned, this report describes a six-step chemical synthesis that exploits the symmetric properties of intermediates having the D-*manno* configuration (Scheme 1). D-(1,2-$^{13}C_2$)Mannose (**1**) was oxidized to D-(1,2-$^{13}C_2$)mannuronic acid (**4**), and **4** was reduced to D-(5,6-$^{13}C_2$)mannonic acid (**5**). Ruff degradation of **5** gave D-(4,5-$^{13}C_2$)arabinose (**6**), from which were generated **7** and D-(1,5,6-$^{13}C_3$)mannose (**8**). Compound **8** was converted to D-(2,5,6-$^{13}C_3$)glucose (**9**) by molybdate-catalyzed epimerization[10]. The labeled intermediates **4**, **5** and **6**, and the products **7** and **9**, were characterized by ^1H- and ^{13}C-n.m.r. spectroscopy, revealing unexpected results for D-mannuronic acid (**4**).

EXPERIMENTAL

Materials. — D-Arabinose, D-glucose, D-mannose, H_2O_2 (3%), and ion-exchange resins were purchased from Sigma Chemical Company. Platinum-on-activated carbon (Pt–C, 5%) was purchased from Aldrich Chemical Company. Potassium (^{13}C)cyanide ($K^{13}CN$, 99 atom% ^{13}C) and deuterium oxide (^2H_2O, 99 atom% ^2H) were obtained from Cambridge Isotope Laboratories.

D-(1-^{13}C)Arabinose was prepared from D-erythrose[11] and K^{13}CN as previously described[9,12]. The cyanohydrin reduction method[3,9,12] was used to prepare D-(1,2-$^{13}C_2$) mannose (**1**) from D-(1-^{13}C)arabinose and K^{13}CN.

Instrumentation. — ^{1}H-decoupled ^{13}C-n.m.r. spectra (75 MHz) and ^{1}H-n.m.r. spectra (300 MHz) were obtained at ~ 20° on a General Electric GN-300 (300 MHz) F.t.-n.m.r. spectrometer equipped with quadrature-phase detection and a 293B pulse programmer. ^{13}C-N.m.r. spectra were referenced externally to the C-1 signal of α-D-(1-^{13}C)mannopyranose (95.5 p.p.m.), ^{13}C chemical shifts are accurate to ±0.1 p.p.m., and ^{13}C–^{13}C spin-coupling constants are accurate to ±0.1 Hz. ^{1}H-N.m.r. spectra were referenced to the internal HO^{2}H signal (4.80 p.p.m.). ^{1}H chemical shifts are accurate to ±0.01 p.p.m., and ^{1}H–^{1}H spin-coupling constants are accurate to ±0.1 Hz.

Conversion of D-*(1,2-$^{13}C_2$)mannose* (**1**) *to methyl* D-*(1,2-$^{13}C_2$)mannopyranosides* (**2**). — D-(1,2-$^{13}C_2$)Mannose (**1**) (1.8 g, 10 mmol) was dissolved in anhydr. MeOH (50 mL), and the solution was evaporated to a syrup at 30° *in vacuo*. This treatment was repeated twice more to remove residual H_2O. The resulting stiff syrup was dissolved in anhydr. MeOH (120 mL) and conc. H_2SO_4 (0.5 mL) was added with efficient stirring. The reaction mixture was refluxed for 5 h, cooled to room temperature, and the H_2SO_4 was neutralized by batchwise addition of Amberlite IRA-68 [OH^-] anion-exchange resin (40 g). The methanolic solution was concentrated to ~ 5 mL at 30° *in vacuo* and loaded on a column (2.8 i.d. × 80 cm) containing Dowex-1 × 2 (200–400 mesh) [OH^-] anion-exchange resin[13]. The column was eluted with CO_2-free distilled H_2O at a flow rate of 1 mL/min, and fractions (18 mL) were assayed[14] with phenol–H_2SO_4. Fractions containing each glycoside were pooled and concentrated to syrups at 35° *in vacuo*. The elution order and yields of D-(1,2-$^{13}C_2$)mannosides were as follows: β-pyranoside (fractions 10–16, 0.2 g, 10%), α-pyranoside (fractions 18–32, 1.6 g, 82%), β-furanoside (fractions 160–182, 20 mg, 1%), α-furanoside (fractions 183–210, 0.12 g, 6%). The ^{13}C-n.m.r. spectral characteristics of the four labeled methyl mannosides were consistent with those previously reported[15a,b].

Conversion of methyl D-*(1,2-$^{13}C_2$)mannopyranosides* (**2**) *to* D-*(1,2-$^{13}C_2$)mannuronic acid* (**4**). – Methyl α- and β-D-(1,2-$^{13}C_2$)mannopyranosides (**2**) (0.98 g, 5 mmol) were dissolved in distilled H_2O (100 mL) at pH ~ 7.5 in a side-arm flask. Sodium hydrogencarbonate ($NaHCO_3$, ~ 100 mg) and 5% Pt–C (300 mg, pre-reduced with H_2) were added, the reaction flask was placed in an oil bath at 50°, and stirring was provided magnetically. Oxygen was bubbled through the solution at a rate of ~ 250 mL/min. As the reaction proceeded, the solution pH decreased due to the production of acidic products. Therefore, the solution pH was maintained at pH ~ 7 with periodic batchwise additions of $NaHCO_3$ (80 mg) to facilitate the reaction and prevent glycoside hydrolysis. The reaction was terminated when no further pH change was observed (10–14 h), and the catalyst was removed by vacuum filtration through Whatman GF/B and Millipore AW filters. ^{13}C-N.m.r. analysis of the filtrate indicated that ~ 70% of **2** was converted to **3**. The filtrate was applied to a column (2.8 i.d. × 35 cm) containing DEAE-Sephadex A-25 [HCO_3^-] anion exchange resin, and the uronides were eluted with a 3-L linear gradient (0.01–0.08M) of $NaHCO_3$ (pH 8.5) at a flow rate of 0.8

mL/min. Fractions (18 mL) were collected and assayed[14] with phenol–H_2SO_4. Fractions 7–14 contained unreacted methyl D-mannopyranosides (**2**). Fractions 40–73 containing the uronides **3** were pooled, and the solution was treated batchwise with excess Dowex-HCR-W2 [H^+] ion-exchange resin and concentrated to ~ 10 mL at 30° *in vacuo*.

Purified methyl α- and β-D-(1,2-$^{13}C_2$)mannopyranuronides (**3**, 5 mmol) were dissolved in distilled H_2O (150 mL), conc. HCl (1.5 mL) was added, and the reaction mixture was refluxed for 12–15 h. ^{13}C-N.m.r. analysis of the reaction mixture indicated that >90% of **3** was converted to **4**. After cooling, the solution containing D-(1,2-$^{13}C_2$) mannuronic acid (**4**) was adjusted to pH 2.5 with 4M NaOH and used without further treatment in the $NaBH_4$ reduction step.

Conversion of D-*(1,2-$^{13}C_2$)mannuronic acid* (**4**) *to* D-*(5,6-$^{13}C_2$)mannonic acid* (**5**). — An aqueous solution (100 mL) containing 5 mmol of D-(1,2-$^{13}C_2$)mannuronic acid (**4**) was adjusted to pH 10.7 with 4M NaOH and allowed to stand for 1–2 h at room temperature to hydrolyze bicyclic lactone forms of **4**. An aqueous solution of $NaBH_4$ (0.2M, 25 mL) was then added, and the reaction mixture was incubated for 1.5 h at ~ 23° with stirring. The residual $NaBH_4$ was destroyed by adding excess Dowex HCR-W2 [H^+] ion-exchange resin to the reaction mixture, and the suspension was filtered to remove the resin. ^{13}C-N.m.r. analysis of the filtrate indicated that >95% of **4** was converted to **5**. The filtrate containing **5** was concentrated to dryness at 30° *in vacuo*. Boric acid was removed from the residue by repeated evaporation from MeOH (30 mL) at 30° *in vacuo*.

Ruff degradation of D-*(1,2-$^{13}C_2$)mannonic acid* (**5**). — Ruff degradation of **5** was performed as described by Whistler and BeMiller[15e]. An aqueous solution (100 mL) containing 5 mmol of D-(1,2-$^{13}C_2$)mannonic acid **5** was adjusted to pH 10.8 with 4M NaOH. Calcium carbonate (0.25 g, 2.5 mmol), $Ba(OAc)_2$ (0.13 g, 0.5 mmol), and iron(III) sulfate pentahydrate (88 mg, 0.18 mmol) were added, the reaction mixture was refluxed for ~ 8 min, cooled to 40°, and aq. H_2O_2 (3%, 8.0 mL, 7 mmol) was added with stirring. The appearance of a dark purple color after ~ 30 min indicated that the reaction was complete. The suspension was filtered, and the filtrate was treated batchwise and consecutively with excess Dowex HCR-W2 [H^+] and Dowex 1 × 8 [AcO^-] (20–50 mesh) ion-exchange resins. The deionized solution was concentrated at 30° *in vacuo* to ~ 10 mL and applied to a column (4.4 i.d. × 108 cm) containing Dowex-50 × 8 [Ca^{2+}] (200–400 mesh) cation exchange resin[16]. The column was eluted with distilled water at a flow rate of 1 mL/min, and fractions (18 mL) were collected and assayed[14] with phenol–H_2SO_4. Fractions 54–62 containing D-(4,5-$^{13}C_2$)arabinose (**6**) were pooled and concentrated to a syrup at 35° *in vacuo* (yield: 0.38 g, 2.5 mmol, 50%).

Conversion of **6** *to* D-*(1,5,6-$^{13}C_3$)glucose* (**7**) *and* D-*(1,5,6-$^{13}C_3$)mannose* (**8**). — D-(4,5-$^{13}C_2$)Arabinose (**6**) (0.18 g, 1.2 mmol) was converted to a mixture of **7** and **8** by the cyanohydrin reduction method[3,9,12] using K^{13}CN, and **7** and **8** were separated by chromatography on Dowex-50 × 8 [Ca^{2+}] (200–400 mesh)[16]. Yields: **7**, 50 mg, 0.27 mmol, 23%; **8**, 90 mg, 0.49 mmol, 41%. Compound **8** was converted to D-(2,5,6-$^{13}C_3$) glucose (**9**) by molybdate-catalyzed epimerization[10].

Characterization of products. — ^{1}H-Decoupled ^{13}C-n.m.r. spectra of **6–9** were identical to those of authentic D-arabinose, D-glucose, and D-mannose, except for the added signal multiplicity caused by ^{13}C–^{13}C spin-coupling.

RESULTS AND DISCUSSION

The chemical method described in this study uses well established reactions to prepare **7** and **9** in high purity and fair yield from the relatively accessible bis-labeled aldohexose **1**. The underlying strategy is to introduce isotopes into the "top half" of the D-mannose precursor, which is readily accomplished *via* the concerted use of cyanohydrin reduction[3,9,12] and molybdate epimerization[10] reactions, and to translate these labels to the "bottom half" of the D-glucose product by exploiting the symmetry of intermediates having the D-*manno* configuration. This approach may be applied generally to prepare a wide variety of glucoses labeled with ^{13}C, ^{2}H, and/or ^{17,18}O at C-4, C-5, and/or C-6.

Fischer glycosidation of D-mannose was performed using HCl, Dowex HCR-W2 [H^+] ion-exchange resin or H_2SO_4 as the acid catalyst, but the best yield of the desired pyranosides (~ 90%) was obtained with H_2SO_4. Oxidation of C-6 of the methyl mannopyranosides **2** was achieved with Pt–C and O_2, giving **3** in ~ 70% yield. Unprotected glycosides may be used since this reagent is highly selective for primary hydroxyl groups[17]. Oxidation with Pt–C has been used recently to prepare methyl D-pentofuranuronides from corresponding methyl D-pentofuranosides in >90% yield[18]. Stacey and Wilson[19] have reported a preparation of D-mannuronic acid based on the oxidation of methyl-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside with potassium permanganate in an acetone–acetic acid solvent, followed by deacetylation of the product with aqueous barium hydroxide. This method, however, requires the protection of secondary hydroxyl groups to prevent their oxidation and is thus less attractive for the preparation of labeled compounds.

Longer reaction times were required to hydrolyze methyl D-mannopyranuronides (**3**) to D-mannuronic acid (**4**) relative to those used for methyl D-mannopyranosides and methyl D-pentofuranuronides[18]. The replacement of a CH_2OH group by a COOH group at C-6 is expected to reduce reaction rates by inhibiting O-1 (or O-5) protonation during H^+-catalyzed glycosidic bond hydrolysis. The lower rate of pyranoside hydrolysis compared to furanoside hydrolysis has been previously documented[20].

The reduction of D-(1,2-$^{13}C_2$)mannuronic acid (**4**) with $NaBH_4$ gave D-(5,6-$^{13}C_2$) mannonic acid (**5**) in high yield (Fig. 1A) with no or little reduction of the carboxyl group, which would produce undesired labeled mannitol. Ruff degradation of **5** gave D-(4,5-$^{13}C_2$)arabinose (**6**) in fair yield (50%). The ^{13}C-n.m.r. spectrum of **6** (Fig. 1B) contained eight doublets attributed to C-5 and C-6 of α-arabinopyranose **6a**, β-arabinopyranose **6b**, α-arabinofuranose **6c**, and β-arabinofuranose **6d**. $^{1}J_{C\text{-}4,C\text{-}5}$ for the α- and β-D-arabinofuranoses (~ 42 Hz) was found to be ~ 4 Hz larger than $^{1}J_{C\text{-}4,C\text{-}5}$ in the α- and β-D-arabinopyranoses (~ 38 Hz) (Fig. 1B).

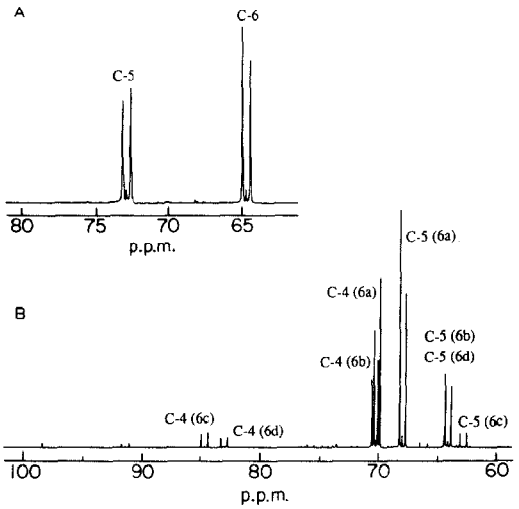

Fig. 1. (A) The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of D-(5,6-$^{13}C_2$)mannonate (**5**) obtained after the $NaBH_4$ reduction of **4** ($^1J_{C\text{-}5,C\text{-}6}$ 41.3 Hz), showing only the enriched carbons. (B) The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of D-(4,5-$^{13}C_2$)arabinose (**6**) obtained after Ruff degradation of **5**, showing only the enriched carbons. Assignments of the signals to the α-pyranose **6a**, β-pyranose **6b**, α-furanose **6c**, and β-furanose **6d** are as indicated. $^1J_{C\text{-}4,C\text{-}5}$ values (Hz) were as follows: **6a**, 37.7; **6b**, 37.6; **6c**, 42.4; **6d**, 42.1.

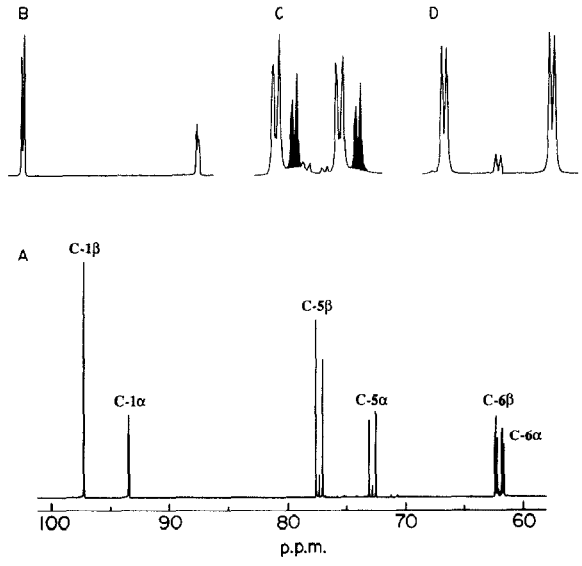

Fig. 2. (A) The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of D-(1,5,6-$^{13}C_3$)glucose (**7**) showing only the enriched carbons for the α-pyranose **7a** and β-pyranose **7b**. (B–D) Expanded signals showing splittings due to ^{13}C–^{13}C spin-coupling at C-1 (B), C-6 (C; filled signals, **7a**; normal signals, **7b**), and C-5α (D) (see text for $J_{C,C}$ values).

The observed ^{13}C–^{13}C spin-coupling constants in **7** and **9** agree with those previously published[21,22] and serve to confirm, along with the observed chemical shifts, the structure and sites of (^{13}C)-substitution. The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of **7** (Fig. 2A) contains three enriched carbon signals for both pyranose anomers. The C-1 signal of the α-pyranose **7a** appears as an unresolved quartet due to ^{13}C–^{13}C spin-coupling to C-5 (1.7 Hz) and C-6 (3.2 Hz), whereas C-1 of the β-pyranose **7b** appears as a doublet since C-1 is coupled only to C-6 (4.3 Hz) (Fig. 2B). The C-6 signals appear as quartets containing $^{1}J_{C\text{-}5,C\text{-}6}$ (**7a**, 43.3 Hz; **7b**, 42.3 Hz) and $^{3}J_{C\text{-}6,C\text{-}1}$ (Fig. 2C). The C-5 signal of **7a** appears as a quartet due to coupling to C-1 and C-6 (Fig. 2D), while that of **7b** appears as a doublet due to coupling to C-6. In **9**, C-2 is not coupled to C-5 or C-6 and thus produces a single signal, while C-5 and C-6, being mutually coupled, appear as doublets (Fig. 3).

The formation of D-(1,5,6-$^{13}C_3$)mannose (**8**) in the cyanohydrin reduction reaction lowers the initial yield of **7**, but **8** is readily converted to **7** by phenylborate-mediated base isomerization[23,24] or to D-(2,5,6-$^{13}C_3$) glucose (**9**) by molydate-catalyzed epimerization[10] (Fig. 3). In addition, D-(4,5-$^{13}C_2$)arabinose (**6**) may be epimerized[10] to give the more biologically relevant D-(4,5-$^{13}C_2$)ribose.

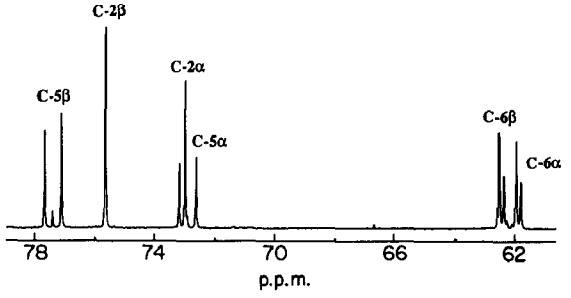

Fig. 3. The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of D-(2,5,6-$^{13}C_3$)glucose (**9**) showing only the enriched carbons of the α-pyranose **9a** and β-pyranose **9b**. See text for ^{13}C–^{13}C spin-coupling constants.

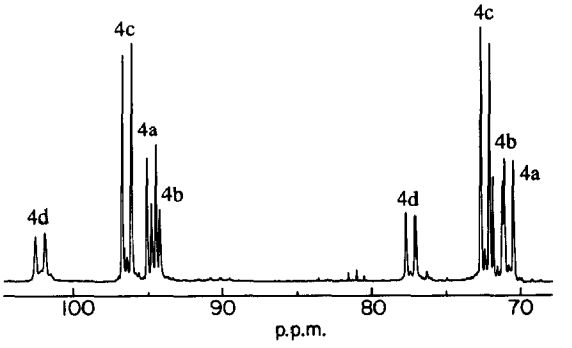

Fig. 4. The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of D-(1,2-$^{13}C_2$)mannuronic acid (**4**) at pH ~ 2.5 obtained after the acid hydrolysis of **3**, showing only the enriched carbons. Four forms are present. Signals **4a** and **4b** are assigned to the α- and β-mannopyranuronic acids, respectively, and **4c** and **4d** are tentatively assigned to lactone forms (see Discussion section). $^{1}J_{C\text{-}1,C\text{-}2}$ values (Hz) are as follows: **4a**, 46.5; **4b**, 42.8; **4c**, 44.6; **4d**, 46.7.

Solution properties of D-*mannuronic acid* (**4**). — The ^{13}C-n.m.r. spectrum of D-(1,2-$^{13}C_2$) mannuronic acid (**4**) (pH 2.5) obtained after the acid hydrolysis of **3** contains four C-1 signals between 94–103 p.p.m. and four corresponding C-2 signals between 70–78 p.p.m. (Fig. 4). Two of the C-1 signals may be assigned to α-D-mannopyranuronic acid (**4a**) (94.8 p.p.m.) and β-D-mannopyranuronic acid (**4b**) (94.5 p.p.m.) by analogy to the C-1 chemical shifts of the α- and β-D-mannopyranoses[22] and on the basis of their relative signal intensities[25] ($\alpha p/\beta p \approx 2.1$). The observed C-2 (**4a**, 70.8 p.p.m.; **4b**, 71.6 p.p.m.), and H-1 (**4a**, 5.43 p.p.m.; **4b**, 5.19 p.p.m.) chemical shifts, and the values of $^3J_{H\text{-}1,H\text{-}2}$ (**4a**, 2.5 Hz; **4b**, < 1.2 Hz), $^1J_{C\text{-}1,H\text{-}1}$ (**4a**, 171.4 Hz; **4b**, 161.7 Hz) and $^1J_{C\text{-}1,C\text{-}2}$ (Fig. 4) are also similar to corresponding parameters in the D-mannopyranoses[21,22].

The two remaining C-1 signals at 96.5 (**4c**) and 102.3 p.p.m. (**4d**) are due to forms present in high proportion (~ 48%), and their corresponding C-2 signals resonate at 72.4 and 77.4 p.p.m., respectively (Fig. 4). $^1J_{C\text{-}1,H\text{-}1}$ values for **4c** and **4d** were 176.4 Hz and 171.5 Hz, respectively. These forms, as well as **4a** and **4b**, reacted with $NaBH_4$ to yield the same product, D-(5,6-$^{13}C_2$)mannonate (**5**) (Fig. 1A). By analogy to the solution behavior of D-mannose, **4c** and **4d** are not likely to be the α- and β-D-mannofuranuronic acids because of their high abundance (furanoses are a minor component of aqueous solutions of D-mannose[25]). Furthermore, α-D-mannofuranuronic acid having O-1 and O-2 *trans* is expected to be more abundant than β-D-mannofuranuronic acid having O-1 and O-2 *cis*, and thus the more deshielded C-1 signal at 102.3 p.p.m. arising from the O-1, O-2 *trans* anomer[15b] should be more intense than that at 96.5 p.p.m.. The opposite is observed.

The proportions of **4c** and **4d** decrease as solution pH increases, and both forms are converted completely into **4a** and **4b** at pH 11. This behavior is consistent with that of aldonolactones, which hydrolyze to aldonates in alkaline solution. Like D-glucuronic acid[26,27], **4** reportedly cyclizes to form furanurono-6,3-lactones[28], and thus the C-1 signals at 96.5 and 102.3 p.p.m. may be assigned to the β- and α-D-mannofuranurono-6,3-lactones, **4c** and **4d**, respectively. This assignment indicates, however, that the β anomer is considerably more favored than the α anomer, which is contrary to expectations based on anomeric distributions observed in simple furanoses having the D-*lyxo* configuration[29]. It is likely that the proposed assignment of the C-1 signal at 102.3 p.p.m. is correct, but the origin of the 96.5 p.p.m. signal is less certain. This signal could be due to alternate lactone forms of **4**, including a pyranurono-6,2-lactone, pyranurono-6,3-lactone, or furanurono-6,2-lactone, but further study will be required to firmly establish its identity.

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REFERENCES

1 (a) J. A. den Hollander, K. L. Behar, and R. G. Shulman, *J. Magn. Reson.*, 57 (1984) 311–313. (b) K. J. Neurohr, E. J. Barrett and R. Shulman, *Proc. Natl. Acad. Sci. U.S.A*, 80 (1983) 1603–1607.
2 (a) J. B. Aguayo, M. P. Gamcsik, and J. D. Dick, *J. Biol. Chem.*, 263 (1988) 19 552–19 557. (b) J. B. Aguayo, I. J. McLennan, C. Graham, Jr., and H.-M. Cheng, *Exp. Eye Res.*, 47 (1988) 337–343.
3 A. S. Serianni, T. Vuorinen, and P. B. Bondo, *J. Carbohydr. Chem.*, 9 (1990) 513–541.
4 (a) J. R. Brainard, R. S. Downey, D. M. Bier, and R. E. London, *Anal. Biochem.*, 176 (1989) 307–312. (b) T. E. Walker and R. E. London, *Appl. Environ. Microbiol.*, 53 (1987) 92–98. (c) C. J. Unkefer, R. E. London, R. D. Durbin, T. S. Uchytil, and P. J. Langston-Unkefer, *J. Biol. Chem.*, 262 (1987) 4994–4999. (d) D. Gagnaire and F. R. Taravel., *Eur. J. Biochem.*, 103 (1980) 133–143. (e) S. J. Gould and D. E. Cane, *J. Am. Chem. Soc.*, 104 (1982) 343–346. (f) M. Ikura, L. E. Kay, and A. Bax, *Biochemistry*, 29 (1990) 4659–4667. (g) E. R. P. Zuiderweg, L. P. McIntosh, F. W. Dahlquist, and S. W. Fesik, *J. Magn. Reson.*, 86 (1990) 210–216.
5 V. H. Kollman, J. L. Hanners, R. E. London, E. G. Adame, and T. E. Walker, *Carbohydr. Res.*, 73 (1979) 193–202.
6 V. H. Kollman, J. L. Hanners, J. Y. Hutson, T. W. Whaley, D. G. Ott, and C. T. Gregg, *Biochem. Biophys. Res. Commun.*, 50 (1973) 826–831.
7 P. B. Kingsley-Hickman, B. D. Ross, and T. Krick, *FASEB J.*, 4 (1990) A1923.
8 A. S. Serianni, E. Cadman, J. Pierce, M. L. Hayes, and R. Barker, *Methods Enzymol.*, 89 (1982) 83–92.
9 A. S. Serianni and R. Barker, in E. Buncel and J. Jones (Eds.), *Isotopes in the Physical and Biomedical Sciences*, Elsevier, 1987, 211–236.
10 M. L. Hayes, N. J. Pennings, A. S. Serianni, and R. Barker, *J. Am. Chem. Soc.*, 104 (1982) 6764–6769.
11 A. S. Perlin, *Methods Carbohydr. Chem.*, 1 (1962) 64–66.
12 A. S. Serianni, H. A. Nunez, and R. Barker, *Carbohydr. Res.*, 72 (1979) 71–78.
13 P. W. Austin, F. E. Hardy, J. C. Buchanan, and J. Baddiley, *J. Chem. Soc.*, (1963) 5350.
14 J. E. Hodge and B. T. Hofreiter, *Methods Carbohydr. Chem.*, 1 (1962) 380–394.
15 (a) A. S. Perlin, B. Casu, and H. J. Koch, *Can. J. Chem.*, 48 (1970) 2596–2606. (b) R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, *Can. J. Chem.*, 53 (1975) 1424–1433. (c) R. L. Whistler and J. N. BeMiller, *Methods Carbohydr. Chem.*, 2 (1963) 79–80.
16 S. J. Angyal, G. S. Bethell, and R. Beveridge, *Carbohydr. Res.*, 73 (1979) 9–18.
17 C. L. Mehltretter, B. H. Alexander, R. L. Mellies, and C. E. Rist, *J. Am. Chem. Soc.*, 73 (1951) 2424–2427.
18 J. Wu and A. S. Serianni, *Carbohydr. Res.*, 210 (1991) 51–70.
19 M. Stacey and P. I. Wilson, *J. Am. Chem. Soc.*, 66 (1944) 587–588.
20 F. Shafizadeh, *Adv. Carbohydr. Chem.*, 13 (1958) 9–61.
21 T. E. Walker, R. E. London, T. W. Whaley, R. Barker, and N. A. Matwiyoff, *J. Am. Chem. Soc.*, 98 (1976) 5807–5813.
22 M. J. King-Morris and A. S. Serianni, *J. Am. Chem. Soc.*, 109 (1987) 3501 3508.
23 S. A. Barker, P. J. Sommers, and R. R. Woodbury, German Pat., DT 2 726 535 (1977).
24 T. E. Walker, C. J. Unkefer, and D. S. Ehler, *J. Carbohydr. Chem.*, 7 (1988) 115–132.
25 (a) S. J. Angyal, *Angew. Chem., Int. Ed. Engl.*, 8 (1969) 157–166. (b) S. J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 15–68.
26 K. Dax and H. Weidmann, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 189–234.
27 P. E. Pfeffer, K. M. Valentine, and F. W. Parrish, *J. Am. Chem. Soc.*, 101 (1979) 1265–1274.
28 H. S. Isbell and H. L. Frush, *J. Res. Natl. Bur. Stds.*, 37 (1946) 1–7.
29 (a) A. S. Serianni, J. Pierce, and R. Barker, *Biochemistry*, 18 (1979) 1192–1199. (b) J. R. Snyder and A. S. Serianni, *Carbohydr. Res.*, 163 (1987) 169–188.