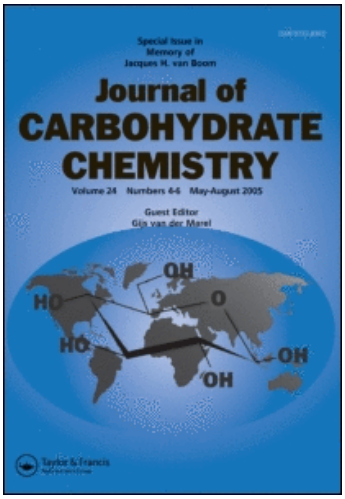


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

The Synthesis of Carbon-13 Enriched Monosaccharides Derived from Glucose and Mannose

Thomas E. Walker[a]; Clifford J. Unkefer[a]; Deborah S. Ehler[a]
[a] Los Alamos National Laboratory, University of California, Los Atamos, NM

To cite this Article Walker, Thomas E. , Unkefer, Clifford J. and Ehler, Deborah S.(1988) 'The Synthesis of Carbon-13 Enriched Monosaccharides Derived from Glucose and Mannose', Journal of Carbohydrate Chemistry, 7: 1, 115 — 132
To link to this Article: DOI: 10.1080/07328308808058907
URL: http://dx.doi.org/10.1080/07328308808058907

J. CARBOHYDRATE CHEMISTRY, 7(1), 115-132 (1988)

THE SYNTHESIS OF CARBON-13 ENRICHED MONOSACCHARIDES DERIVED FROM GLUCOSE AND MANNOSE

Thomas E. Walker*, Clifford J. Unkefer and Deborah S. Ehler

Los Alamos National Laboratory
University of California
INC-4 MS-C345
Los Alamos, NM 87545

Received May 29, 1987 - Final Form January 8, 1988

ABSTRACT

A modified Kiliani-Fischer reaction is used to prepare multigram quantities of [1-^{13}C]-enriched glucose and mannose which are converted chemically or enzymatically into other labeled monosaccharides. The simplest conversion is the synthesis of labeled fructose from labeled glucose using commercially available immobilized glucose isomerase. The equilibrium for this reaction provides a 1:1 mixture of glucose and fructose which can be separated by chromatography. The equilibrium can be shifted toward fructose by treating the reaction with germanate ion. [1-^{13}C]Mannose can be converted into more useful sugars using a modification of the Lobry de Bruyn-Alberda van Ekenstein transformation. In this reaction, D-[1-^{13}C]mannose is treated with an aqueous solution of dilute alkali and phenylboronate to form a mixture of labeled fructose, mannose and glucose. Fructose can be converted to a mixture of methyl fructofuranosides by using trifluoroacetic acid in methanol. [2-^{13}C]Dihydroxyacetone can be prepared from methyl D-[2-^{13}C]fructose by treatment with periodate followed by reduction with borohydride and acid hydrolysis.

INTRODUCTION

Carbon-13 enriched D-glucose can be easily synthesized with a variety of labeling patterns and as such makes an ideal precursor from which other ^{13}C-enriched sugars can be prepared. Barker and coworkers[1,2] have described an elegant technique for the incorporation of [^{13}C]cyanide into the C-1 position of monosaccharides. This approach involves the stabilization of the nitriles in the Kiliani reaction followed by catalytic reduction to an epimeric mixture of two aldoses which can be separated by chromatography on AG 50. For the synthesis of D-[1-^{13}C]glucose, arabinose is reacted with [^{13}C]cyanide and ultimately results in a C-2 epimeric mixture of primarily D-[1-^{13}C]mannose, which is not particularly useful. Barker's group has developed techniques for converting D-[1-^{13}C]mannose into D-[1-^{13}C]glucose[3] enzymatically or into D-[2-^{13}C]glucose chemically.[4,5] Williams and Whaley[6] have described the synthesis of D-[6-^{13}C]glucose from 1,2,5,6-diisopropylidene glucose. The synthesis of uniformly ^{13}C-labeled glucose from the blue-green alga *Agmenellum quadruplicatum* has been described by Kollman *et al.*[7]

We have exploited several chemical and enzymatic methods for the conversion of labeled mannose and glucose into other useful labeled sugars. In this laboratory, we routinely prepare D-[1-^{13}C]glucose from 50 g of arabinose, which has lead to a surplus of D-[1-^{13}C]mannose amounting to hundreds of grams. We use a modification of the Lobry de Bruyn-Alberda van Ekenstein transformation to convert mannose into a mixture of glucose, fructose, and mannose. King-Morris and Serianni[8] have reported that the potassium hydroxide-catalyzed isomerization of D-[1-^{13}C]mannose under anaerobic conditions leads to label scrambling from C-1 to C-6 and that the reaction cannot be used for the preparation of [1-^{13}C]glucose having isotopic integrity. However, we find that the presence of phenylboronate in the reaction mixture protects the sugars from the undesired side reactions leading to the [6-^{13}C]-enriched derivatives.

We have prepared labeled fructose enzymatically from glucose using immobilized glucose isomerase, a reaction which can be shifted toward

fructose by the addition of germanate. We have prepared methyl fructofuranosides from fructose using trifluoroacetic acid in methanol, a previously unreported method which preferentially leads to furanosides rather than pyranosides. A mixture of the labeled fructofuranosides can then be used for the synthesis of labeled dihydroxyacetone.

RESULTS AND DISCUSSION

Large-Scale Preparation of [^{13}C]-Enriched Cyanide and Sugars. We have prepared potassium [^{13}C]cyanide directly rather than isolating the intermediate ammonium cyanide as described by Ott *et al.*[9] The scale of the reaction can be readily increased or decreased; however, we have observed that higher concentrations of potassium hydroxide froth badly. The above procedure is also applicable without modification for the preparation of potassium [^{13}C,^{15}N]cyanide since only a small excess of [^{15}N]ammonia is used in the reaction.

Monosaccharides labeled at C-1 are conveniently prepared from potassium [^{13}C]cyanide as described by Serianni *et al.*;[2] a large-scale reaction is described in the methods section in which [^{13}C]cyanide is the limiting reactant. Unreacted [^{13}C]cyanide is not recovered; the yield of labeled sugars from potassium [^{13}C]cyanide is 59%.

Synthesis of [^{13}C]-Enriched Fructose. Carbon-13 enriched D-fructose can be readily prepared with any labeling pattern available in D-glucose. The interconversion reaction for the two sugars is catalyzed by the enzyme glucose isomerase[10] which is commercially available as an immobilized preparation. This reaction will reach an equilibrium consisting of about 50% fructose but can be forced to over 80% by the addition of sodium germanate, which binds fructose[11] preferentially to glucose at the pH of the reaction. The time course for the reaction with and without germanate as monitored by ^{13}C NMR spectroscopy is shown in Fig. 1. The reaction was followed by withdrawing samples at intervals and quenching the reaction with acetic acid. If insufficient acetic acid was used, a broad peak of residual

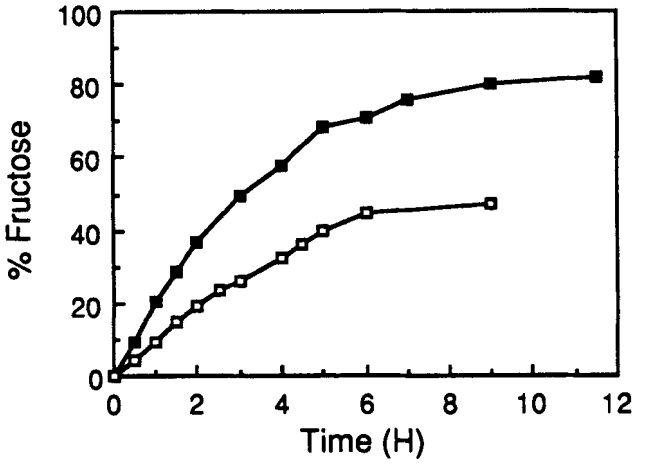


FIG. 1. Time course for the glucose isomerase ("Sweetzyme")-catalyzed isomerization of D-[2-^{13}C]glucose to D-[2-^{13}C]fructose with germanate (closed squares) and without germanate (open squares). The percent D-[2-^{13}C]fructose was determined by ^{13}C NMR spectroscopy and may be underestimated. Details are given in the text.

fructose bound to germanate appeared in the region of fructose C-2. The acidified reaction mixture containing germanate was applied to a column of AG 2 OAc^- and eluted with 0.1 M acetic acid to remove germanate. Sodium acetate was eluted first, followed immediately by a mixture of glucose and fructose; the germanate eluted later as a broad peak. Sodium acetate separated only partially from the sugars, but the germanate was completely removed. Labeled fructose can also be prepared from mannose as described below.

Isomerization of D-[1-^{13}C]Mannose to D-[1-^{13}C]Glucose and D-[1-^{13}C]Fructose. The Lobry de Bruyn-Alberda van Ekenstein reaction is a well known alkali-catalyzed degradation reaction of free sugars[12,13] which is difficult to control for the synthesis of other carbohydrates unless the reaction can be moderated. King-Morris and Serianni[8] have described an application of this reaction in which D-[1-^{13}C]mannose is incubated for several days at pH 11.5 and 25 °C. Under these conditions, they observed by ^{13}C NMR that in addition to [1-^{13}C]-enriched sugars, a small proportion (2-3%) of

[6-^{13}C]-enriched sugars were generated. They postulated that the small amount of label at C-6 indicated a symmetrical 3,4-enediol intermediate and that the [6-^{13}C]glucose was in the L- configuration. They concluded that the Lobry de Bruyn-Alberda van Ekenstein reaction could not be used to prepare D-[1-^{13}C]glucose with isotopic integrity.

We have found that barium acetate (and other metal salts) will catalyze the isomerization of glucose and mannose to fructose, a reaction which has been observed previously by Speck.[13] Although this reaction does not require alkaline conditions, the yield of fructose from the reaction is not as high as could be obtained by alternative methods.

Phenylboronate in the presence of mild alkali has been shown to be an excellent moderator for the isomerization of glucose to fructose for the production of high fructose corn syrup.[14,15] The protection afforded by the phenylboronate allows the reaction to be run at higher pH and temperature and consequently leads to a better yield of fructose in a shorter time. The time course for the isomerization of mannose in a reaction mixture which contains equimolar amounts of phenylboronate and mannose at pH 12.8 and 50 °C is illustrated in Fig. 2. After 8 h, 75% of the mannose has been converted to fructose compared to about 25% observed by King-Morris and Serianni[8] after 7 days at pH 11.5 and 25 °C. Another important benefit of phenylboronate is that it protects the sugars from those reactions which lead to label scrambling.

Our ^{13}C NMR spectrum of an equilibrium mixture of α- and β-[1-^{13}C]glucose derived from [1-^{13}C]mannose shows no evidence for enrichment at C-6. The resonance from natural abundance ^{13}C at C-6 (Fig. 3A) of the [1-^{13}C]glucose is a doublet as a result of ^{13}C-^{13}C spin-spin coupling between C-1 and C-6. The small center peak arises from species that have ^{13}C at C-6 and not at C-1 and is either due to residual ^{12}C at C-1 in 99+% enriched [1-^{13}C]glucose or due to C-1 to C-6 label scrambling. Although too small to be integrated, the small singlet in Fig. 3A represents ^{12}C at C-1 and is the intensity expected for 99+% ^{13}C at C-1. For high enrichments at C-1, the amount of [6-^{13}C]glucose in the sample can be calculated from equation 1 where X is the fraction of material which has

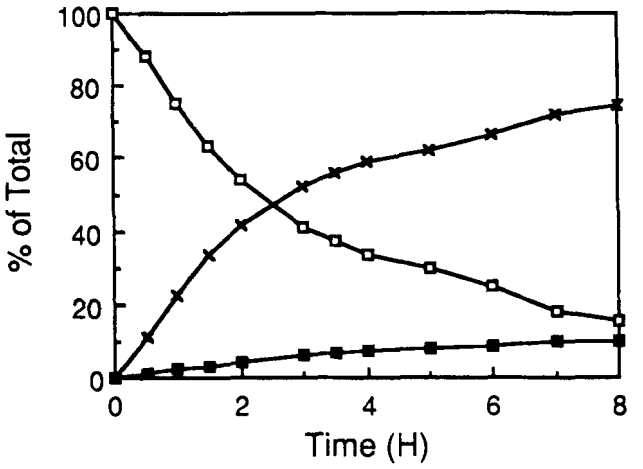


FIG. 2. Time course for the alkali-catalyzed isomerization of D-[1-^{13}C]mannose to a mixture of D-[1-^{13}C]mannose (open squares), D-[1-^{13}C]glucose (closed squares), and D-[1-^{13}C]fructose (x). The relative concentrations were determined by ^{13}C NMR which somewhat overestimates the amount of fructose. Details are given in the text.

undergone label scrambling and S and D are the integrals for the singlet and

$$X = 0.011S/D \quad (1)$$

doublet, respectively. In order to better illustrate the sensitivity of the method, a sample of [1-^{13}C]glucose was spiked with [6-^{13}C]glucose (0.1% in Fig. 3B and 0.2% in Fig. 3C). Using equation 1, we calculated the amount of [6-^{13}C]glucose present as 0.096% in Fig. 3B and 0.22% in Fig. 3C. The appearance of the singlet in Fig. 3B demonstrates that 0.1% [6-^{13}C]glucose can be readily detected and that the amount of label scrambling to C-6 in our reaction is clearly much less than 0.1%. Using a similar anaylsis, we find no evidence for scrambling to C-6 in either [1-^{13}C]fructose or [1-^{13}C]mannose isolated from the isomerization reaction. These results suggest that the phenylboronate-sugar complex prevents the formation of the symmetrical 3,4-enediol intermediate which leads to label scrambling as described by King-Morris and Serianni[8].

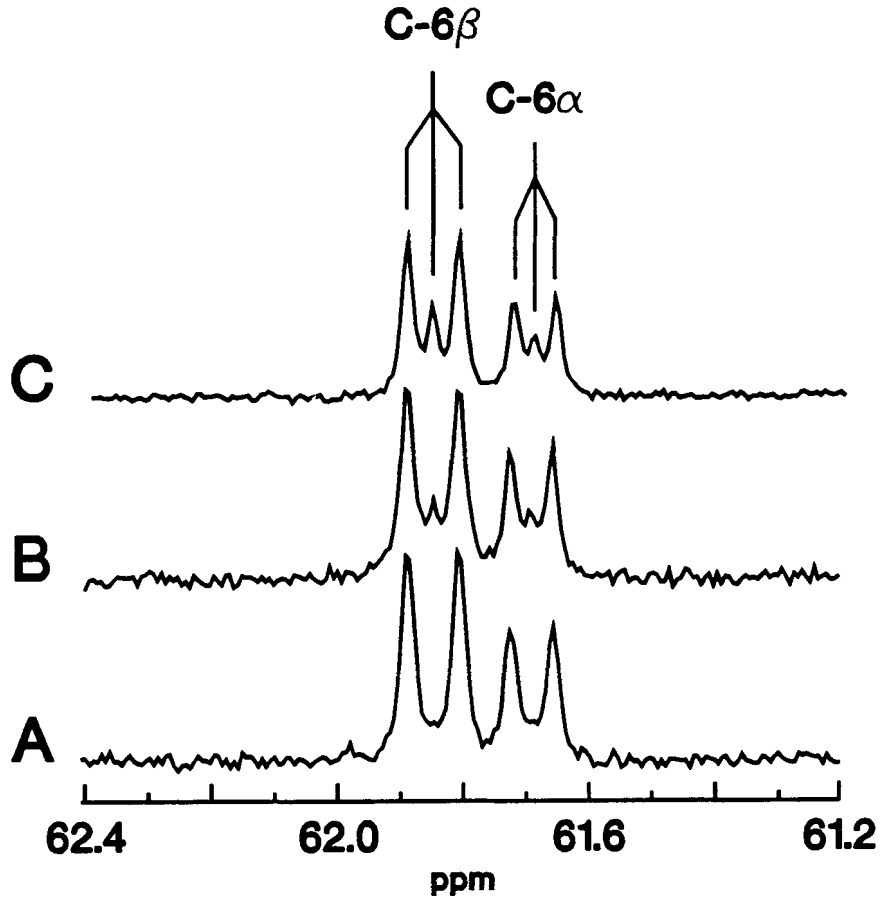


FIG. 3. Proton-decoupled ^{13}C NMR spectra of the C-6 region of [1-^{13}C]glucose prepared from [1-^{13}C]mannose using the alkali-phenylboronate reaction. The singlets in the center of the C-6 α and β doublets represent the amount of ^{12}C at C-1 and the amount of [6-^{13}C]glucose in the sample (see text). A. [1-^{13}C]Glucose B. Sample A spiked with 0.1% [6-^{13}C]glucose C. Sample A spiked with 0.2% [6-^{13}C]glucose. A relaxation time of 7 s was used with a 3.129 acquisition time to give a spectral resolution of 0.32 Hz/point The spectra were not resolution-enhanced or sensitivity-enhanced and integrals were determined using a Lorentzian line shape analysis (see methods).

Using the same singlet/doublet analysis described for C-6, we find label scrambling from C-1 to C-2 in both [1-^{13}C]glucose and [1-^{13}C]fructose derived from [1-^{13}C]mannose. The levels of scrambling for [1-^{13}C]glucose and [1-^{13}C]fructose as calculated from equation 1 are quite different, being 0.4% in [1-^{13}C]glucose and 0.1% in [1-^{13}C]fructose. We believe that the scrambling in [1-^{13}C]fructose is less because it is better protected as a complex with phenylboronate whereas [1-^{13}C]glucose is in better dynamic equilibrium with [1-^{13}C]mannose. It is likely that the scrambling occurs solely in the [1-^{13}C]mannose in a side reaction similar to that catalyzed by molybdate. This explanation would suggest that fructose and glucose formed during the latter part of the reaction would exhibit more of the ^{13}C-2 product than that formed early in the reaction. The low level of label scrambling observed in [1-^{13}C]fructose (0.1%) is not a serious problem. The major disadvantage is that the enrichment at C-1 can no longer be calculated based on the C-2 singlet.

King-Morris and Serianni[8] do not mention scrambling from C-1 to C-2, although careful inspection of their ^{13}C NMR spectrum of [1-^{13}C]glucose suggests that a small amount of [2-^{13}C]glucose might be present. The scrambling from C-1 to C-2 which we observe is probably due to a reaction similar to the molybdate-catalyzed isomerization of sugars described by Hayes *et al.*,[5] but catalyzed by phenylboronate or by an impurity. To test for the possibility of a metal ion contaminant, we treated one sample of phenylboronate with Chelex resin to remove residual metal ions; the same degree of rearrangement was found with the Chelex-treated and untreated samples. Analysis of the phenylboronic acid for metal ions by Inductively Coupled Plasma–Mass Spectrometry (ICP–MS) demonstrated that metal ion contamination was below about 20 ppm.

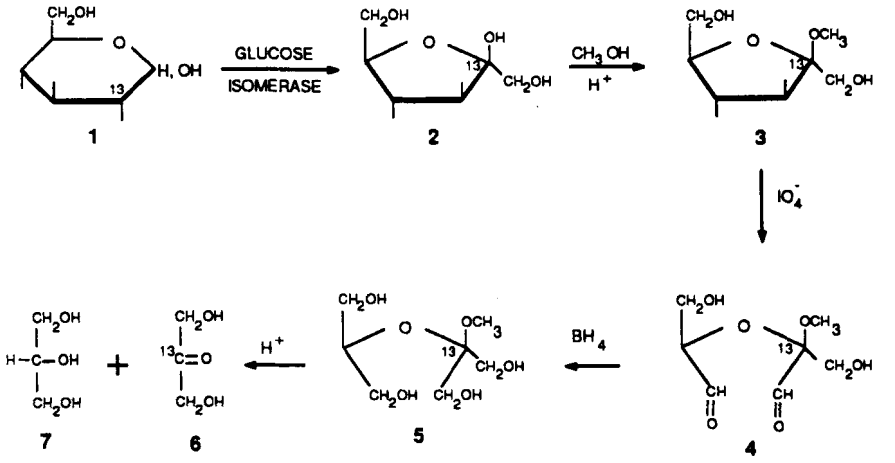
The mixture of sugars from the isomerization reaction could be either separated by chromatography or treated with glucose isomerase as described above to improve the yield of [1-^{13}C]glucose. Overall recovery of labeled sugars was 80%. At higher pH or temperature and for longer times, an increase in by-products (lactic acid and saccharinic acids) resulted in decreased recovery of desired products. Fortunately, the by-products are

readily removed from the neutral sugars using anion exchange resin. We have also carried out the isomerization reaction on [1-^{13}C]mannose containing unlabeled arabinose with no problems other than an increase in easily removed by-products. In a separate experiment, we determined that under the conditions of the isomerization reaction, arabinose was largely converted to a compound which appeared by ^{13}C NMR to be a deoxy-aldonate.

Synthesis of Methyl [2-^{13}C]Fructosides. The methyl glycosidation reaction was carried out in anhydrous methanol containing [2-^{13}C]fructose and 1% trifluoroacetic acid; the resultant product was found by ^{13}C NMR to consist of 81.8% of the methyl furanosides, 10.3% of the methyl pyranosides, and 7.9% of residual free fructose. For the preparation of methyl furanosides of fructose and many other sugars, we have found this technique to be vastly superior to the more traditional techniques using anhydrous acids such as HCl which yield much higher ratios of the pyranosides. All four methyl glycosides were resolved by chromatography on AG 1X8 OH^-.

Synthesis of [2-^{13}C]Dihydroxyacetone. Scheme I illustrates the synthetic route for the synthesis of [2-^{13}C]dihydroxyacetone (**6**) from D-[2-^{13}C]glucose (**1**). As described above, D-[2-^{13}C]glucose (**1**) is converted to D-[2-^{13}C]fructose (**2**) followed by conversion to a mixture of methyl fructosides (**3**). Although the β-furanoside is depicted in the scheme, the crude product from the glycosidation (81.8% furanosides) was used for the periodate reaction. Further purification of the fructoside mixture is unnecessary because all four of the fructosides will yield the same labeled product, and the free fructose will be destroyed. The periodate reaction gives the dialdehyde (**4**) which is reduced with borohydride to give the mixed acetal of dihydroxyacetone (**5**). Hydrolysis of this product leads to a mixture of [2-^{13}C]dihydroxyacetone (**6**) and unlabeled glycerol (**7**). This mixture can be used for many subsequent synthetic reactions with no problems or the mixture can be separated by chromatography on AG 50 Ba^{++}.

^{13}C NMR Spectra of [^{13}C]-Enriched Fructose. The ^{13}C-^{13}C coupling constants observed in the ^{13}C NMR spectra of [1-^{13}C] and



SCHEME I

[2-^{13}C]fructose are shown in Table 1. These couplings aid in the assignments of many of the resonances as has been shown for aldoses.[16] The chemical shifts observed in the spectra of the labeled fructose are consistent with the resonance assignments reported by Angyal and Bethell[17] and by Pfeffer *et al.*[18] using deuterated sugars. Three of these assignments have been reported to be incorrect, with the new assignments based on shift changes observed in the spectrum of 3-*O*-methyl-D-fructose.[19] The reassignments involved interchanging C-3 and C-4 of both the β-furanose and the β-pyranose, and C-1 and C-6 of the β-pyranose. In all three cases, a one-bond ^{13}C-^{13}C coupling from our spectrum of [2-^{13}C]fructose makes the assignment unambiguous and illustrates that the reassignments are incorrect. In addition, the intensity of C-1 in our spectrum of [1-^{13}C]fructose reinforces the original assignment for the β-pyranose.[17,18] This assignment problem emphasizes the hazards of making resonance assignments based on chemical derivatives, particularly with closely spaced lines. Unambiguous assignments can best be made by observing the effect of isotopic substitution on the ^{13}C NMR spectra.[16-18,20] The resonance assignments from the enriched fructose are also largely in agreement with those reported by Funcke

TABLE 1. ^{13}C - ^{13}C Couplings in [^{13}C]Fructose[a]

A. [1-^{13}C]Fructose

Fructose Configuration	^{13}C - ^{13}C Coupling to Carbon [b,c]				
	2	3	4	5	6
β-Pyranose	49.8	2.1	2.7	d	br
β-Furanose	51.0	3.0	d	1.5	d
α-Furanose	51.1	br	d	1.7	e
α-Pyranose	49.4				
Open-chain	41.2				

B. [2-^{13}C]Fructose

Fructose Configuration	^{13}C - ^{13}C Coupling to Carbon [b,c]				
	1	3	4	5	6
β-Pyranose	49.8	47.5	3.9	d	d
β-Furanose	51.1	45.5	4.3	d	d
α-Furanose	51.2	47.1	d	d	e
α-Pyranose	49.8				

a. Spectra were taken on a Bruker AM-200 spectrometer at 25 °C
b. Coupling constants are in Hz
c. Broadened peaks are designated br; no table entry means the peaks were not observed
d. No observed coupling
e. This resonance overlaps with the labeled α-pyranose C-1 and is not resolved

et al.,[21] although their work was done at 80 °C to better observe resonances from the less abundant isomers, particularly the open-chain form.

^{13}C NMR Spectra of [^{13}C]-Enriched Methyl Fructosides. Our chemical shift assignments for three of the four methyl fructosides agree with those of Angyal and Bethell[17] whose assignments were based on deuterium substitution. The α-pyranoside was not observed by the earlier workers in a spectrum of unfractionated material; our assignments are reported in Table 2. The ^{13}C-^{13}C coupling constants for the four compounds are reported in Table 3.

EXPERIMENTAL

General. Phenylboronic acid and germanium dioxide were purchased from Aldrich Chemical Co. D-[2-^{13}C]Glucose was prepared as described by Hayes *et al.*[5] Glucose isomerase ("Sweetzyme") was a gift of Novo Laboratories of Wilton, Ct. Ion exchange resins (AG 50, AG 1, and AG 2) were purchased from Bio-Rad, Richmond, CA. Liquid chromatography was carried out at 85 °C using a 300 x 7.8 mm column of Aminex HPX–87P from Bio-Rad and a Model 1770 refractive index monitor. Column fractions were detected using a refractive index detector (Waters R404) connected to the column and were identified either by liquid chromatography or ^{13}C NMR. A Radiometer PHM 85 pH meter with an Orion Ross combination electrode was used to adjust the initial pH of the [1-^{13}C]mannose isomerization reaction mixture. The electrode was calibrated between pH 10.0 and 12.0 using standard buffers; sample pH was adjusted with 3N, 1N, and 0.1N sodium hydroxide.

Proton-decoupled ^{13}C NMR spectra were obtained on aqueous samples containing 5-10% deuterium oxide using a Bruker AM-200 spectrometer operated at 25 °C and with a typical spectral width of 3500 Hz. The peak integrals used to monitor reaction products were determined by the Bruker integrate routine. Peak integrals used to calculate the extent of label scrambling were determined by Lorentzian line shape analysis (fit to the sum of Lorentzian lines) carried out on a VAX 11/780 computer using a modified Levenberg-Marquart algorithm implemented by the NMR1 software package

Table 2. Chemical Shift Assignments for 1-*O*-Methyl α-D-Fructopyranoside[a]

Fructose Configuration	C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-Fructopyranoside	58.2	101.6	68.3	71.4	65.3	61.3	48.8

a. Spectra were taken on a Bruker AM-200 spectrometer at 25 °C. Chemical shifts are relative to TMS as an external standard.

Table 3. ^{13}C - ^{13}C Couplings in 1-*O*-Methyl D-[2-^{13}C]Fructoside[a]

Fructose Configuration	^{13}C - ^{13}C Coupling to Carbon [b]					
	2	3	4	5	6	OMe
β-Pyranose	50.9	48.5	c	c	1.3	2.4
β-Furanose	52.3	46.3	3.9	0.9	c	2.3
α-Furanose	52.8	48.3	2.9	c	2.1	2.1
α-Pyranose	51.3	49.2	c	c		1.9

a. Spectra were taken on a Bruker AM-200 spectrometer at 25 °C
b. Coupling constants are in Hz
c. No observed coupling

supplied by the National Institutes of Health Resource for NMR Data Analysis (Syracuse, NY).

Large-Scale Preparations. We prepared potassium [^{13}C]cyanide at a 2 mole per day rate from [^{13}C]methane and ammonia using a modification of the procedure described by Ott *et al.*[9] [^{13}C]Methane (10.2 L/h) and excess ammonia (10.8 L/h) were passed through a quartz reaction tube (2.8 X 100 cm) containing 250 g of platinum gauze and crumpled platinum foil spread along 61 cm within the tube and heated to 1000 °C with an electric furnace. The product gases (hydrogen [^{13}C]cyanide + hydrogen) were passed through a sparger into a stirred solution of carbonate-free 1 M potassium hydroxide in absolute ethanol contained in a 2-L graduated cylinder. A small trap at the outlet of the reaction tube collected the small amount of brown polymeric material formed in the reaction. Most of the potassium [^{13}C]cyanide precipitated in the ethanol and was filtered in an inert atmosphere, washed with ethanol and ether and dried *in vacuo* at 40 °C. Evaporation of the filtrate *in vacuo* resulted in crystallization of residual potassium [^{13}C]cyanide, which was then recovered by filtration. Carbon deposits which accumulated on the surface of the platinum were removed by flowing oxygen through the reaction tube at 500 °C. The oxygen was flushed from the reactor with helium prior to the next reaction.

We prepared D-[1-^{13}C]glucose and D-[1-^{13}C]mannose from D-arabinose (47 g, 310 mmoles) and potassium [^{13}C]cyanide (18 g, 272 mmoles) essentially as described by Serianni *et al.*[1,2] The nitriles were stabilized at pH 4.0 with acetic acid and were reduced to aldoses with hydrogen (40 psi) and 5% Pd/$BaSO_4$ (18 g). The solution was treated with AG 50 H^+ to remove cations, evaporated *in vacuo* to remove acetic acid, and dried overnight in a desiccator to give 52.7 g of a thick syrup. The epimers were separated by chromatography on AG 50 Ba^{++} (200 - 400 mesh) in a column 9 cm x 140 cm to give D-[1-^{13}C]glucose (10.9 g, 55.1 mmoles, 20.2%) and 41.0 g of a mixture containing D-[1-^{13}C]mannose (20.9 g, 105.5 mmoles, 38.8%) and unreacted arabinose (20.0 g, 108.7 mmoles). The recovery of sugars from the column

was 98.5%, and the recovery of ^{13}C in labeled glucose and mannose from potassium [^{13}C]cyanide was 59.0%.

[^{13}C]Fructose. The conversion of labeled glucose to fructose is the same regardless of the label; the conversion of the [2-^{13}C]-enriched sugar is described. D-[2-^{13}C]Glucose (29.5 g, 149.0 mmoles) and 120 mg of $MgSO_4$ were dissolved in 250 mL of water and the pH adjusted to 8 with sodium hydroxide and maintained between 7.8 and 8.5 with sodium hydroxide or acetic acid as necessary. The solution was stirred at 65 °C with 12 g of "Sweetzyme", and samples were withdrawn at half-hour or hour intervals for ^{13}C NMR analysis. When the proportion of fructose leveled off, the solution was filtered through Celite, treated with AG 50 H^+ and chromatographed on AG 50 Ba^{++} to separate glucose and fructose. Yield: D-[2-^{13}C]glucose 11.0 g, 55.5 mmoles (37.2%); D-[2-^{13}C]fructose 11.8 g, 59.6 mmoles (40.0%). The overall recovery, including 1.5 g of unresolved sugars, was 84.8%.

The reaction could also be carried out using sodium germanate to improve the conversion of glucose to fructose. D-[2-^{13}C]Glucose (5.0 g, 25.2 mmoles) in water (30 mL) was treated with germanium dioxide (1.42 g, 13.6 mmoles) as described by Barker *et al.*[11] to form a glucose-germanate mixture. To this solution was added immobilized glucose isomerase (2 g) and anhydrous magnesium sulfate (20 mg); the temperature was raised to 60 °C and the pH adjusted to 8.0. After 7 h, the level of fructose had peaked and the reaction was quenched by the addition of acetic acid to pH 4 followed by filtration to remove the enzyme. The pH was then adjusted to 3.6 with acetic acid, and the solution was evaporated *in vacuo* to a syrup which was chromatographed on a 5 x 40 cm column of AG 2 OAc^- using 0.1 M acetic acid as an eluant. The sugars, which were partially separated from sodium acetate and completely from germanate, were desalted using AG 50 H^+ and separated on a 5 x 85 cm column of AG 50 Ba^{++}. Yield: D-[2-^{13}C]glucose 0.6 g, 3.0 mmoles (12.0%); D-[2-^{13}C]fructose 4.1 g, 20.7 mmoles (82.1%).

Isomerization of D-[1-^{13}C]Mannose. To a solution of D-[1-^{13}C]mannose (52.0 g containing 9.0 g of D-arabinose and 43.0 g of

D-[1-^{13}C]mannose) in 900 mL of water was added phenylboronic acid (35.2 g, 0.29 moles). The pH was raised to 11.8 with sodium hydroxide and the solution kept at 50 °C under a nitrogen atmosphere. Samples taken for ^{13}C NMR analysis (at intervals of 0.5 or 1 h) were acidified with glacial acetic acid to ca. pH 4. After 8 h, the entire reaction mixture was acidified with glacial acetic acid and the solution kept at 4 °C overnight. The precipitate of phenylboronic acid was removed by filtration and washed with cold water, and the filtrate extracted three times with ether to remove residual phenylboronic acid. The ether layer was back-extracted with water, and the combined aqueous phases were evaporated *in vacuo* to remove any excess ether, followed by batchwise treatment with AG 50 H^+ resin to remove cations. The acidic solution was evaporated *in vacuo* to remove acetic acid, and the resultant syrup was found by ^{13}C NMR to consist of a mixture of D-[1-^{13}C]glucose (10%), D-[1-^{13}C]mannose (15%), and D-[1-^{13}C]fructose (75%). This mixture was treated with glucose isomerase to partially convert the fructose to glucose, and the resulting syrup (36 g) was chromatographed on AG 50 Ba^{++} to separate the three sugars. Yield: D-[1-^{13}C]glucose, 11.0 g (21.1%); D-[1-^{13}C]fructose, 13.5 g (26.2%); D-[1-^{13}C]mannose 8.9 g (17.3%). The overall recovery of labeled sugars (including 1.4 g of unresolved sugars) was 80.9%.

Methyl D-[^{13}C]Fructosides. Dry D-[2-^{13}C]fructose (10.6 g, 59.0 mmole) was dissolved in a solution of dry methanol (250 mL) containing trifluoroacetic acid (2.5 mL), and the mixture stored at room temperature. After 12 h, the solvent was removed by evaporation *in vacuo*, and residual trifluoroacetic acid was removed by the addition and evaporation of dry dioxane. The syrup (15.4 g), which contained residual dioxane, could be used directly for the preparation of D-[2-^{13}C]dihydroxyacetone, or the methyl fructosides could be resolved by chromatography on AG 1 OH^-.

D-[2-^{13}C]Dihydroxyacetone. Methyl D-[2-^{13}C]fructofuranoside syrup (12.5 g containing 55.5 mmole of sugar and residual dioxane) was dissolved in 200 mL of water in an ice bath. The solution was stirred in an ice bath under a nitrogen atmosphere, and a 1.28 molar excess of sodium *meta*-periodate

(15.2 g, 71.1 mmole) was added in ten equal portions over a period of 2.5 h. After stirring for an additional hour in the cold, the reaction mixture was treated with a solution containing an excess of barium acetate in 50 mL of cold water. The resulting precipitate was filtered through Celite to remove the insoluble barium salts and the filtrate titrated to pH 8 with sodium hydroxide. Sodium borohydride (5.4 g) was added, and after 3 h at room temperature, the solution was titrated with AG 50 H^+ resin to pH 2.4. The resin was then removed by filtration, and the filtrate was concentrated *in vacuo* to a syrup. Boric acid was removed as methyl borate by the addition of methanol followed by evaporation *in vacuo*. Two similar treatments with water were used to remove acetic acid. The syrup was dried overnight in a desiccator after which it weighed 8.5 g and contained equimolar amounts of the desired D-[2-^{13}C]dihydroxyacetone (4.25 g, 47.2 mmole, 85.0% yield) and unlabeled glycerol. The syrup could be used directly for subsequent syntheses or chromatographed on AG 50 Ba^{++}.

ACKNOWLEDGMENT

We gratefully acknowledge Ellen Stallings of Los Alamos National Laboratory for the ICP-MS analysis of the phenylboronate. This work was supported in part by U. S. P. H. S. Grant RR 02231 and carried out under the auspices of the Department of Energy/OHER, Stable Isotopes Program.

REFERENCES

1. A. S. Serianni, H. A. Nunez, M. L. Hayes, and R. Barker, *Methods in Enzymology,* **89,** 64-73 (1982).

2. A. S. Serianni, H. A. Nunez, and R. Barker, *Carbohydr. Res.,* **72,** 71-78 (1979).

3. A. S. Serianni, E. Cadman, J. Pierce, M. L. Hayes, and R. Barker, *Methods in Enzymology,* **89,** 83-92 (1982).

4. E. L. Clark, M. L. Hayes, and R. Barker, *Carbohydr. Res.,* **153,** 263-270 (1986).

5. M. L. Hayes, N. J. Pennings, A. S. Serianni, and R. Barker, *J. Amer. Chem. Soc.*, **104,** 6764-6769 (1982).

6. D. L. Williams and T. W. Whaley, *J. Labelled Compd. Radiopharm.*, **19,** 669-679 (1982).

7. V. H. Kollman, J. L. Hanners, R. E. London, E. G. Adame, and T. E. Walker, *Carbohydr. Res.*, **73,** 193-202 (1979).

8. M. J. King-Morris and A. S. Serianni, *Carbohydr. Res.*, **154,** 29-36 (1986).

9. D. G. Ott, V. N. Kerr, T. G. Sanchez, and T. W. Whaley, *J. Labelled Compd. Radiopharm.*, **17,** 255-262 (1980).

10. S. A. Barker and J. A. Shirley, *Econ. Microbiol.*, **5,** 171-226 (1980).

11. S. A. Barker, H. Pelmore, and P. J. Somers, *Enzyme Microb. Technol.*, **5,** 121-124 (1983).

12. C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec trav. chim.*, **14,** 203 (1895).

13. J. C. Speck, *Adv. Carb. Chem.*, **13,** 63-103 (1958).

14. S. A. Barker, A. K. Chopra, B. W. Hatt, and P. J. Somers, *Carbohydr. Res.*, **26,** 33-40 (1973).

15. S. A. Barker, B. W. Hatt, and P. J. Somers, *Carbohydr. Res.*, **26,** 41-53 (1973).

16. T. E. Walker, R. E. London, T. W. Whaley, R. Barker, and N. A. Matwiyoff, *J. Amer. Chem. Soc.*, **98,** 5807-5813 (1976).

17. S. J. Angyal and G. S. Bethell, *Aust. J. Chem.*, **29,** 1249-1265 (1976).

18. P. E. Pfeffer, K. M. Valentine, and F. W. Parrish, *J. Amer. Chem. Soc.*, **101,** 1265-1274 (1979).

19. T. A. W. Koerner, Jr., R. J. Voll, L. W. Cary, and E. S. Younathan, *Biochem. Biophys. Res. Commun.*, **82,** 1273-1278 (1978).

20. R. Barker and T. E. Walker, *Methods Carb. Chem.*, **8,** 151-165 (1980).

21. W. Funcke, C. von Sonntag, and C. Triantaphylides, *Carbohydr. Res.*, **75,** 305-309 (1979).