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Note

On the mechanism of conversion of hexoses into 5-(hydroxymethyl)-2-furaldehyde and metasaccharinic acid*†

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The decomposition of hexoses such as D-glucose to 5-(hydroxymethyl)-2-furaldehyde (**5**) in acid solution and to metasaccharinic acid (**4**) in alkali represent two well-known reactions. It is generally accepted[1,2] that 3-deoxy-D-*erythro*-hexosulose (**2a**) or an enolic derivative therof (**2**) functions as an important intermediate in the formation of both compounds. In both acidic and basic solution, the initial reaction is thought to involve conversion of the hexose into its corresponding 1,2-enediol (**1**), which then undergoes dehydration to give **2** and **2a**. Although this conversion is normally envisaged as either an acid-catalyzed dehydration or, in base, as a β-elimination reaction, it has recently been suggested[3] that **2a** could alternatively be produced directly from D-glucose 3-phosphate and certain Amadori products via hydride-shift mechanisms. The acidic dehydration reaction has been suggested[2] to involve the further dehydration of **2** or **2a** to **3** followed by conversion of **3** into **5**. Anet[4], using synthetically prepared **2a** and **3**, has recently concluded that, for the dehydration of D-fructose in oxalic acid at 100°, about 50% of the total **5** formed could have arisen via **2** without prior equilibration, but that a substantial amount of **5** is formed via **2a**. In alkali, **2a** is thought to undergo a benzilic acid type of rearrangement to give **4** directly. Consistent with such proposals are the facts (*a*) that the aldehyde carbon atom **5** of corresponds to C-1 of the hexose from which it is derived[5], (*b*) that formation of metasaccharinic acid is favored by introduction of certain 3-*O*-substituents[6], (*c*) that **2a** has been produced by treatment of D-fructose with acid[7], and (*d*) that **2a** is readily converted into **4** and **5** by treatment with acid or base under comparatively mild conditions[8].

In this work, the importance of 3-deoxyaldosuloses (**2a**) in such reactions was examined by converting D-glucose and D-fructose into **5** in acidified deuterium oxide

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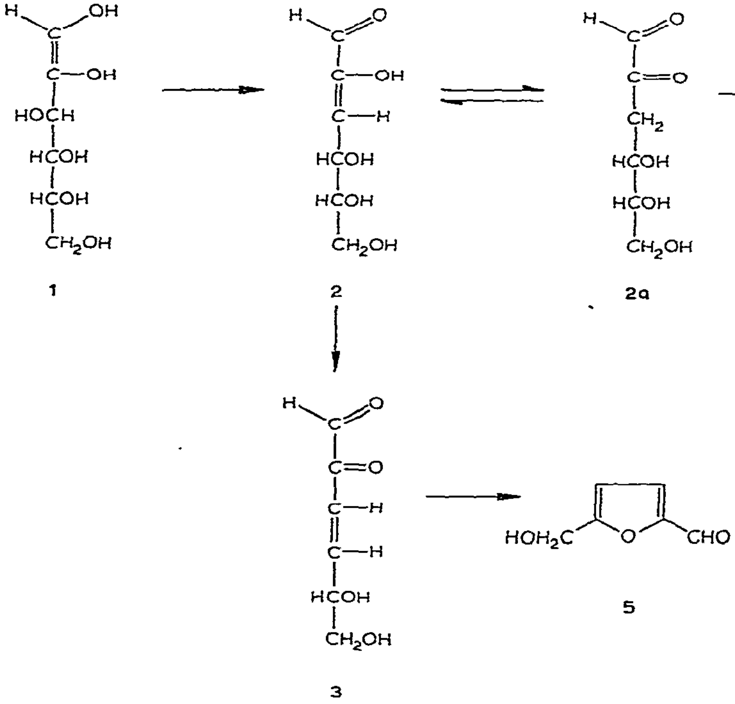
solution. The possibility that a 2,3-hydride-shift mechanisms might operate in the formation of **2a** was examined by preparing D-glucose-2-*d* and converting *it into* **5** in acidified water.



The relevance of **2a** as an intermediate in alkaline reactions was examined by converting D-glucose into metasaccharinic acid in alkaline deuterium oxide. In each case the amount of deuterium incorporated at either position 3 of **5** or at C-3 of **4** should reflect the extent of participation of **2a** as an intermediate. Such data, besides providing mechanistic information, should be of value in comparing the **5** derived from D-glucose and from D-fructose, since the resistance of the former sugar to dehydration compared with the latter suggests possible differences in reaction pathways[9]. For the acid-catalyzed dehydration reactions, D-glucose and D-fructose were converted into **5** under conditions (15 mM sulfuric acid at 250°) that give rise to maximum yields of **5** from D-glucose. For comparative purposes, D-fructose was converted into **5** under conditions used by Anet (10 mM oxalic acid at 100°) in his kinetic study. The **5** was isolated after the dehydration reactions after oxidation to the crystalline 2-furoic acid, which was then converted into the methyl ester and the extent of deuterium incorporation was measured by n.m.r. spectroscopy. A 60-MHz n.m.r. spectrum of methyl 5-(hydroxymethyl)-2-furoate in chloroform-*d* containing tetramethylsilane showed doublets at δ 7.75 (J 3.5 Hz, H-3), and at δ 6.96 (H-4), a singlet at δ 5.05 (CH_2), and the methyl ester resonance as a singlet at δ 4.19.

Measurements of deuterium incorporation were made relative to the latter signal and, in all experiments, no measurable incorporation could be detected.

The **5** derived from D-glucose-2-*d* by treatment with 15 mM aqueous sulfuric acid at 250° was, again, devoid of measurable carbon-bound deuterium. The recovery of unreacted D-glucose-2-*d* at the end of the experiment further showed that loss of deuterium did not occur by a reversible equilibration of the sugar with solvent protons.

Pure samples of crystalline "α"- and "β"-glucometasaccharinic acid lactones were isolated after the alkaline degradation of D-glucose[10]. N.m.r. spectra of these materials as well as that of a pure α,β misture showed signals for the C-3 protons as a complex multiplet at δ 2.0–2.5. Measurements of diminution of n.m.r. signals for a pure "α,β" mixture produced when an alkaline solution in deuterium oxide was used gave a product that contained 80–90% deuterium at C-3.

The possibility of primary and solvent deuterium isotope-effects must be taken into consideration when the collected data are used to interpret reaction mechanisms. For any isotope-exchange experiments, such effects will affect the validity of a direct comparison of reactions in water with similar ones in deuterium oxide. Thus, the extent of equilibration of hexose with **1**, and **2** with **2a** might be substantial in water and much less so in dueterium oxide. Furthermore, the rate of decomposition of **2** and **2a** might also be affected by isotope effects.

In alkaline dueterium oxide solution, however, the evidence clearly indicates the participation of **2a** as as reaction intermediate and further points to the presence of substantial equilibration between **2** and **2a**, since a direct conversion would result in only 50% incorporation. The data, which are accurate within ±0.1 atom of deuterium per molecular position, are best explained by a reaction sequence involving hexose, **1**, **2**, **2a**, and **4**. The data do not, however, substatiante the presence of such compounds in the corresponding acid-catalyzed, dehydration reaction, although the possibility cannot be excluded that **2a** is a minor contributor (that is, it is responsible for less than 20% of the total yield of **5**), or that it is formed by mechanism as yet not considered. Formation by hydride-shift mechanisms seems unlikely, and it is noteworthy that, in addition to this work, the suggestion[3] that Amadori products give rise to **2a** by a 1,3-hydride-shift type of mechanisms has recently been refuted[11].

The acid-catalyzed dehydration reaction, as it occurs in deuterium oxide, is best explained on the basis of an essentially irreversible sequence that involves hexose, **1**, **2**, **3**, and **5**. The recent reports that D-fructose does not equilibrate with its 1,2-enediol in acidic deuterium oxide solution[12] (but does so to a considerable extent in base), and that **2a** is converted into **5** in acidified deuterium oxide solution with no measurable incorporation of carbon-bound deuterium[13], are also pertinent

EXPERIMENTAL

Materials and methods. — T.l.c. was performed on plates coated with silica gel by using either *A*, 9:1 benzene–methanol or *B*, 4:1:1 ethyl acetate–acetic acid–water, as irrigants. Spots were visualized by spraying the plates with 10% sulfuric acid in ethanol followed by charring at 110°, or, in the case of furan derivatives, by short-wave u.v. irradiation in conjunction with plates coated with silica gel HF. Paper chromatography was performed by the descending method, with either aniline hydrogen phthalate[14] or silver nitrate[15] spray reagents. Gas-chromatographic separations of saccharinic acids involved initial conversion into the trimethylsilyl ethers[16], and were made on 10% EGS columns. N.m.r. spectra were measured on 10% solutions with a Varian A-60 spectrometer. Furan derivatives and sugar acetates were measured in 4,4-dimethylchloroform-*d* and metasaccharinic acids in deuterium oxide. Tetramethylsilane and sodium 4,4-dimethyl-4-silapentane-1-sulfonate, respectively, were used as internal standards. All enzymes and phosphorylated substrates were obtained from Sigma Chemical Co.

Conversion of D-*glucose and* D-*fructose into 5-(hydroxymethyl)-2-furaldehyde* (5) *in deuterium oxide solution.* — To 20 ml of deuterium oxide (99%) that was 15 mM in sulfuric acid was added 2.0 g of D-glucose. The resulting solution was sealed in 1-ml portions into 5×20-mm glass tubes, which were then immersed in a potassium nitrate bath for 90 sec at 250°. At the end of this time the reaction was stopped by plunging the tubes into an ice–water bath. The tube contents were combined, neutralized with barium carbonate, and the **5** isolated by continuous extraction with ether for 5 h. The **5** present in the ether phase was readily detected by t.l.c. with irrigant *A*, and spectral measurements at 280 nm indicated an overall yield of 20%. To the ether extract was added 30 ml of water and, after evaporation of the ether, the aqueous phase was added to 170 ml of water containing 2.0 g of sodium hydroxide and 2.5 g of freshly prepared silver oxide. The suspension was stirred rapidly for 2 h while air was bubbled through it. The resulting filtrate was passed through Dowex-50 (H^+) and the acidic effluent was evaporated to dryness, to give crystalline 5-(hydroxymethyl)-2-furoic acid (225 mg) which, after recrystallization from acetic acid–chloroform, had m.p. and mixed m.p. 164°, identical with an authentic sample by t.l.c. (irrigant *A*).

D-Fructose was converted in the same way into **5** (in deuterium oxide which was 15mM in sulfuric acid at 250°), or with 10 mM oxalic acid for 16.5 h at 100°.

The methyl ester of the acid was prepared by adding ethereal diazomethane slowly with stirring to 100 mg of the crystalline acid, until the sample had dissolved completely. Evaporation of the ether gave an oil that contained no unreacted acid and gave a single spot by t.l.c. (irrigant *A*). This material was used directly for measurements of deuterium incorporation.

Preparation of D-*glucose-2-*d. — The procedures described by Topper[17] were modified and scaled up for this preparation. To 100 ml of deuterium oxide was added 15 g of barium D-fructose 6-phosphate, 150 mg of crude D-glucose phosphate isomerase, and a seed crystal of barium D-glucose 6-phosphate. After 10 h at 37° the crystalline barium D-glucose 6-phosphate resulting was filtered and washed with methanol to give 7.8 g of product. After recrystallization by dissolution in dilute hydrochloric acid and neutralization with barium hydroxide, the material was dissolved in a carbonate–hydrogen carbonate buffer at pH 10 and was treated with alkaline phosphatase for 6 h at 37°. The mixture was then filtered through Celite 536, treated with Dowex-50 (H^+) and Dowex-1 (CO_3^{-2}), and the neutral solution was evaporated to give a glassy solid (1.44 g). Both paper chromatography and t.l.c. indicated a glucose as the only detectable sugar present. A portion of the material was converted into the crystalline α-D pentaacetate by using zinc chloride and acetic anhydride as described in an earlier report[18]. N. m.r. spectra in chloroform-*d* showed the signal of the anomeric proton at δ 6.23 as a singlet instead of as the normally[19] observed doublet.

Conversion of D-*glucose-2-*d *into* **5**. — Approximately 1.0 g of D-glucose-2-*d* was converted into **5** in 15 mM aqueous sulfuric acid as described above, and the **5** was isolated as the furoic acid in the usual fashion. Unreacted D-glucose-2-*d* was isolated by preparative, paper chromatography on Whatman 3 mm paper by using 10:3:3 butyl alcohol–pyridine–water as irrigants, and was identified as such by n.m.r. spectrometry after conversion into the crystalline α-D pentaacetate as well as by comparison of its chromatographic mobility in irrigant *A* and its m.p. and mixed m.p. (110°).

Conversion of D-*glucose into the metasaccharinic acids* (**4**) *in deuterium oxide.* — To 21.5 g of deuterium oxide was added 6 g of sodium hydroxide, and the resulting solution was heated to boiling. A solution of 5 g of D-glucose in 23.2 g of deuterium oxide was added slowly at a rate sufficient to maintain the temperature above 95°. After the addition, the solution was refluxed for 1 h, cooled, passed through a column containing 180 ml of Dowex-50 (H^+), and evaporated to a syrup. T.l.c. (irrigant *B*) indicated that the syrup contained, in addition to a number of unidentified materials, lactic acid, 2,4-dihydroxybutyrolactone, and "α"- and "β"-metasaccharinic acid lactones. The syrup was blended with 10 ml of 4:1:1 chloroform–acetic acid–methanol and added to a 2.5 × 50-cm column of silica gel. The column was eluted with the same solvent and 3-ml fractions were collected. A mixture of pure "α"- and "β"-glucometasaccharinic acid lactones, as evidenced by t.l.c. (irrigant *B*) and gas–liquid chromatographic comparison with authentic samples, was obtained in fractions 140–200.

Although this mixture was used directly for measurements of deuterium incorporation, the pure "α" and "β" isomers were further resolved on a cellulose column by using butanone saturated with water, to give the crystalline compounds having m.p. 108° ("α") and m.p. 82° ("β").

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