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SYNTHESIS OF METHYL α- AND β-D-XYLOPYRANOSIDE-5-^{18}O*

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ABSTRACT

Methyl α- and β-D-xylopyranoside-5-^{18}O (**5** and **6**) were prepared by way of oxygen exchange between ^{18}O-water and the periodate-oxidation product (**1**) obtained from 1,2-*O*-isopropylidene-α-D-glucofuranose. The isotopic enrichment of **5** and **6** was determined by hydrolysis of each to D-xylose-5-^{18}O (**3**), conversion of the sugar into 1,2,3,4-tetrakis-*O*-(*tert*-butyldimethylsilyl)-β-D-xylopyranose-5-^{18}O (**7**), and determination of the ^{18}O content of the latter by use of a quadrupole, mass spectrometer.

INTRODUCTION

To determine kinetic isotope-effects in the hydrolysis of pyranosides at their ring-oxygen atom, we needed methyl α- and β-D-xylopyranoside-5-^{18}O (**5** and **6**). We describe here the synthesis of the labeled glycosides, and the determination of their ^{18}O content by quadrupole, mass spectrometry.

The sequence of reactions used to synthesize **5** and **6** is shown in Scheme 1. The anhydrous, crystalline, so-called "dimer" bis(1,2-*O*-isopropylidene-α-D-xylopentodialdose) 3,5′:5,5′-cyclic acetal† (**1**), prepared by a modification of the procedure described by Schaffer and Isbell[1], was exchanged at its acetal oxygen atoms with ^{18}O-water in boiling tetrahydrofuran saturated with trimethylamine. After removal of the solvents, the ^{18}O-enriched product (**1**) was reduced with lithium aluminum hydride to give chromatographically pure 1,2-*O*-isopropylidene-α-D-xylofuranose-5-^{18}O (**2**) in 88% yield. Methanolysis of **2** (92% yield), followed by esterification of the mixture of glycosides (**3**) with phenylboronic acid gave the 2,4-phenylboronates (**4**)

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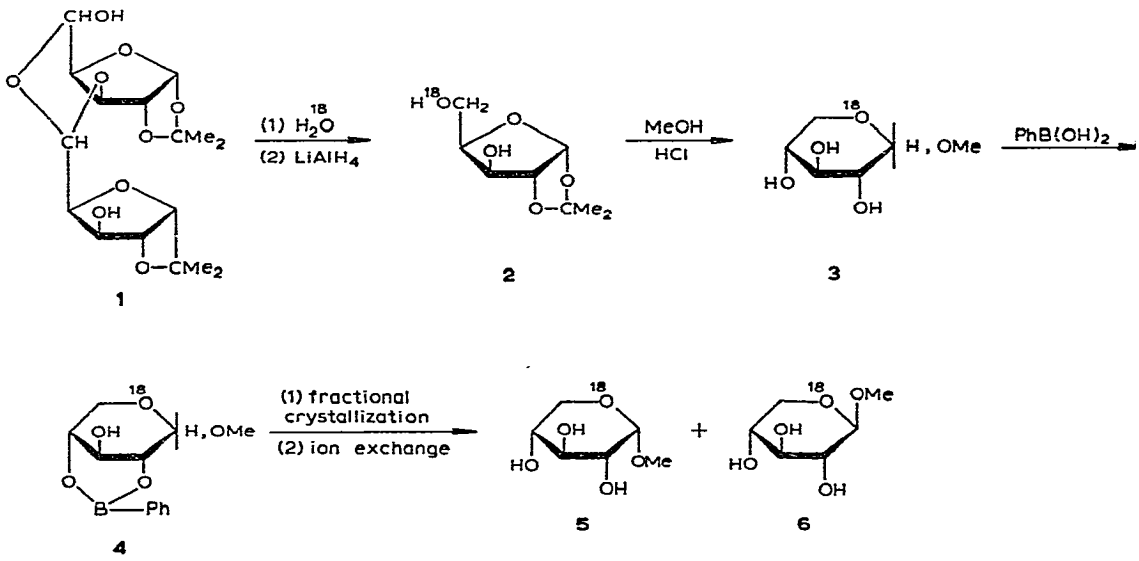
†Systematic name: 1,2-*O*-isopropylidene-3,5-*O*-[4,5-*O*-isopropylidene-(L-*gluco*-tetrahydro-3,4,5-trihydroxyfurfurylidene)]-(5-hydroxy-D-xylofuranose).



Scheme 1

of the pyranosides, which were separated by fractional recrystallization[2] of the α anomer. Removal of the phenylboronic group gave pure methyl α-D-xylopyranoside-*5*-^{18}O (**5**) and pure methyl β-D-xylopyranoside-*5*-^{18}O (**6**), each in ~7% yield from **1**. The low yield of isolated glycosides was due almost entirely to the fractional recrystallization step.

To measure the kinetic isotope-effect of the ring-oxygen atom during hydrolysis of **5** or **6**, we needed to measure the levels of ^{18}O in the D-xylose-*5*-^{18}O; therefore, we decided to measure ^{18}O enrichment in **5** and **6** through that in their hydrolysis product. In the assay procedure, D-xylose-*5*-^{18}O was treated with a mixture of *tert*-butylchlorodimethylsilane and imidazole[3]. The reaction mixture was subjected to preparative gas–liquid chromatography (g.l.c.) to isolate *tert*-butyldimethylsilyl 2,3,4-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-xylopyranoside-*5*-^{18}O (**7**), and the ^{18}O content of the latter compound was determined directly with an interfaced gas–liquid chromatograph–quadrupole mass spectrometer. The advantages of the quadrupole, mass spectrometer for determining isotopic composition have been discussed by Caprioli *et al.*[4]. D-Xylose released by 5% hydrolysis of **5** or **6** can be readily freed from large proportions of unreacted glycoside by g.l.c. of the etherified mixture. Quadrupole, mass spectrometry has already been used[4,5] to measure the abundance of ^{18}O in specifically ^{18}O-labelled D-glucose and D-fructose with a precision of 0.2–0.4% and an accuracy of 0.3–0.6%.

Three criteria had to be met[5] before mass spectrometry could be used to determine the isotopic composition of D-xylose-^{18}O: (*i*) it was necessary to prepare from the sugar a volatile derivative whose mass spectrum contained an ion of definite elemental composition that included O-5, (*ii*) that ion could not have peaks of signi-

ficant intensity that were one or two mass units adjacent to the ion being measured, and (*iii*) the intensity of the ion had to be high enough to provide precise data. These criteria were satisfied by one ion-fragment (*m/e* 549) in the mass spectrum of compound **7**. The corresponding pertrimethylsilylated trimethylsilyl glycoside was examined, and no peaks in its spectrum could be used.

The molecular ion of **7** (*m/e* 606) was very weak, but a relatively strong ion-fragment was observed at *m/e* 549, corresponding to the loss of a *tert*-butyl group ($M^+ - 57$). No peaks of significant intensity were observed at *m/e* 547 or 548 when the mass spectrum was measured at high attenuation. In addition, the intensities of the peaks at *m/e* 550 and 551 in the unlabelled compound agreed with those expected from the natural abundances of heavier isotopes. To determine the level of ^{18}O enrichment in the D-xylopyranoside **5**, the relative intensities of the peaks at *m/e* 549 and 551 in an unenriched sample of compound **7** were compared with the intensities of those peaks in the ^{18}O-labelled sample. Each sample of **7** was analyzed by three separate injections into the g.l.c.–mass spectrometer, followed by triplicate countings of the ion-intensities at *m/e* 549 and 551. Thus, the measurement of the abundance of the ($M^+ + 2$) ion from **7** was the average of nine separate determinations. The g.l.c.–m.s. assay was also repeated on a duplicate sample of **7** prepared by hydrolysis of **5**. The abundances of *m/e* 551 relative to the ion-intensities counted at *m/e* 549 are given in Table I. From these data, the enrichment of D-xylose with oxygen-18 was calculated to be 37.3 ±0.2 atom-% excess. The duplicate analysis value of 37.6 ±0.6 atom-% excess agreed closely.

TABLE I

ISOTOPIC ABUNDANCES OF ION FRAGMENTS OF *m/e* 551 FROM LABELLED AND UNLABELLED *tert*-BUTYLDIMETHYLSILYL 2,3,4-TRIS-*O*-(*tert*-BUTYLDIMETHYLSILYL)-β-D-XYLOPYRANOSIDE (**7**)

Sample of hydrolyzed glycoside	*Identity*	*Area of* m/e *551 in mass fragmentogram*[a]	^{18}O *enrichment (atom-% excess)*
1-U	unlabelled **7**	28.61 ±0.23	—
1-L	labelled **7**	147.0 ±0.6	37.3 ±0.2
2-U	unlabelled **7**	24.90 ±0.92	—
2-L	labelled **7**	135.6 ±0.2	37.6 ±0.6

[a]Area relative to area of *m/e* 549 at 100.00; average of nine determinations.

The ^{18}O assay on D-xylose is precise enough to detect a ring-oxygen isotope-effect of ⩾1% during the hydrolysis of **5** or **6**. The maximal kinetic isotope-effect expected is[6] ~6%, provided that the ring-oxygen atom is involved in the rate-limiting step. The ^{18}O content of D-xylose-5-^{18}O was 58% of the value calculated had the exchange reaction involving **1** achieved equilibrium. We found that the exchange reaction had to be terminated short of completion, because prolonged heating of **1** in the basic medium gradually destroyed it, as evidenced by t.l.c. and by lowered yields of compound **2**.

We assumed that all of the oxygen-18 introduced into the D-xylose was situated at O-5; the synthesis should have been specific, in that primary and secondary alcohols are known to exchange extremely slowly with water under acidic or basic conditions[7,8]. Although this point will eventually be checked quantitatively, preliminary verification was obtained by using a high-resolution, mass spectrometer. The mass spectrum of the bis(trimethylsilyl) ether of compound **2** gave strong peaks[9] at *m/e* 319 and 103. The fragment at *m/e* 319 is due to the loss of a methyl group from the molecular ion; the latter fragment, $[CH_2OSiMe_3]^+$, which contains O-5, is formed by cleavage of the C-4–C-5 bond. Within experimental error, the ratio of the intensity of the peak at *m/e* 105 to that at *m/e* 103 equalled the ratio of the intensity of that at *m/e* 321 to that at *m/e* 319.

EXPERIMENTAL

General. — Solutions were evaporated under diminished pressure below 40°. Melting points, determined on a Fisher–Johns melting-point apparatus, were not corrected. G.l.c. was conducted with a Hewlett–Packard Model 5750 gas chromatograph equipped with a column (1.83 m × 6.35 mm) packed with 3% of Dexsil 300 on Chromosorb W (80–100 mesh: Analabs, Inc., New Haven, Conn.). An automated collection-unit, Model 5795A, was used for preparative g.l.c. The column was operated isothermally at 230° at a gas (helium) flow-rate of 150 ml.min^{-1}. Mass spectra were also obtained with an MS-9 double-focusing, high-resolution, mass spectrometer (Model MS 902, Associated Electrical Industries, Ltd., Urmston, Manchester, England).

1,2-O-Isopropylidene-α-D-xylofuranose-5-^{18}*O* (**2**). — Bis(1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdofuranose) 3,5′:5,5′-cyclic acetal (**1**) was prepared by a modification of the method described by Schaffer and Isbell[1]. To a solution of sodium periodate (22 g) in water (150 ml) was added, dropwise, a solution of 1,2-*O*-isopropylidene-α-D-glucofuranose (22 g) in water (22.5 g), and the mixture was stirred for 30 min at 25° and then extracted with chloroform (5 × 300 ml). The extracts were combined, dried (anhydrous sodium sulfate), and evaporated to a syrup which was dissolved in benzene (250 ml), and the solvent slowly removed (10 h) by distillation in a Dean–Stark apparatus. During the distillation, paraformaldehyde solidified on the condenser. The last traces of formaldehyde in **1** were removed by crystallization from water. The crystalline monohydrate of **1** (m.p. 175–178°) was dissolved in absolute ethanol, the solution dried with 4A molecular sieves, the suspension filtered, and the filtrate evaporated to dryness; dry benzene was added to and evaporated from the syrup. The anhydrous form of **1** crystallized from benzene in an overall yield of 52%, m.p. 180–182° (lit.[1] m.p. 182–184°).

The anhydrous dimer (**1**; 2.19 g, 5.8 mmoles) was dissolved in dry tetrahydrofuran (60 ml) presaturated with trimethylamine at 25°. After the addition of ^{18}*O*-water (0.5 g; 25.1 mmoles; 95 atom-% ^{18}O; BioRad Laboratories, Richmond, California), the mixture was refluxed for 9 h with protection from atmospheric

moisture. The solvent was removed by use of a mechanical vacuum-pump, and the syrup was dried by three successive additions and evaporations of dry benzene (20 ml). T.l.c. on silica gel with 9:10 dichloromethane–methanol showed that the exchanged product contained two components whose mobilities were identical to those of the two components in the crystalline starting-material (**1**). Small amounts of other components were visible (lower R_F values than those of the two main components) in the reaction mixture.

The ^{18}O-enriched dimer (2.19 g, 11.6 mmoles) was dissolved in dry tetrahydrofuran (20 ml), and the solution was added dropwise, with stirring, to a mixture of lithium aluminum hydride (0.41 g, 11.6 mmoles) and tetrahydrofuran (15 ml). After being refluxed for 20 min, the mixture was cooled, and water (2 ml) was slowly added, with stirring, After 1 h, the inorganic salts were removed by filtration, the filtrate was evaporated, and the resulting syrup was dried by four successive dissolutions in and evaporations of absolute ethanol. T.l.c. then showed that the syrupy product (**2**) (1.94 g, 88.2%) contained only one component, having a mobility identical to that of a crystalline sample of known 1,2-*O*-isopropylidene-α-D-xylofuranose[10].

Methyl α- and β-D-*xylopyranoside*-5-^{18}O (**5** *and* **6**). — Prior to methanolysis, the syrupy **2** (1.94 g) was repeatedly dissolved in dry methanol and the solution evaporated to remove all traces of ethanol. A solution of the acetal **2** in methanolic hydrogen chloride (0.14M; 20 ml) was boiled under reflux for 8 h, and then evaporated to a thin syrup, which was passed through a column of Dowex AG-1(OH^-), a strongly basic anion-exchange resin. Evaporation of the effluent gave 1.55 g (92% based on **2**) of a mixture of glycosides which, on dissolution in 2:1 ethanol–ethyl acetate (15 ml), followed by cooling, deposited crystals (164 mg) of pure methyl β-D-xylopyranoside-5-^{18}O (**6**), m.p. 153–156°; lit.[11] m.p. 157°. The mother liquors from the crystallization were placed in a flask containing glass beads, and evaporated to dryness; phenylboronic acid (1.35 g, Aldrich Chemical Co., Milwaukee, Wisconsin) and benzene were added as described by Ferrier *et al.*[2]. Slow distillation of the benzene during 4 h removed the water released by esterification. The mixture was then filtered, and the filtrate concentrated to 15 ml. Petroleum ether was added; on cooling, the solution deposited crystals of methyl α-D-xylopyranoside-5-^{18}O 2,4-phenylboronate, m.p. 172–175° (lit.[2] m.p. 175–176°). Two crops of crystals (totaling 962 mg) were collected. The phenylboronate was de-esterified by dissolving in acetone (5 ml), adding water (10 ml) containing 4 ml of Dowex AG-1 (OH^-), and stirring for 1 h. The solution was then passed through a column containing a mixture of strongly basic and strongly acidic ion-exchange resins, namely, Amberlite MB-3 (H^+, OH^-). The effluent was evaporated to dryness, and the resulting syrup was dried by repeated addition and evaporation of absolute ethanol. Two recrystallizations from ethanol gave 244 mg (6.5% from **1**) of pure methyl α-D-xylopyranoside-5-^{18}O (**5**) having m.p. 88–91°; lit.[11] m.p. 90–92°.

The mother liquors obtained from the fractional recrystallization of the methyl α-D-xylopyranoside-5-^{18}O 2,4-phenylboronate contained mainly the 2,4-phenyl-

boronic ester of the β-D-pyranoside; this was de-esterified as just described, and pure methyl β-D-xylopyranoside-5-^{18}O (**6**) (112 mg) was obtained by crystallization from ethanol. The total yield of the β-D anomer **6** was 276 mg (7.6% from **1**).

Measurement of ^{18}O-enrichment. — A solution of **5** (10 mg) in 0.5M aqueous sulfuric acid was boiled under reflux for 1 h; the hydrolyzate, which contained no glycoside (as evidenced by paper chromatography), was treated in the usual way, and the solution was evaporated to dryness. The residue was treated with a mixture (2.0 ml) of *tert*-butylchlorodimethylsilane (2 mmoles) and imidazole (5 mmoles) in *N,N*-dimethylformamide (Applied Science, Inc., State College, Pennsylvania), and the mixture was heated for 30 min at 60°, cooled, stirred for 24 h at 25°, and then added to ether–water. The organic phase was washed with water, and dried (magnesium sulfate). The slowest-moving peak in a gas chromatogram was identified as that of *tert*-butyldimethylsilyl 2,3,4-tris-*O*-(*tert*-butyldimethylsilyl)-β-D-xylopyranoside (**7**). Samples of the pure compound were collected neat in a U-tube, and stored at 0° prior to mass-spectral analysis.

A Finnigan Model 3200 GC/MS instrument equipped with a 6100 data system (Finnigan Corp., Sunnyvale, California) was used to determine isotopic enrichment. Samples (1 μl) of **7** were dissolved in spectral-grade acetone (5 ml), and aliquots (1 μl) of the solution were injected into the GC/MS instrument. Continuous, selected ion-monitoring was used, with isotope ratios determined by computer calculation of the areas under the peaks in the resulting fragmentogram[4]. An ionizing voltage of 70 eV was used in the mass spectrometer, with the analyzer at 100°, and the separator at 270°. A glass column (2 × 200 mm) packed with Dexsil 300 was employed in the gas–liquid chromatograph, with the injector at 200° and the oven at 180°.

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