*Carbohydrate Research*, 54 (1977) 209-215

# THE ACID-CATALYZED DEHYDRATION OF 1-BENZYLAMINO-1-DEOXY-D-*threo*-PENTULOSE, 1-DIBENZYLAMINO-1-DEOXY-D-FRUCTURONIC ACID, AND D-GLUCURONIC ACID*

KEVIN B HICKS AND MILTON S FEATHER†

*Department of Biochemistry, University of Missouri-Columbia*
*Columbia, Missouri 65201 (U S A )*

(Received May 25th 1976 accepted for publication with revisions July 25th, 1976)

## ABSTRACT

Three compounds, 1-benzylamino-1-deoxy-D-*threo*-pentulose (**1**), 1-dibenzylamino-1-deoxy-D-fructuronic acid (**2**), and D-glucuronic acid (**3**) were converted into 2-furaldehyde in acidified tritiated water In the latter system, the 2-furaldehyde derived from **1** contained 13% of the activity of the solvent at the aldehyde carbon and 9% at positions 3-5 of the furan ring, that from **2** contained 8% at the aldehyde carbon and 29% at positions 3-5, and that from **3** contained 18% at positions 3-5 In deuterium oxide, the 2-furaldehyde derived from **1** contained 14 atom % of deuterium at position 3, 5% at position 4, and 0% at position 5 That from **2** contained 50% at position 3, 44% at position 4, and 7% at position 5 That from **3** contained 35% at position 3, 15% at position 4, and 5% at position 5 The data for **1** are discussed relative to prior data on incorporation collected for D-xylose Incorporation data for both **2** and **3** are qualitatively consistent with a decarboxylation step involving a $\beta,\gamma$-unsaturated, carboxylic acid intermediate A mechanism for the decarboxylation of hexuronic acids is presented

## INTRODUCTION

Earlier work[1] in this laboratory has involved studies on the mechanism of dehydration of various sugars and derivatives thereof by means of isotope-exchange experiments By converting a sugar into the appropriate dehydration product in isotopically labeled water (either acidified deuterium oxide or tritiated water) and then measuring the distribution of carbon-bound isotope in it, it has been possible to distinguish between various mechanisms currently under consideration The present study involves similar experiments on Amadori products derived from D-xylose (1-benzylamino-1-deoxy-D-*threo*-pentulose, **1**), and from D-glucuronic acid (1-di-

---

*Journal Paper No. 1547 of the Missouri Agricultural Experiment Station
†To whom inquiries should be addressed

benzylamino-1-deoxy-D-fructuronic acid, 2), and on D-glucuronic acid itself (3). In earlier experiments with hexoses and Amadori products derived from them, both classes of compound were found to give rise to 5-(hydroxymethyl)-2-furaldehyde on treatment with acid[2] For the three compounds under consideration here, similar results were observed, namely, all give rise in strongly acidic media to 2-furaldehyde as the major dehydration-product

In the case of D-glucuronic acid, and, presumably, Amadori products derived from it the reaction involves a decarboxylation step, and C-1 of the acid corresponds to the aldehyde carbon of 2-furaldehyde[3] In spite of the many earlier studies[4-7] on the dehydration and decarboxylation of hexuronic acids, the precise mechanism for the reaction is still obscure

Anet[8] has previously shown that, for hexoses and Amadori products derived from them, the mechanism for the dehydration reactions are very similar (Chart 1) Thus, the Amadori product (4) undergoes 1,2-enolization to 5, which then undergoes β-elimination to give 6, which, on hydrolysis of the amine substituent, is converted into the enolic form (7) of a 3-deoxyglycosulose intermediate (8). Either 7 or 8 then loses a further molecule of water to give 9, which is then converted into the appropriate 2-furaldehyde Evidence for this mechanism stems from the isolation of both 7 (ref 9) and 9 (ref. 10) (R = $-CH_2OH$) from acid-treated solutions of D-fructose as well as from "difructose glycine"[8], an Amadori product. Although other systems

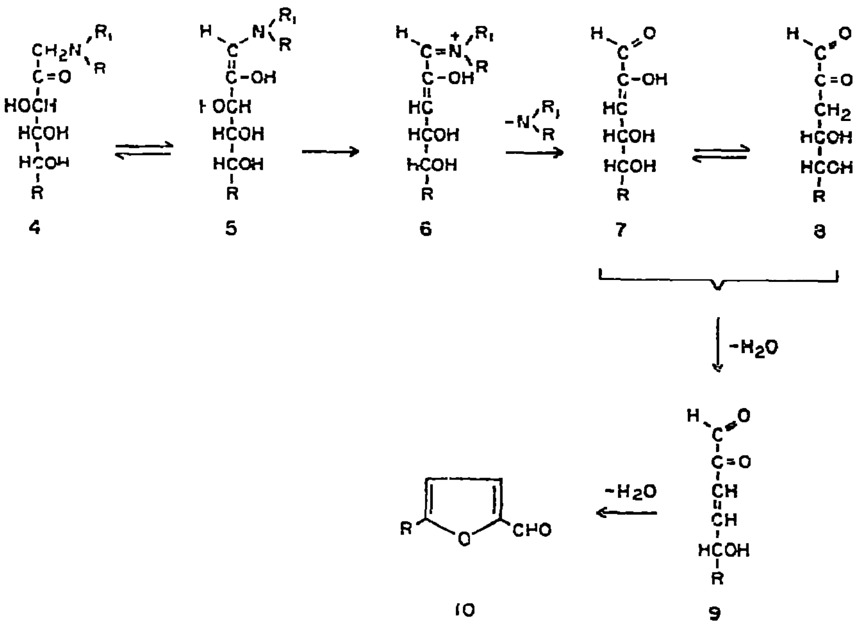


Chart 1

have not been extensively investigated, it seems likely that other sugars are dehydrated via the same pathways.

## RESULTS AND DISCUSSION

The experiments involved both deuterium- and tritium-exchange measurements. The carbohydrate was treated with refluxing acid and the 2-furaldehyde produced was distilled from the solution as it was formed. The resulting 2-furaldehyde was readily identified from its u.v. spectrum ($\lambda_{max}$ 280 nm) and from the melting point of its phenylhydrazone. Typical yields, determined spectrophotometrically, were 41% for **1**, 15% for **2**, and 5% for **3**. For the case of deuterium exchange, the 2-furaldehyde was oxidized to 2-furoic acid and then converted into methyl 2-furoate, and deuterium incorporation in the ring at position 3, 4, and 5 was measured by measurements of n.m.r. signal diminution, relative to the proton signals of the methyl ester. The n.m.r. spectrum (in chloroform-*d*) of methyl 2-furoate shows signals at $\delta$ 3.88 (singlet, methyl protons), 7.20 (multiplet, position 3), 6.51 (multiplet, position 4), and 7.58 (multiplet, position 5), relative to tetramethylsilane.

For the tritium-exchange experiments, a portion of the sample was converted into 2-furaldehyde phenylhydrazone and the latter was counted. A further aliquot was oxidized to 2-furoic acid and the latter sublimed to constant radiochemical activity. By difference, the incorporation at the aldehyde carbon versus that on the furan ring could be calculated. The collected data are presented in Tables I and II.

TABLE I

CARBON-BOUND TRITIUM INCORPORATED[a] INTO 2-FURALDEHYDE FROM REACTANTS

| *Compound* | *Aldehyde carbon* | *Positions 3–5* |
|---|---|---|
| **1** | 13% | 9% |
| **2** | 8% | 29% |
| **3** | | 18%[b] |

[a] Expressed as % activity of the solvent. [b] This figure represents an average of results obtained from four different reaction parameters. See text for a description.

TABLE II

DEUTERIUM INCORPORATED[a] INTO THE FURAN RING OF 2-FURALDEHYDE FROM REACTANTS

| *Compound* | *Position* | | |
|---|---|---|---|
| | *3* | *4* | *5* |
| **1** | 14% | 5% | 0% |
| **2** | 50% | 44% | 7% |
| **3** | 35% | 15% | 5% |

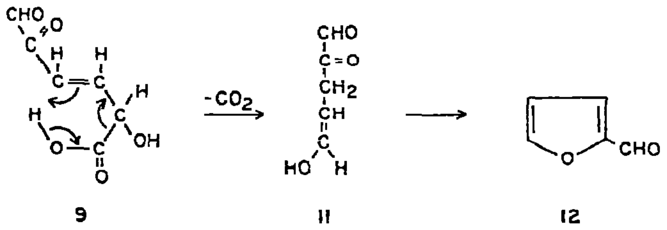
[a] Expressed as atom % of deuterium.

It may be seen that there are significant differences between the amount of exchange for tritium versus deuterium for the same compound under the same reaction conditions. This is presumably because of isotope effects operating during the reaction Additionally, because of such effects, it is impossible to relate the amount of isotope incorporated to that which would have been incorporated by hydrogen exchange in the same reaction For these reasons, the results can only be interpreted in qualitative terms as regards the possible mechanism

For compound **1**, however, significant incorporation occurs at both the aldehyde carbon and (largely) at position 3 of the furan ring This behavior is in contrast to the reaction of D-xylose[1 11] itself, which is converted into 2-furaldehyde with essentially no exchange at any position This result is taken to indicate that, during the dehydration of **1**, both intermediates **4** and **5**, and **7** and **8** (Chart 1, R = H), undergo substantial, reversible equilibration Similar observations have been reported for Amadori products derived from aldohexoses[2] Compound **3**, and the **2** derived from it, both incorporate substantial isotope into the furan ring, largely at positions 3 and 4 Compound **3** was included in the study because in an earlier report[11] it was erroneously concluded, (based on both deuterium- and tritium-exchange measurements) that negligible incorporation is observed when **3** is converted into 2-furaldehyde This error was corrected for tritium exchange in a later report[1], which showed that the 2-furaldehyde ring (positions 3–5) contains 19 8% the activity of the solvent, a figure that agrees well with the data in Table I Because of the discrepancies observed in prior experiments with D-glucuronic acid, a detailed study was made of this sytem, in order to rule out possible variations in incorporation resulting from variations in reaction conditions Parameters considered were (*a*) the concentration of isotope used, (*b*) the concentration of carbohydrate in the reaction solution, (*c*) the form of the sugar used, namely D-glucuronic acid versus D-glucofuranurono-6,3-lactone, and, (*d*) temperature parameters. A yield study showed that the yields of 2-furaldehyde remained the same, starting from either D glucuronic acid or its 6,3-lactone, and no yield change was observed when samples were added to the acidic solution at 25°, followed by a heat-up time to reflux of 30 min, or when they were added to the solution that was already at reflux. Similarly identical results (Table II) were obtained for deuterium incorporation, starting with either the lactone or the acid, using both a heat-up time of 0 5 h as well as by dropping the samples directly into a refluxing solution of acidified deuterium oxide As expected, and contrary to our earlier report[11], significant incorporation was observed in the furan ring, largely at positions 3 and 4 These experiments were performed because variable incorporation might have occurred as a result of prolonged contact with solvent, during which time exchange might occur prior to dehydration This possibility does not appear to be the case nor does the form of the starting material [the lactone (in the furanose form) versus the acid in the pyranose form] seem to have any effect on the incorporation data

In a final series of experiments, the amount of tritium incorporated into the furan ring during the dehydration reactions was examined in detail by using D-glucuronic acid as a starting material with two levels of solvent activity and two levels of sugar concentration. The resulting 2-furoic acid obtained by using acidified, tritiated water having specific activity 1.9 μCi per mmole at 0.6% sugar concentration contained 16.3% of the activity of the solvent, whereas, when using a 5% concentration of sugar, the activity was 14.0%. Repetition of these experiments with water having an activity of 0.4 μCi per mmole showed that, at 0.6% sugar concentration, the 2-furoic acid contained 21.2% of the solvent activity, whereas at 5% concentration the value was 19.5%. Although these differences are small compared to the overall incorporation observed, they appear to be significant and cannot be attributed either to addition of water to the medium because of exchangeable hydrogen atoms in the starting sugar molecule or to water introduced as a result of the dehydration reactions. Nevertheless, the data show that substantial incorporation occurs in the furan ring during the reaction. Because there is no explanation for these slight differences readily apparent, all four numbers were given equal statistical weight and are shown in Table I as an average value.

A number of mechanisms have been suggested in the past for the decarboxylation of hexuronic acids and their resultant conversion into 2-furaldehyde[4–6]. Isbell proposed a mechanism for the dehydration (and decarboxylation) of D-galacturonic acid to 2-furaldehyde as well as to reductic acid. It is noteworthy that the isotope-incorporation data collected in this study are consistent with his proposal and could have occurred *via* equilibration of keto–enol forms during the dehydration reaction. Although the Isbell mechanism is justified on an electronic basis, inspection of the proposed dehydration pathway (Chart 1) for the dehydration of a hexuronic acid or an Amadori product derived from it (R = $CO_2H$) shows that intermediate **9** is a β,γ-unsaturated acid, this class of compounds is known to undergo decarboxylation readily. Thus as an alternative to the decarboxylation step proposed by Isbell, compound **9** (R = $CO_2H$), on decarboxylation by the mechanism generally accepted[1,2] for the decarboxylation of a β,γ-unsaturated acid, (Chart 2), would give rise to an isomer (**11**) of 2-ketopentanedial, which could cyclize to 2-furaldehyde (**12**). Such a mechanism is attractive because it involves the formation of a stable, hydrogen-bonded, six-membered ring, this, through a concerted shift of electrons, could eliminate carbon dioxide. Such a mechanism would predict incorporation at position 3



9 11 12

Chart 2

of the furan ring as a result of proton transfer during decarboxylation, at position 4 through equilibration of **11** with its keto form prior to cyclization and dehydration and possibly at the aldehyde carbon as a result of reversible equilibration of the starting material with its 1,2-enediol The mechanism is qualitatively consistent with the labeling patterns observed

Further support for such a decarboxylation mechanism is found in a report describing the dehydration of L-*xylo*-5-hex-ulosonic acid (L-sorburonic acid, 5-keto-D-gluconic acid") in methanolic hydrogen chloride[7,13]. In this system, the carboxyl group is esterified and hence cannot participate in a decarboxylation reaction by the foregoing pathway. The major product of this reaction is methyl 5-formyl-2-furoate, a product that would be predicted if the D-glucuronic acid underwent dehydration in a manner analogous to that of hexoses and pentoses, instead of undergoing decarboxylation

## EXPERIMENTAL

*Materials and methods.* — U.v spectra were recorded with a Perkin–Elmer model 124 recording, double-beam spectrophotometer Radiochemical determinations (except for phenylhydrazones) were performed with a Packard model 3003 TRI-CARB liquid scintillation counter using a scintillant composed of 2 parts of a solution composed of 2 liters of toluene, 8 25 g of 2,5-diphenyloxazole (PPO), 0 25 g of 1,4-bis 2-(4-methyl-5-phenyloxazolyl)-benzene ($Me_2$POPOP), and 1 part of Triton X-100 (v/v). Efficiencies were determined by using tritiated toluene as an internal standard. Phenylhydrazine derivatives were combusted and analyzed radiochemically by the Pemlab Company N m r spectral measurements were made with a Varian T-60 spectrometer Chemical shifts are reported relative to tetramethylsilane.

*Preparation of starting materials.* — D-Glucuronic acid and D-glucofuranurono-6,3-lactone were obtained commercially Preparation of 1-dibenzylamino-1-deoxy-D-fructuronic acid (m p 150°) and 1-benzylamino-1-deoxy-D-*threo*-pentosulose (m p 135°) followed the procedures of Heyns and Baltes[14] and Micheel and Hagemann[15], respectively

*Conversion of carbohydrate into 2-furaldehyde.* — Dehydration reactions were conducted in 1 25M sulfuric acid, except for studies involving **3** or its γ-lactone, in which 3M acid was used Carbohydrate concentrations were adjusted so that 200–400 mg of 2-furaldehyde were produced. For deuterium-exchange experiments, the solutions contained 96 0 ±0.1 atom percent deuterium. In a typical experiment involving tritium exchange, 8 g of **2** was placed in a 100-ml, round-bottomed flask equipped with a condenser and receiving flask. After adding 75 ml of tritiated water that was 1 25M in sulfuric acid, the solution was distilled and 60 ml of distillate was collected during a 3-h period The distillate was divided into two equal portions, with the 2-furaldehyde therein being converted into the phenylhydrazone or into 2-furoic acid as described next For deuterium-exchange experiments, a similar procedure was used, with the resulting 2-furaldehyde being converted first into 2-furoic acid and finally into methyl 2-furoate as described next

*Conversion of 2-furaldehyde into the phenylhydrazone.* — Phenylhydrazones were prepared by adding an excess of phenylhydrazine to one portion of the distillate. The resulting crystals were isolated on a filter, washed with water, and recrystallized from ethanol–water to constant specific activity (m.p. 93°).

*Oxidation of 2-furaldehyde to 2-furoic acid.* — In a typical experiment, 2.5 g of freshly prepared silver oxide was added to a sample of distillate containing 200–400 mg of 2-furaldehyde. The suspension was aerated, stirred, and maintained at pH 10 by the dropwise addition of M sodium hydroxide for the duration of the reaction. U.v. spectra revealed the absence of 2-furaldehyde after 30 min, at which time the mixture was centrifuged, filtered, and the filtrate treated with an excess of Dowex-50 ($H^+$) and finally evaporated to dryness. The resulting 2-furoic acid was redissolved in methanol and evaporated to dryness two times, and then sublimed (60° and 0.25 mm) to constant radiochemical activity (m.p. 131°).

*Preparation of methyl 2-furoate.* — For deuterium-exchange investigations, 200 mg of 2-furoic acid was esterified by the dropwise addition of ethereal diazomethane. After evaporation at room temperature, the resulting viscous liquid was dissolved in 1.5 ml of chloroform-*d* and used for n.m.r. measurements.

## REFERENCES

1 M. S. Feather, D. W. Harris, and S. D. Nichols, *J. Org. Chem.*, 37 (1971) 1605–1606, and references therein.
2 M. S. Feather and K. R. Russell, *J. Org. Chem.*, 34 (1969) 2650–2652.
3 W. A. Bonner and M. R. Roth, *J. Am. Chem. Soc.*, 81 (1959) 5454–5456.
4 D. M. W. Anderson and S. Garbutt, *J. Chem. Soc.*, (1963) 3204–3210.
5 H. S. Isbell, *J. Res. Natl. Bur. Stand.*, 33 (1944) 45–61.
6 E. Stutz and H. Deuel, *Helv. Chim. Acta*, 41 (1958) 1722–1730.
7 M. S. Feather and J. F. Harris, *J. Org. Chem.*, 31 (1966) 4018–4021.
8 E. F. L. J. Anet, *Adv. Carbohydr. Chem.*, 19 (1964) 181–218, and references therein.
9 E. F. L. J. Anet, *Chem. Ind. (London)*, (1962) 262.
10 E. F. L. J. Anet, *Aust. J. Chem.*, 16 (1963) 270–277.
11 M. S. Feather, *Tetrahedron Lett.*, (1970) 4143–4145.
12 E. S. Gould, *Mechanism and Structure in Organic Chemistry*, Holt, Rinehart and Winston, New York, 1959, p. 352.
13 E. Votoček and A. Kroslak, *Collect. Czech. Chem. Commun.*, 10 (1938) 259–263; *Chem. Abstr.*, 32 (1938) 8410.
14 K. Heyns and W. Baltes, *Chem. Ber.*, 93 (1960) 1616–1631.
15 F. Micheel and G. Hagemann, *Chem. Ber.*, 92 (1959) 2836–2840.