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DECOMPOSITION REACTIONS OF AMINO SUGARS: THE DEHYDRATION OF 2-AMINO-2-DEOXY-D-GLUCOSE*

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ABSTRACT

The stability of the title compound (**1**) was investigated at 100° in acidified aqueous solutions containing, in some instances, glycine or pyridine. In strong acid (3M hydrochloric acid), the sugar was relatively stable, and no identifiable decomposition-products were observed. In less-acidic solutions (≤0.5M hydrochloric acid) in the presence of glycine, substantial decomposition occurred with the production of 5-(hydroxymethyl)-2-furaldehyde (**2**) in 0.5–5.2% yield. The major dehydration products, however (up to 18% of the starting sugar), were pyrazine derivatives bearing dissimilar, four-carbon, acyclic-sugar side-chains attached to C-2 and C-5 of the ring, respectively, arising, most probably, from C-3–C-6 of the original sugar molecules. When the conversions were performed in deuterium oxide solution, carbon-bound isotope was observed in **2** (at the aldehyde carbon and at C-3) and, in the pyrazine derivatives, on the ring (positions 3 and 6), and on the sugar-derived, side-chains.

INTRODUCTION

Although 2-amino-2-deoxy-D-glucose (**1**) undergoes numerous unique reactions attributable to its amino group, it is normally considered inert in acidic systems when compared with aldoses and ketoses. Under these conditions, the protonated amino group disfavors any acid-catalyzed enolization and subsequent elimination reactions. Although relatively little information concerning stability is available for this sugar, several reports have indicated that, under certain conditions, **1** may decompose under unexpectedly mild conditions[1,2]. A study of the stability of **1** in water at pH 7 showed that it disappears rapidly with the evolution of ammonia[3]. Other decomposition-products were not absolutely identified in this study, but the

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data presented showed that some of these were u.v.-absorbing compounds. Soon and Chichester[4], in a kinetic study of the stability of sugars in acidic solution and sugars in the presences of amines (glycine), also observed that **1** decomposes very rapidly as compared with D-glucose, but, again, detailed studies on the nature of the products were not performed.

It is well known that non-nitrogenous sugars readily decompose in the presence of amines by forming intermediate, Amadori-rearrangement products (1-amino-1-deoxy-2-ketoses), which then undergo dehydration to furans, furanones, and related compounds[5] depending on the reaction conditions. Such amino sugars as **1** are not normally included as reactants in such schemes, even though part of the molecule is, in effect, a catalyst for the reaction.

This paper reports on a systematic study surveying the extent of dehydration reactions of compound **1** in aqueous systems containing amines (glycine and pyridine). Where the yield of dehydration products is maximized, a detailed product-analysis was undertaken, as well as deuterium-incorporation studies, to elucidate the mechanism.

RESULTS AND DISCUSSION

In most experiments, **1** was treated for 24 h at 100° in acidified, aqueous solutions containing variable amounts of reagents. At the end of this time, the solution was exhaustively extracted with ethyl acetate and the extracts were dried and evaporated. The major organic-extractable product was found to be 5-(hydroxymethyl)-2-furaldehyde (**2**), which was readily identified by t.l.c. comparison with an authentic sample, by its 60-MHz ^{1}H-n.m.r. spectrum, and by its characteristic u.v. absorption maxima. A number of experiments were made at various acidities, as well as ratios of **1** versus glycine. For each instance, the color of the solution was visually noted and the yield of ethyl acetate-extractable **2** was determined by isolation (see

TABLE I

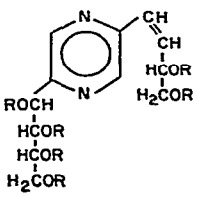
YIELD DATA FOR DECOMPOSITION[a] OF 2-AMINO-2-DEOXY-D-GLUCOSE HYDROCHLORIDE

Reagents	*Solvent*	*Color after 24 h*	*Yield (%) of compound* **2**
None	3M HCl	Light brown	0
Glycine[b] (1 mol)	0.5M HCl	Black	5.2
Glycine (0.1 mol)	0.5M HCl	Light brown	0.5
Glycine (1.0 mol)	H_2O	Black	3.9
Glycine (0.1 mol)	H_2O	Black	4.1
	H_2O	Black	2.2
pH 7[c]	H_2O	Black	3.6
Pyridine (0.1 mol)	H_2O	Black	4.1
Pyridine (1.0 mol)	H_2O	Black	~0.2

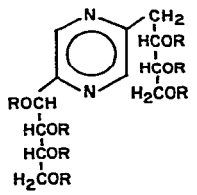
[a]For 24 h at 100°. [b]Per mole of starting sugar. [c]Adjusted by addition of 6M sodium hydroxide.

Table I). These results show that **1** is remarkably stable in strong (3M hydrochloric) acid but, upon lowering the acidity and/or adding glycine, substantial browning and concomitant production of **2** is observed. The fact that **2** is itself unstable and decomposes under these conditions[6], and therefore has an effect on measured yields, is important. The yields found, therefore, may be termed significant even though the maximum is 5.2%. Based on yields of **2** obtained from **1**, a catalytic effect by glycine may be inferred in both water, the pH of which was 4.5, and in 0.5M hydrochloric acid. These data are generally consistent with those reported by Soon and Chichester for a similar system[4].

The aqueous phase of the mixture was acidified and evaporated. The solids were extracted with methanol, leaving behind the hydrochloride salt of **1**, amine salts, and inorganic salts (as evidenced by n.m.r. measurements). Analysis (n.m.r.) of the methanol extract showed no starting material, but absorptions in the region δ 8.3–6.2, indicated the presence of aromatic and alkenic compounds. The methanol extract was acetylated (pyridine–acetic anhydride) and the acetylated materials were separated by column chromatography. Two major compounds were isolated: **3a**, a



3a R= Ac
3b R=H



4a R=Ac
4b R=H

crystalline hexaacetate in 2.5% yield; and **4a**, a syrupy heptacetate in 13% yield, together with two additional compounds (**5a** and **6a**) that are isomeric with **4a** (0.07% yield each) and were not positively identified. On *O*-deacetylation, compounds **3a**, **4a**, and **5a** yielded crystalline, hydroxyl derivatives (compounds **3b**, **4b**, and **5b**, respectively). The physical properties of compounds **4a–6a** (u.v., mass spectrum, n.m.r., and elemental analysis) establish their general structure as disubstituted pyrazines having dissimilar, acetylated sugar side-chains, one of which contains a methylene group. Data on the *O*-deacetylated derivative **4b** (m.p., n.m.r. spectrum, elemental analysis) (see Experimental section) are identical with those reported by Kuhn and coworkers for the same compound[7]. This was confirmed by preparing **4b** according to Kuhn's procedure. This product was identical with **4b**, as evidenced by an unchanged mixed m.p., and conversion into a heptaacetate having properties identical to those of **4a**.

The physical data obtained for compound **3a** (m.p. 96–97°, ν_{max} 236 and 295 nm, M^+ 538), indicate that it is structurally similar to **4a**, but further dehydrated. On *O*-deacetylation, the resulting **3b** had m.p. 185–185.5°, ν_{max} 238 and 299 nm, and an n.m.r. spectrum showing absorbances for two aromatic and two alkenic protons at

TABLE II

DEUTERIUM CONTENT OF **2** PRODUCED FROM **1** IN DEUTERATED SOLVENTS[a]

Conditions		*C-1 (%)*	*C-3 (%)*
Glycine[b] (1 mol)	0.5M DCl D_2O	34	88
Glycine (0.1 mol)	D_2O	13	48
	D_2O	16	33
pH 7[c]	NaOD–D_2O	17	39

[a]After 24 h of reaction at 100°. [b]Per mol of starting material. [c]Adjusted to pH 7 with 6M NaOD.

δ 8.38 and 6.56, respectively. These physical data are consistent with the proposed structure for **3a**. In addition, the elemental analyses obtained for both **3a** and **3b** show that the empirical formulas differ from those found for **4a** and **4b** by one molecule of acetic acid and one molecule of water, respectively. Additional support for this assignment is the fact that further acid treatment of **4b** converts it into **3b**, consistent with it's being a further dehydration product. It is noteworthy that elimination reactions of this type are known[6] to proceed during the dehydration of aldoses under similar conditions. Such a structure, therefore, would not be unexpected. It is also significant that, during this conversion, no furan derivatives are produced.

In order to gain some insight into the mechanism of the dehydration reaction, deuterium-exchanged **1** was converted into **2** in deuterium oxide solution. Incorporation was observed only at the aldehyde carbon of **2** and at C-3 (corresponding, to C-1 and C-3 of **1**). The results (Table II) show substantial incorporation under conditions where significant yields of **2** were observed. These results are similar to those observed for the conversion of some D-glucose-derived Amadori compounds into **2** in a prior study[8].

Both compounds **3a** and **4a** were isolated from isotope-incorporation reactions after acetylation, and the amount of deuterium exchange that occurred during the reaction was measured by integration of the appropriate signals in the n.m.r. spectra. For **3a**, 50% incorporation into the pyrazine ring (at position 3 and 6) and 45% incorporation at the alkanic carbons of the side chain were noted. For **4a**, 34% of exchange was measured at ring positions **3** and **6** (in unequal proportions), as well as 40% at the methylene carbon atom of the side chain.

It is noteworthy that, for the numerous acid-catalyzed processes involving conversions of sugars into 2-furaldehyde derivatives that have been studied in the past, solvent-proton exchange (as evidenced by both deuterium- or tritium-exchange experiments) has generally not been observed[6]. In contrast, when Amadori compounds are converted into 2-furaldehydes in isotopically labeled water, the resulting products have been observed to be extensively labeled at both the aldehyde carbon atom as well as position 3 of the furan ring[7], as was found for the **2** investigated in this study.

By analysis of Table I, it appears that the addition of amines to the reaction does not have the same kind of catalytic effect as on the decomposition reaction of aldoses and ketoses. As evidenced by the increasing instability of **1** with decreasing acidity of the reaction media, it is probable that the driving force of the decomposition of **1** is the liberation of the free amino group from the hydrochloride salt. Isolation of significant yields of such pyrazines as compounds **4a–6a** indicate that dehydration reactions analogous to those of aldohexoses and aldopentoses are further disfavored by irreversible cyclization–aromatization reactions involving the amino and electrophilic carbonyl groups at C-2 and C-1, respectively. The deuterium-incorporation data suggest that some classical dehydration-reactions occur via a pathway similar to that of Amadori compounds.

The results clearly show that **1** does not appear to be as stable in solution, particularly in the presence of amines, as might be expected. The data indicate that care should be exercised when manipulating samples containing 2-amino-2-deoxy-aldoses, particularly when amino acids or protein is present in the sample. Losses of amino sugars could well occur during hydrolysis of glycoproteins and related materials prior to sugar analyses.

EXPERIMENTAL

Materials and methods. — Thin-layer and column chromatography was performed with silica gel. Melting points were determined with a Thomas–Hoover Uni-Melt device and are uncorrected. Optical rotations were measured with a Schmidt–Haentsch polarimeter and i.r. spectra with a Perkin–Elmer 237-B spectrometer. Proton n.m.r. spectra were recorded with a Varian T-60 instrument. Mass spectra were determined with a CEC model 2031 spectrometer by direct insertion. U.v. spectra were recorded on a Perkin–Elmer, Coleman 124 double-beam spectrophotometer. Chromatographically homogeneous oils were purified for analysis by Kuhlrohr distillation.

General method for decomposition reactions. — A stirred, aqueous solution (50 mL) of 2-amino-2-deoxy-D-glucose hydrochloride **1** (10 g, 46.4 mmol) and the appropriate reagent (see Table I) was kept for 24 h at 100°. The mixture was cooled, filtered to remove solids, and extracted with ethyl acetate (6 × 30 mL). The combined organic phases were dried (sodium sulfate) and evaporated under diminished pressure, weighed, and examined by ^{1}H-n.m.r. spectroscopy.

Isotope-incorporation reactions were conducted in deuterium oxide solution, exactly as just described except that all reagents and starting materials were first freeze-dried twice from deuterium oxide. Deuterium incorporation was determined by integration of the appropriate signals in the n.m.r. spectrum.

Large-scale decomposition reaction. — Compound **1** (50 g, 232 mmol) and glycine (17.4 g 232 mmol) were heated in 0.5M hydrochloric acid (250 mL) for 24 h at 100°. The cooled, filtered mixture was extracted with ethyl acetate (6 × 150 mL). The organic phase gave 1.65 g (5.2%) of 5-(hydroxymethyl)-2-furaldehyde which was

identical to an authentic sample as evidenced by t.l.c. and ^{1}H-n.m.r. comparison with an authentic sample. The aqueous phase was evaporated and gave an oil. Addition of methanol caused precipitation of solids that were recovered by filtration. Repetition of the foregoing process until no more solids were obtained gave 53 g of a mixture of compound **1** and glycine. The residue was acetylated with pyridine–acetic anhydride and gave, after chromatography with 1 : 1 ethyl acetate–hexane as eluent, compound **3a**; yield 1.56 g (2.5%), m.p. 96–97°, $[\alpha]_D^{20}$ +46° (*c*, 1.0, chloroform); *m/e* 538 (M^+); ν_{max} 1750 cm^{-1} (C=O); ν_{max}^{EtOH} 268, (log ε 4.19), 275 (4.17), and 295 nm (3.62); n.m.r. ($CDCl_3$): δ 8.50 (2 H, s, H-aromatic) 6.80 (2 H, m, H-olefinic), 6.18 (1 H, d, *J* 2.5 Hz), 5.70 (2 H, m), 5.3 (1 H, m), 4.3 (4 H, m), 2.22, 2.18, 2.15, 2.11, and 2.00 (18 H, 5s, 6 Ac).

Anal. Calc. for $C_{24}H_{30}N_2O_{12}$: C, 53.5; H, 5.8; N, 5.2. Found: C, 53.5; H, 5.8, N, 4.9.

Further elution gave compound **4a** (9 g, 13%) as an oil, $[\alpha]_D^{20}$ −18° (*c*, 1.0, chloroform); ν_{max} 1750 cm^{-1} (C=O); ν_{max}^{EtOH} 268 (log ε 4.12), 275 (4.09), and 295 nm (3.36); *m/e* 598 (M^+); n.m.r. ($CDCl_3$): δ 8.44 and 8.38 (2 H, 2d, *J* 1 Hz, H-aromatic), 6.17 (1 H, d, *J* 2.5 Hz) 5.8–5.1 (4 H, m), 4.4–4.1 (4 H, m), 3.10 (2 H, m, -C*H*$_2$-) 2.20, 2.05, 2.03, 2.00, 1.84, and 1.82 (21 H, 6s, 7 Ac).

Anal. Calc. for $C_{26}H_{34}N_2O_{14}$: C, 52.2; H, 5.7; N, 4.7. Found: C, 52.3; H, 5.9; N, 4.7.

Further elution gave compound **5a** (50 mg, trace) as an oil; $[\alpha]_D^{20}$ −9.0° (*c*, 0.5, chloroform), ν_{max} 268 (log ε 4.19), 275 (4.17), and 295 nm (3.62); *m/e* 598 (M^+); n.m.r. ($CDCl_3$): δ 8.35 (2 H, s, H-aromatic), 6.16 (1 H, d, *J* 2.5 Hz), 5.8–5.1 (4 H, m), 4.5–4.1 (4 H, m), 3.1 (2 H, m -C*H*$_2$-), 2.16, 2.15, 2.08, 2.06, 2.04, 1.96, and 1.90 (21 H, 7s, 7 Ac).

Further elution gave the final product, compound **6a** (48 mg, trace), m.p. 139–140°, $[\alpha]_D^{20}$ −25° (*c*, 0.5, chloroform); ν_{max} 1750 cm^{-1} (C=O); ν_{max}^{EtOH} 269 (log ε 4.62), 275 (4.63), 275 (4.63), and 298 nm (4.30); *m/e* 598 (M^+); n.m.r. ($CDCl_3$): δ 8.50 and 8.44 (2 H, 2s, H-aromatic), 6.18 (1 H, d, *J* 2.5 Hz), 5.8–5.1 (4 H, m), 4.6–3.8 (4 H, m), 3.1 (2 H, m, -C*H*$_2$-), 2.23, 2.17, 2.13, 2.10, 2.02, and 1.95 (21 H, 6s, 7 Ac).

O-Deacetylation of compounds **3a**, **4a**, **5a**, *and* **6a**. — The aforementioned acetates were dissolved in anhydrous methanol in which a catalytic amount of sodium had been added. After being stirred for 16 h at 25°, the products were removed by filtration and recrystallized from methanol. In this manner, compound **3a** gave compound **3b** (94%), m.p. 185–185.5°, $[\alpha]_D^{20}$ −150° (*c* 1.0, water); ν_{max} 3450 cm^{-1} (OH); $\nu_{max}^{H_2O}$ 238 (log ε 4.20), and 299 nm (3.85); n.m.r. (D_2O): δ 8.40 (2 H, s, H-aromatic), 6.45 (2 H, m, H-alkenic), 4.80 (1 H, s), 4.4–4.0 (2 H, m), and 3.7–3.2 (5 H, m).

Anal. Calc. for $C_{12}H_{18}N_2O_6$: C, 50.3; H, 6.3; N, 9.8. Found: C, 50.3; H, 6.4; N, 9.4.

By the same method, compound **4a** gave compound **4b** (88%); m.p. 152–153°; ν_{max} 3500 cm^{-1} (OH); $\nu_{max}^{H_2O}$ 273 nm (log ε 4.21); n.m.r. (D_2O) δ: 8.35, 8.15 (2 H, 2s, H-aromatic) 4.75 (1 H, s), 4.0–3.1 (8 H, m), and 2.8 (2 H, m, -C*H*$_2$-).

Anal. Calc. for $C_{12}H_{20}N_2O_7$: C, 47.4; H, 6.6; N, 9.2. Found: C, 47.3, H, 6.6, H, 9.0.

By the same method, compound **5a** gave compound **5b**; yield 78%; m.p. 201–202°, $\nu_{max}^{H_2O}$ 274 nm (log ε 4.8); by the same method, compound **6a** gave compound **6b** (81%) as an oil, $\nu_{max}^{H_2O}$ 274 nm (log ε 4.4).

Interconversion of compounds **3b** *and* **4b**. — Compound **4b** (100 mg, 0.3 mmol) in hydrochloric acid (10 mL, 0.1M) was heated for 23 h at 100°. The colorless mixture was cooled, made neutral, extracted with ethyl acetate, evaporated, acetylated with acetic anhydride–pyridine, and purified by chromatography to give compound **3a** (44 mg, 23%) and compound **4a** (229 mg 66%). The ethyl acetate extract was evaporated, but gave no residue.

Alternative preparation of compound **4a**. — Compound **1** was converted into the free base by the action of sodium methoxide–methanol. This product (3.4 g, 18.9 mmol) was dissolved in acetic acid (34 mL) and heated for 30 min at 100°. The cooled mixture was evaporated and a portion of the resultant light-brown solid recrystallized from methanol to give the known[7] compound **4b**, m.p. 152–153°, mixed m.p. unchanged. The bulk of the impure product was acetylated to give compound **4a** (4.83 g, 85%).

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