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ACID-CATALYZED ISOMERIZATION AND DEHYDRATION OF DL-GLYCERALDEHYDE AND 1,3-DIHYDROXY-2-PROPANONE*

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ABSTRACT

The interconversion of 2,3-dihydroxypropanal (**1**) and 1,3-dihydroxy-2-propanone (**3**) and their dehydration to pyruvaldehyde (**5**) has been studied in 0.5M sulfuric acid at 100°. The conversion of **1** and **3** into **5** under these conditions, in tritiated water, was monitored by isolation of **5** and determination of the distribution of carbon-bound tritium (namely, that at the aldehyde and methyl groups). This was accomplished by conversion of **5** into the phenylosazone and counting, and by conversion of **5** into pyruvic acid (isolated as the *p*-nitrophenylhydrazone), counting, and a difference calculation. In all cases negligible activity (about 0.8% the activity of the solvent) was found at the aldehyde carbon of **5**, derived from either **1** or **3**, and parallel chromatographic studies indicated only a small extent of interconversion. As **5** incorporated tritium under conditions of its formation, plots of incorporation versus time were made and the curves extrapolated to zero time, in order to determine initial activity at the methyl group of **5**, derived from either **1** or **3**. This method showed that the **5** derived from **3** contained 10% of the activity of the solvent at the methyl group as it was produced, whereas the **1**-derived **5** contained no activity. In another experiment, [2-^{3}H]**1** was prepared and converted into **5**. The resulting **5** was found to be labeled at C-3, indicating at C-2 → C-3 intramolecular, hydrogen-transfer reaction during the conversion of **1** into **5**.

INTRODUCTION

In prior studies[1] in this laboratory dealing with the acid-catalyzed interconversion of D-glucose, D-mannose, and D-fructose, it was found that, in some respects, the data collected for the reaction are not consistent with the generally accepted mechanism involving a 1,2-enediol intermediate. For example, the conversion of D-glucose into D-fructose in acid involves a quantitative, intramolecular, hydrogen-transfer from C-2 of the aldose to C-1 of the ketose and, in the reverse case, a similar transfer occurs from C-1 of the ketose to C-2 of each of the epimeric aldoses[1]. Such

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observations are similar to those observed for enzyme-catalyzed isomerizations[2].

In order to examine the extent of such transfer reactions during acid-catalyzed isomerization as well as dehydration reactions, an investigation was undertaken of the interconversion and dehydration of DL-2,3-dihydroxypropanal (**1**) and 1,3-dihydroxy-2-propanone (**3**) in acid solution. The studies involved qualitative chromatographic studies of their interconversion, as well as isotope-acquisition measurements during which the compounds were converted, in acidified, tritiated water, into their common dehydration product, pyruvaldehyde (**5**).

Although the pure trioses have been available for a number of years, less is known concerning the mechanism of their interconversion and dehydration than for the higher sugars. Isotope-exchange studies appear not to have been made in this system and, based on kinetic studies[3,4], a difference of opinion exists as to whether triose interconversion is subject to general acid–base catalysis or specific base-catalysis. In addition, under strongly acidic conditions, it has been suggested that the mechanisms for the interconversion may change, as dehydration to pyruvaldehyde becomes dominant at the expense of interconversion.

RESULTS AND DISCUSSION

Fig. 1 shows the generally accepted mechanism for the interconversion of **1** and **3** via the enediol (**2**). In addition to serving as the hypothetical intermediate for this interconversion, **2** is also thought to undergo dehydration, via beta elimination, to the enolic form (**4**) of pyruvaldehyde (**5**). Under the conditions of this study (0.5 M sulfuric acid at 100° for times up to 24 h), compound **5** was the major product. Based on paper chromatography, only traces of **3** were observed arising from **1** and vice versa. Chromatographic studies also indicated that **3** was dehydrated much faster than **1**, an observation consistent with known facts concerning higher sugars.

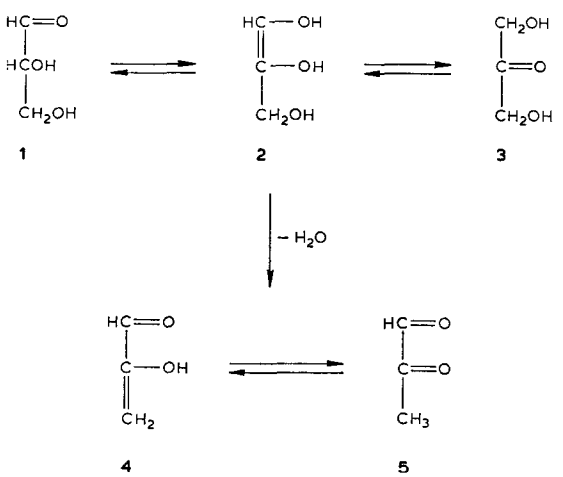

Fig. 1. The generally accepted mechanism of the interconversion of DL-glyceraldehyde and 1,3-dihydroxy-2-propanone and their mutual dehydration.

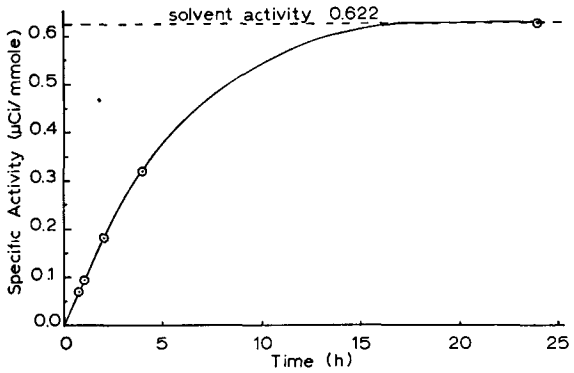

Fig. 2. The incorporation of tritium by pyruvaldehyde during reflux in tritiated water 0.5M in sulfuric acid.

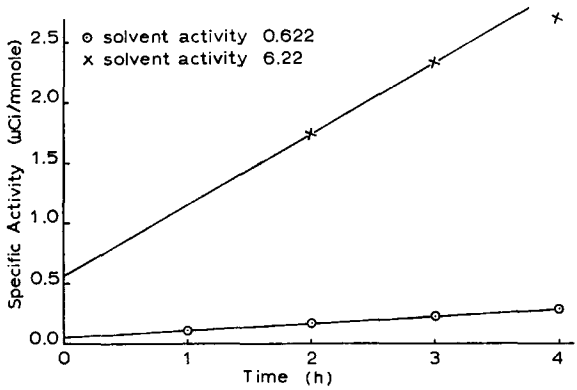

Fig. 3. The incorporation of tritium by pyruvaldehyde during reflux in tritiated water 0.5M in sulfuric acid, as produced from 1,3-dihydroxy-2-propanone.

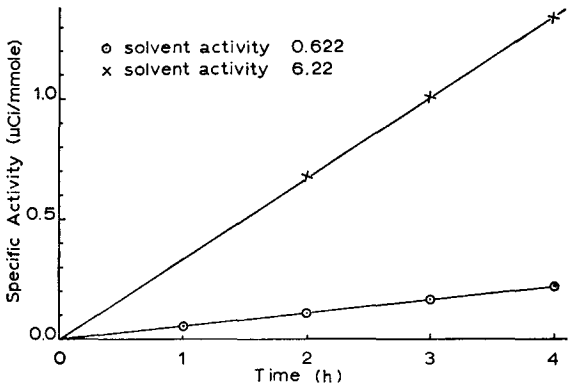

Fig. 4. The incorporation of tritium by pyruvaldehyde during reflux in tritiated water 0.5M in sulfuric acid, as produced from DL-glyceraldehyde.

To provide more-precise information on the mechanism of interconversion of **1** and **3** as well as their dehydration, **1** and **3** were converted into **5** in acidified, tritiated water, and the distribution of carbon-bound tritium in **5** was determined. Total radiochemical activity of **5** was determined by conversion into the crystalline phenylosazone and counting. The activity at the aldehyde carbon versus that on the methyl group was determined by converting **5** into pyruvic acid [isolated as its crystalline (*p*-nitrophenyl)hydrazone], followed by counting and a comparison of the activities of the two compounds.

In all instances, the aldehyde carbon atom of **5** contained negligible activity. In six determinations, the average incorporation at this position was 0.8% of the activity of the solvent. This supports the observation, based on paper chromatography, that little interconversion occurred and that the primary reaction was conversion into **5**. This conclusion is based on the mechanistic pathway shown in Fig. 1 and does not include possible interconversions based on intramolecular hydrogen-transfer reactions that would permit interconversion without substantial acquisition of isotope at the aldehyde carbon.

Examination of the reactivity of **5** indicates that it is capable of incorporating solvent tritium at the methyl group under conditions of its formation. Thus, the extent of tritium incorporation into **5** increased with time during the reaction. In order to determine the initial activity contained in the methyl group of **5**, some rate studies were undertaken. The extrapolation of a plot (Fig. 2) of tritium incorporation at the methyl carbon atom of **5** versus time, when it is treated in acidified, tritiated water under conditions of its formation from **1** or **3** shows, as expected, an intercept of zero incorporation at zero time. This result indicates that such an extrapolation might be useful for estimating the amount of tritium incorporated at the methyl group of **5** during its formation from either **1** or **3**. As the **5** contained relatively low levels of activity at the aldehyde carbon atom, it was not converted into pyruvic acid for determination of the amount of isotope incorporated at the methyl carbon atom. At equilibrium, the radiochemical activity of the methyl group of **5** was equal to that of the solvent. For the case of **5** derived from **3** similar plots (Fig. 3), using two different tritium-ion concentrations show that the initially formed **5** appears to be labeled at the methyl carbon atom and that it contains 10% the activity of the solvent. In the case of **5** derived from **1**, however, (Fig. 4) the initially produced **5** appears to be radiochemically inert.

Because data are not available concerning possible isotope-effects operative during the reaction, it is impossible to relate a 10% incorporation at the methyl group of **3**-derived **5** to the extent of solvent–proton exchange during the reaction. The data are, nevertheless, qualitatively consistent with the classical enediol-mediated reaction, as shown in Fig. 1. Although not entirely conclusive, the kinetic data shown in Fig. 4 suggest that the **5** derived from **1** might not be formed by solvent–proton exchange via a classical enediol intermediate, and that an intra- or intermolecular hydrogen transfer might be involved. As already stated, intramolecular hydrogen-transfer reactions have been observed in related reactions, such as the

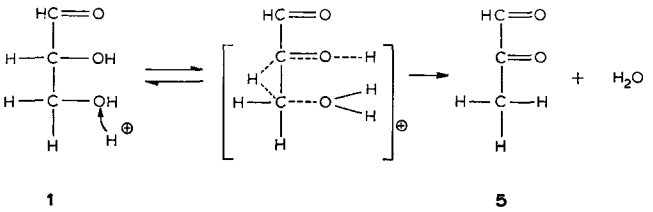

Fig. 5. A proposed aldose 1,2-hydride-shift mechanism operable in acid solution.

acid-catalyzed interconversion of D-mannose, and D-fructose[1] and during the interconversion of D-[2-^{3}H]ribose and D-arabinose in alkaline solution[5]. Although the major portion of these involve a transfer from[2] a carbon atom α to a carbonyl function, a measurable portion of the hydrogen transferred has, in some instances, been found elsewhere in the molecule. For example, during the conversion of D-[1-^{3}H]fructose into D-mannose, 23% of the tritium retained by the D-mannose was found[1] to be at C-3–C-6. Gleason and Barker[5] also reported similar results in the conversion of D-[2-^{3}H]Ribose into D-arabinose.

In order to examine the possibility of such a transfer occurring during the conversion of **1** into **5**, D-glyceraldehyde specifically tritiated at C-2 was prepared and converted into **5** in ordinary, acidified water under the conditions used to collect the data presented in Figure 4. The starting [2-^{3}H]**1** had a specific activity of 1.5×10^{-2} μCi per mmol. The pyruvaldehyde obtained from it (isolated as the phenylosazone) had a specific activity of 2.2×10^{-3}, and the pyruvic acid [isolated as the (*p*-nitrophenyl)hydrazone] had a specific activity of 2.1×10^{-3} μCi per mmol. Thus, during the dehydration of **1** to **5**, 14% of the activity at C-2 of **1** is transferred to C-3 of **5**.

Because of the complexity of the reaction, and isotope effects that almost certainly affect the extent of incorporation, the results should be interpreted only in qualitative terms. Nevertheless the data indicate that, during the conversion of **1** into **5**, an intramolecular transfer of hydrogen from C-2 of **1** to C-3 of **5** is involved, at least in part. A mechanism such as is shown in Fig. 5 cannot be excluded as a possibility. It is noteworthy that such a mechanism cannot operate for the conversion of **3** into **5**, as, in this case, C-2 of **3** is devoid of carbon-bound hydrogen.

EXPERIMENTAL

Material and methods. — Paper chromatograms were developed with 18:4:1:3 (v/v) ethyl acetate–acetic acid–formic acid–water as irrigant. Spots were visualized by the silver nitrate–sodium hydroxide dip-reagent[6]. T.l.c. was performed on silica gel impregnated with sodium acetate[7], and plates were developed with 4:5:1 (v/v) 1-butanol–ether–water as irrigant. Spots were visualized by spraying with 10% ethanolic sulfuric acid followed by heating for 10 min at 110°. A Waters Associates Model 201 liquid chromatograph, employing a water-jacketed, 0.9 × 60 cm column

maintained at 75° and packed with Aminex Q-15s (calcium form) was also used for separations. The retention times for **1** and **3** were 22 and 27 min, respectively, when the system was operated at approximately 500 lb.in^{-2}, with distilled water (1 ml per mm) as the eluent.

Tritiated water and D[2-^{3}H]mannose were purchased from New England Nuclear Co. Chromatographically pure DL-glyceraldehyde, 1,3-dihydroxy-2-propanone, and pyruvaldehyde were purchased from Sigma Chemical Company. The activity of the tritiated samples was determined by custom oxidation of the crystalline samples in a Pemlab autoxidizer, followed by scintillation counting of the water produced in the oxidation. The oxidation, counting, and computer-data processing were performed by Pemlab., Brookfield, Illinois. Elemental analyses were made by Clark Microanalytical, Urbana, Illinois.

Conversion of **1** *and* **3** *into pyruvaldehyde* **5**. — In a typical experiment, 10 g of **1** or **3** was boiled under reflux for 4 h in 50 ml of tritiated water 0.6222 μCi per mmol) that was 0.5M in sulfuric acid. The reaction was stopped by cooling the solution to 15° and adjusting to pH 4–5 by the addition of 10M sodium hydroxide. The **5** contained in a 20-ml aliquot of this mixture was isolated by distillation under diminished pressure. Water was added to the distillation vessel during the distillation to keep the total volume in the flash near 20 ml, until a total of 100 ml of distillate had been collected[8,9].

Conversion of **5** *into the phenylosazone.* — A 10-ml aliquot of the foregoing distillate was treated with an excess of phenylhydrazine and the resulting phenylosazone was isolated and recrystallized from ethanol to constant radiochemical activity, m.p. 146°, lit.[10,11] m.p. 145°.

Anal. Calc. for $C_{15}H_{16}N_4$: C, 71.4; H, 6.35; N, 22.2. Found: C, 70.8; H, 6.50; N, 21.7.

Conversion of **5** *into pyruvic acid.* — The remaining 90 ml of the pyruvaldehyde distillate was treated with 0.5 ml of bromine and 3.0 g of sodium acetate[12] and allowed to react for 48 h at 25°. The solution was then passed through a column containing 75 ml of Dowex-50 (H^+) and the effluent through a column containing 60 ml of Dowex-1 (COeE). After washing the column with 3 volumes of water, the pyruvic acid was displaced from the resin by stirring with 5 ml of 6M sulfuric acid. After filtration, the addition of (*p*-nitrophenyl)hydrazine in ethanol gave the crystalline hydrazone. After recrystallization from methanol–water, the material had m.p. 222°, lit.[12] m.p. 217°. The product was recrystallized from methanol–water to constant specific activity.

Kinetic methods. — The amount of tritium incorporated into pyruvaldehyde during the formation from **1** or **3** was determined by removing aliquots at various times during reflux. These aliquots were separately treated as already described. The specific activity of the resulting pyruvaldehyde phenylosazones was plotted versus time of reflux. The best line was drawn through the points, and the extrapolated zerointercept was used as a measure of the amount of bound isotope incorporated by pyruvaldehyde during its formation. Pyruvaldehyde was also refluxed in acidified,

tritiated water in the same manner. Aliquots were taken, treated with phenylhydrazine, and the pyruvaldehyde phenylosazone so formed recrystallized to constant specific activity. A plot of the specific activity of the pyruvaldehyde phenylosazone versus reflux time was also made.

Preparation of D-[2-^{3}*H*]*glyceraldehyde.* — Approximately 20 g of [2-^{3}H] mannose was converted into D-[2-^{3}H]mannitol as described by Wolfrom and Thompson[13], and thence into the 1,2:5,6-di-*O*-isopropylidene derivative[14]. This material (m.p. 118°, lit.[14] m.p. 122°) was then converted into 2,3-*O*-isopropylidene-D-[2-^{3}H]glyceraldehyde and thence into D-[2-^{3}H]glyceraldehyde by the procedures of Fischer and Baer[14], which involve oxidation with lead tetraacetate, followed by hydrolysis. The syrupy D-[2-^{3}H]glyceraldehyde was diluted with 10 g of DL-glyceraldehyde and 130 ml of water. After concentration to a light syrup, the resulting DL mixture crystallized. Recrystallization to constant radiochemical activity gave a preparation that was chromatographically pure, having a specific activity of 9.2×10^{-2} μCi per mmol.

REFERENCES

1 D. W. Harris and M. S. Feather, *J. Am. Chem. Soc.*, 97 (1975) 178–181, and references therein.
2 I. A. Rose, *Enzymes*, 2 (1970) 281–313.
3 J. C. Speck, Jr., *Adv. Carbohydr. Chem.*, 13 (1958) 63–99.
4 M. Fedoroňko and J. Konigstein, *Collect. Czech. Chem. Commun.*, 34 (1969) 3881–3894.
5 W. G. Gleason and R. Barker, *Can. J. Chem.*, 49 (1971) 1432–1440.
6 W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, 166 (1950) 444–445.
7 M. Lato, B. Brunelli, and G. Ciuffini, *J. Chromatogr.*, 39 (1969) 407–417.
8 M. Ikawa and K. P. Link, *J. Am. Chem. Soc.*, 72 (1950) 4287–4288.
9 C. Neuberg, E. Farber, A. Levite, and E. Schwenk, *Biochem. Z.*, 83 (1917) 264–268.
10 F. R. Japp and F. Klingemann, *J. Chem. Soc.*, 53 (1888) 519–544.
11 H. V. Pechmann, *Ber.*, 20 (1887) 2539–2544.
12 C. Neuberg and G. Gorr, *Biochem. Z.*, 166 (1925) 442–443.
13 M. L. Wolfrom and A. Thompson *Methods Carbohydr. Chem.*, 2 (1963) 65–68.
14 E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, 128 (1939) 463–473.