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EVIDENCE FOR A C-2→C-1 INTRAMOLECULAR HYDROGEN-TRANSFER DURING THE ACID-CATALYZED ISOMERIZATION OF D-GLUCOSE TO D-FRUCTOSE*†

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ABSTRACT

In mechanistic studies by isotope-exchange techniques of the conversion of D-fructose and D-glucose into 2-(hydroxyacetyl)furan, it was shown that both sugars are converted in acidified, tritiated water into the furan containing essentially no carbon-bound tritium. As the hydroxymethyl carbon atom of the furan corresponds to C-1 of the hexose, this result suggests that one of the hydrogen atoms in this group, when it is produced from D-glucose, must arise intramolecularly. This hypothesis was verified by synthesizing D-glucose-*2*-^3H and converting it into the furan in acidified water. The 2-(hydroxyacetyl)furan obtained was labeled exclusively on the hydroxymethyl carbon atom, thus showing that intramolecular hydrogen-transfer occurs, during the conversion, from C-2 of D-glucose to the carbon atom corresponding to C-1. The specific activities of the product and reactant permitted calculation of the tritium isotope-effect ($k_h/k_t = 4.4$) for the reaction. The precise step for the transfer from C-2 of the aldose to the carbon atom corresponding to C-1 was found to be during the isomerization of D-glucose to D-fructose, as evidenced by the conversion of D-glucose-*2*-^3H into D-fructose-*1*-^3H in acidified water.

INTRODUCTION

The interconversion of aldoses and ketoses by general acid–base catalysis (the Alberda von Eckenstein–Lobry de Bruyn transformation) is a well known reaction in carbohydrate chemistry[1]. The mechanism of the transformation, as it occurs in alkaline solution, has been extensively studied by isotope-exchange techniques[2,3] and the data are consistent with a reaction involving a 1,2-enediol intermediate, common to both products, which gives rise to the 2-ketose by acceptance of a solvent proton at C-1, or to the appropriate aldose, by protonation at C-2. The mechanism is further supported by observations that sugars such as D-mannose, D-glucose, and D-fructose are interconvertible in alkaline solution. The mechanism for the transformation in acid solution, which requires much more drastic reaction-conditions, has not

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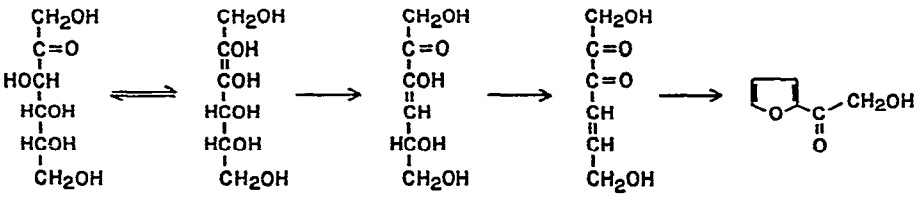
been as extensively studied, but is, nevertheless, thought to proceed via a similar 1,2-enediol intermediate. Isotope-exchange studies have not been performed for the reaction in acid, but observations that D-fructose gives rise to D-glucose[4] on acid treatment, and that D-mannose[5] can also be formed, are consistent with such a mechanism.

The features of the transformation are different in biological systems when it is catalyzed by isomerase enzymes. It has been shown that, for the case of D-glucose phosphate isomerase[6], partial intramolecular hydrogen-transfer from C-2 of the aldose to a stereospecific C-1 position of the ketose occurs. For a number of pentose isomerases, the transfer from C-2 to C-1 is quantitative, that is, solvent protons do not participate in the reaction to the extent that they become carbon-bound[7–9]. Except for the work of Gleason and Barker[10], who showed that partial transfer of hydrogen from C-2 to C-1 occurs during the interconversion of D-ribose and D-arabinose in alkaline solution, intramolecular transfer-reactions have not been reported as features of chemically catalyzed, isomerizations of sugars.

This paper presents evidence for an intramolecular hydrogen-transfer from C-2 to C-1 during the conversion of D-glucose into D-fructose in acid solution. Indirect evidence for this phenomenon was obtained during some prior studies on the conversion of D-glucose and D-fructose into 2-(hydroxyacetyl)furan and this was reported in preliminary form[11]. In this paper, details of these studies are reported, and direct evidence is given for the transfer reaction, the conversion of D-glucose-*2*-^3H into D-fructose-*1*-^3H in aqueous acid.

The probable mechanism for the formation of 2-(hydroxyacetyl)furan (**5**) from D-fructose (**1**) involves the conversion of the ketose **1** into the 2,3-enediol **2**, which undergoes dehydration to the enolic form (**3**) of 4-deoxy-D-*glycero*-2,3-hexodiulose and thence to **5** via the 4-enose **4**. The mechanism is analogous to that proposed[12] for the formation of 2-furaldehydes from hexoses, a reaction that is similar to the one considered here and which has had considerable experimental verification. The fact[13] that **5** is produced in much lower yield from D-glucose than from **1** probably stems from the fact that the aldose must first be converted into the ketose, with subsequent decomposition through intermediates **2**–**4**. During attempts to verify this mechanism by using isotope-exchange techniques, it was found that **5** derived from either D-glucose or D-fructose, after reaction in acidified, tritiated water, was essentially devoid of carbon-bound tritium; it contained less than 6% the activity of the solvent.



1 **2** **3** **4** **5**

As it is generally conceded that the hydroxymethyl carbon atom of **5** corresponds to C-1 of the starting sugar, the results indicate that one of the carbon-bound hydrogen atoms of this group, when it is derived from C-1 of D-glucose, arises from an intramolecular source and not from the solvent. To examine this possibility, D-glucose-*2*-^3H was synthesized by converting D-fructose 6-phosphate into D-glucose-2-^3H 6-phosphate in tritiated water with D-glucose, phosphate isomerase, with subsequent isolation of the D-glucose-*2*-^3H after treatment with alkaline phosphatase. This enzymic process is known[6,14] to give D-glucose specifically tritiated at C-2. When the D-glucose-*2*-^3H was converted into **5** in acidified ordinary water, the **5** so obtained had a specific activity that was 23% that of the starting sugar, and all of the label was located at the hydroxymethyl carbon atom, as evidenced by the conversion (by periodate oxidation) of tritiated **5** into 2-furoic acid that contained less than 3% of the activity of the starting **5**.

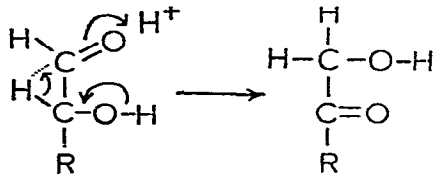
The foregoing data indicate that the extent of intramolecular transfer involved in the conversion of D-glucose into **5** under these conditions (M sulfuric acid at 100°) is nearly complete (as evidenced by negligible isotope incorporation from the solvent when **5** is produced in tritiated water), and that the differences in the specific activities of D-glucose-*2*-^3H and the **5**-^3Hproduced from the reaction are, therefore, the result of a kinetic isotope-effect. The ratio of the activities indicate that the magnitude of this effect (k_h/k_t) is 4.4.

The most logical step for a transfer to occur is in the conversion of D-glucose into D-fructose. This possibility was examined by converting D-glucose-*2*-^3H into D-fructose in ordinary acidified water. It was not feasible to perform the conversion under the same conditions whereby D-glucose-*2*-^3H was converted into **5**, because of the low yields of D-fructose. At lower acidity (pH 3.0), the yields were much improved, and radioactive D-fructose was isolated as its crystalline 2,3:4,5-diisopropylidene acetal after removal of the D-glucose by crystallization and treatment of the residual syrup with D-glucose oxidase. The isotope was shown to be located at C-1 by treatment of the derivative with alkaline permanganate under conditions that convert it into 2,3:4,5-di-*O*-isopropylidene-D-*arabino*-hexulosonic acid. This procedure released all of the tritium into the solvent. The D-fructose-*1*-^3H so obtained contained 12% the activity of the starting D-glucose-*2*-^3H, in contrast to the reaction in stronger acid, which involves nearly complete transfer and gives **5** containing 23% of the activity of the starting D-glucose-*2*-^3H. We cannot account for this discrepancy except to point out that the experimental conditions were different; side reactions, perhaps base-catalyzed, could be more prominent at lower acidity.

The 12% retention of activity observed under these conditions in the conversion of D-glucose-*2*-^3H into D-fructose-1-^3H, in conjunction with the observed isotope-effect for the conversion of D-glucose-*2*-^3H into 5-^3H, indicates that at least 53% of the D-glucose to D-fructose conversion is accomplished with intramolecular transfer of hydrogen when the reactant is ordinary D-glucose rather than D-glucose-*2*-^3H.

Although the intramolecular transfer was only partial at pH 3, it must be nearly complete at higher acidities in order to account for the isotope-exchange data

obtained for the overall dehydration reactions. Thus, in spite of the fact that 1,2-enediols are generally accepted as intermediates in aldose–ketose interconversions, the data obtained here at high acidities are more consistent with a concerted mechanism involving a hydride shift, as illustrated. It is noteworthy that a similar mechanism does not appear to function in basic solution, as Isbell and associates[15] found that the D-fructose produced from D-glucose-*2*-^3H in aqueous base was not radioactive.



The analogy between the acid-catalyzed isomerization and the isomerase-catalyzed reactions is obvious, but may not be complete, because the addition of hydrogen to C-1 of the ketose from C-2 of the aldose is stereospecific in the enzyme-catalyzed reaction and the stereochemistry is not yet known for the acid-catalyzed reaction. Interestingly, the intramolecular hydrogen-transfer involved in the D-glucose phosphate isomerase reaction was originally discussed in terms of a hydride-shift mechanism, but more recent mechanisms[9] suggested for this class of enzymes have involved the idea that classical 1,2-enediols are intermediates in these reactions, and that they may arise via sugars in energetically unfavorable, boat conformations.

EXPERIMENTAL

Materials and methods. — A Packard Tri-Carb scintillation counter was used with a scintillant made up of 2 parts of a solution composed of 2 litres of toluene, 8.25 g of 2,5-diphenyloxazole (PPO), 0.25 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (Me_2POPOP), and 1 part of Triton X-100 (*v*/*v*). Efficiencies were determined by using an internal standard of tritiated toluene. U.v. spectra were obtained with a Coleman Model 124 recording, double-beam, grating spectrophotometer. T.l.c. was performed with silica gel HF support and 19:1 chloroform–methanol as the eluant. Spots were visualized on the plates by u.v. radiation at 254 nm or by spraying with 10% ethanolic sulfuric acid followed by heating for 10 min at 110°. Paper chromatograms were obtained with Whatman No. 1 paper and were developed by the descending method, with 18:1:3:4 (*v*/*v*) ethyl acetate–formic acid–acetic acid–water as irrigant. Spots were visualized with aniline hydrogen phthalate.

Preparation of D-*glucose*-2-^{3}H. — This preparation was similar to that used[14,16] for preparing D-glucose specifically deuterated at C-2. To 100 ml of water containing 1 Ci of tritium was added 15 g of D-fructose 6-phosphate (Ba salt), several seed crystals of barium D-glucose 6-phosphate, and 250 mg of D-glucose phosphate isomerase (Sigma Type III). After incubation for 10 h at 37°, the resulting, crystalline

barium D-glucose 6-phosphate was isolated on a funnel and washed with 10 ml of water. Identical amounts of the foregoing materials were then added to the filtrate, and the process was repeated until a total of 75 g of barium D-fructose 6-phosphate had been passed through. The combined yield of barium D-glucose 6-phosphate-*2*-^3H was dried in a desiccator, and recrystallized by suspending it in 400 ml of water, acidifying with hydrochloric acid until dissolution occurred, and then rendering the solution neutral with a saturated solution of barium hydroxide. These crystals were filtered off, washed with water, and dried *in vacuo* over calcium chloride. For conversion of the phosphate ester into D-glucose, the foregoing preparation was added to 1 liter of carbonate–hydrogen carbonate buffer (pH 10.4) and 500 mg of alkaline phosphatase (Sigma Type III) was added. The suspension was kept for 18 h at 37° and then filtered through Celite. The filtrate was deionized by treatment with Dowex-50 (H^+) and Dowex-1 (CO_3^{2-}) and evaporated to a foam; yield 8.0 g. The preparation had a paper-chromatographic flow-rate identical to that of authentic D-glucose, and no contamination by D-fructose could be detected.

The conversion of sugars into **5** *in tritiated water.* — In a typical experiment, 216 g of D-fructose was dissolved in 2400 ml of water that contained 100 mCi of tritium (as ^3H_2O) and was 0.5 M in sulfuric acid. After heating for 1.5 h at 100°, the solution was cooled and clarified by filtration through Whatman No. 1 paper. The filtrate was extracted with six 200-ml portions of chloroform and the extract was dried (sodium sulfate) and evaporated. The crystalline **5** contained in the residue was isolated by sublimation at 70° and 0.25 mmHg. This material was recrystallized twice from hexane to give 3.1 mg of material having a specific activity of 0.048 μCi per mmole, corresponding to 6% the activity of the water. D-Glucose was converted into **5** in exactly the same way, except that the tritiated water was M in sulfuric acid and the heating time was 3.5 h. The yield of purified **5** was 0.739 mg per experiment, and the material had a specific activity of 0.0405 μCi per mmole. In both examples, the **5** so obtained was identified by its u.v. spectrum (λ_{max} 260 nm) and by the fact that its t.l.c. mobility was identical to that of an authentic standard.

The conversion of D-*glucose*-2-^{3}H *into* **5**. — To 2.4 liters of water was added 216 g of D-glucose-*2*-^3H (prepared by diluting the 8-g sample of the foregoing D-glucose-*2*-^3H). To measure the activity of the starting material accurately, 10 ml of solution was withdrawn, evaporated to dryness, and the residue was converted into α-D-glucose pentaacetate[17]. After 3 recrystallizations from ethanol, the material exhibited a constant specific-activity of 0.916 μCi per mmole. The remainder of the solution was made M with concentrated sulfuric acid and, after heating for 3 h at 100° the resulting **5** was isolated by extraction with chloroform.

The resulting chloroform extract was found to contain 5.0 mg of **5**, as determined by spectrophotometric measurements at 260 nm in conjunction with a standard curve. The **5** was isolated by evaporation of the chloroform solution and was purified by 3 successive thin-layer chromatographic purifications. The resulting material migrated as a single spot on thin-layer chromatograms, had a flow rate identical to that of **5**, and gave a u.v. spectrum identical to that of authentic **5**.

This procedure was repeated 9 times, and each sample (about 5 mg) was separately isolated and purified, and an aliquot containing 2 mg was counted. The first 2 samples had specific activities of 1.56 and 0.54 μCi per mmole, respectively, and were discarded, as they could have been derived from small amounts of contaminating D-fructose-*1*-^3H that may have been present in the preparation as a result of the preparative process. Samples 3–9 had the following specific activities in μCi per mmole: 0.24, 0.14, 0.19, 0.22, 0.24, 0.22, and 0.21. These values indicate an average specific-activity of 0.21 μCi per mmole (23% of the activity of the starting D-glucose-*2*-^3H).

The conversion of tritiated **5** *into non-labeled 2-furoic acid.* — The remainder of samples 3–9 obtained in the preceding experiment were combined, diluted with inert **5**, and crystallized from hexane to give 200 mg of **5**-^{3}H. This sample (which had a specific activity of 2.52×10^{-2} μCi per mmole) was dissolved in 300 ml of water, and 680 mg of sodium metaperiodate was added. After 15 h, t.l.c. indicated the reaction to be complete, as evidenced by the appearance of a spot having a flow-rate identical to that of 2-furoic acid, and the disappearance of the spot corresponding to **5**. This solution was extracted with five 200-ml portions of ether, dried (sodium sulfate), and evaporated. After evaporation of 500 ml of methanol from the residue, the latter was taken up in 500 ml of hexane, the solution evaporated to dryness, and the residue sublimed at 80° and 0.3 mm to give crystalline 2-furoic acid. After recrystallization from hexane, the material (50 mg) had m.p. 130°, a t.l.c. mobility identical to that of authentic material, and a specific activity of 7.49×10^{-4} μCi per mmole (corresponding to 3% the activity of the starting **5**).

Conversion of D-*glucose-2-*^{3}H *into* D-*fructose-1-*^{3}H. — The D-glucose-*2*-^3H used in this experiment was purchased from Amersham–Searle, Inc. and was prepared by reduction of methyl D-*arabino*-hexopyranosid-2-ulose with NaB^3H_4 followed by hydrolysis, and chromatographic purification of the resulting D-glucose-*2*-^3H. The only radioactive component found on paper chromatograms of this preparation were attributable to D-glucose. The sample was diluted with inert D-glucose to give 200 g of sugar in 3500 ml of water. A 20-ml aliquot of this solution was evaporated to dryness and the resulting D-glucose was converted into the α-pentaacetate[17], which was crystallized from ethanol to constant specific-activity (0.900 μCi per mmole). The remaining solution was adjusted to pH 3.0 with sulfuric acid and autoclaved at 15 lb.in^{-2} for 10 h, neutralized with barium carbonate, and the mixture was filtered. The filtrate was then evaporated to a syrup, seeded with crystalline α-D-glucose, and allowed to crystallize. The crystals were separated from the residual syrup, washed with methanol, and the crystallization process repeated with the evaporated washings until no further crystals were obtained. The final syrup, which contained both glucose and fructose (as evidenced by paper chromatography) was set aside and the crystals were redissolved in 3500 ml of acid (pH 3.0) and the process repeated. The process was performed a total of 5 times, and the combined syrup (5 g) was treated with D-glucose as described by Ohno and Ward[4].

After removing contaminating ions and D-gluconic acid by treatment with

Dowex-50 (H^+) and Dowex-1 (CO_3^{2-}), the resulting syrupy, chromatographically pure fructose (150 mg) was converted into the crystalline 2,3:4,5-diisopropylidene acetal[18] which, after 2 recrystallizations from methanol had m.p. 96°, m.m.p. 96°, and a mobility by t.l.c. identical to that of an authentic sample. This material had a specific activity of 0.107 μCi per mmole.

*Permanganate oxidation of 2,3:4,5-di-*O*-isopropylidene-*D*-fructose-1-*^{3}*H.* — The procedure used in this experiment is the same as that used by Ohle and Wolter[19] for the conversion of the acetal into the corresponding 1-carboxylic acid derivative. Thus 5.68 mg of 2,3:4,5-di-*O*-isopropylidene-D-fructose, having a specific activity of 0.107 μCi per mmole, was treated with 2.0 ml of an aqueous solution, containing approximately 5 mg of potassium permanganate and 500 mg of potassium hydroxide, for 36 h at 25°. Subsequently, 1.0 ml of water was obtained from the reaction solution by distillation under diminished pressure, and it was found to contain 1.10 μCi, indicating that 94% of the carbon-bound tritium in the starting product had been released into the solvent by oxidation at C-1 of the D-fructose derivative.

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