*Carbohydrate Research*, 77 (1979) 213–217


## Note

# The alkaline degradation of 2-amino-2-deoxy-D-glucose*

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(Received December 11th, 1978; accepted for publication in revised form, February 28th, 1979)

In strongly acidic solution, 2-amino-2-deoxy-D-glucose (**1**) is stable, because protonation of the amino group affords an intermediate resistant to acid-catalyzed enolization, epimerization, and elimination reactions. In less-acid media, and in the presence of amines, **1** is readily dehydrated to 5-(hydroxymethyl)-2-furaldehyde[1] with concomitant production of disubstituted pyrazines[1–3]. Reports[4] on some reactions of **1** and its oligomers[5] in alkaline solution show that **1** gives rise to u.v.-absorbing products of undetermined structure, and Whistler and BeMiller[5] reported the isolation of saccharinic acids from the alkaline degradation of chitotriose.

This study examines the effect of temperature and alkalinity on the degradation of **1**, and provides a detailed product-analysis of these transformations.

Alkaline solutions of **1** of different basicities were heated for a designated time-period at a particular temperature. The crude mixtures were cooled, acidified, evaporated, dissolved in deuterium oxide, and examined by $^1$H-n.m.r. spectroscopy. The reaction progress was monitored by comparison of the relative areas of the signals for the anomeric protons of **1** ($\delta$ 5.5 and 4.9) to those of other products formed during the reaction. It was found that the disappearance of **1** became more rapid in progressively more-basic solution and at progressively higher temperature. For example, no reaction was observed after 24 h at 27° in 8–16M sodium hydroxide or in saturated calcium hydroxide, but considerable reaction was observed after 0.75 h for **1** in 8M sodium hydroxide at 60°, and complete consumption of **1** occurred in 8M sodium hydroxide after 1 h at 60° and in 16M solution of sodium hydroxide after 10 min at 100°. For completed reactions, the n.m.r. spectrum showed total disappearance of signals for compound **1** and appearance of new resonances in the region $\delta$ 1.4–2.2, indicating the presence of deoxy sugars, or derivatives thereof.

Solutions from completed reaction were made neutral, evaporated to dryness,

*Issued as Journal Paper No. 8256 of the Missouri Agricultural Experiment Station. Acknowledgment is made to the National Science Foundation for financial assistance through Grant Number CHE 76-81022 AO1.
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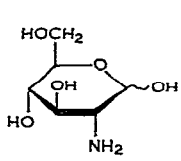


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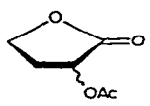


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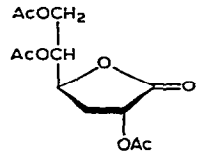


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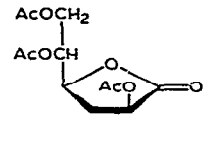


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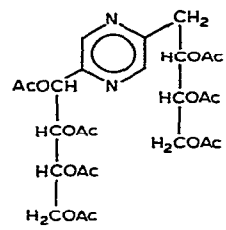


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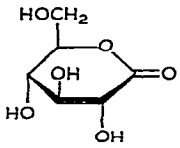


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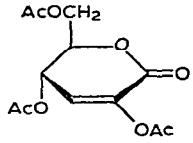


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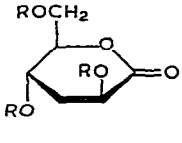
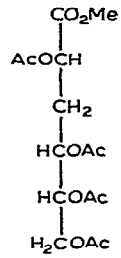



8 R = Ac
10 R = Bz

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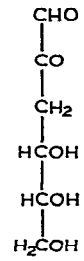


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and the residue acetylated. G.l.c. analyses indicated the presence of three major products (compounds **2**–**4**) in the relative ratio of 15:36:43, respectively. Ten other, minor (<0.2%), components were also detected. It was found that heating the solutions of **1** for prolonged times beyond the disappearance of starting material failed to change the number or relative ratios of any of the decomposition products.

Column chromatography afforded each of the three major products (**2**, **3**, and **4**) in 8.8, 11.2, and 20.4% yields, respectively. A trace of compound **5** was also isolated.

The mass-spectral data, as well as n.m.r. spectra and elemental analyses suggest that **3** and **4** are epimeric, tri-*O*-acetylated deoxyhexonolactones (saccharinic acid derivatives). The alkaline degradation of non-nitrogenated aldoses under similar conditions gives rise to metasaccharinolactones (3-deoxyaldono-1,4-lactones) as major products, and the data collected are consistent with, but do not absolutely prove, that **3** and **4** are tri-*O*-acetylated derivatives of such compounds.

In order to investigate further the structures of **3** and **4**, the syntheses of derivatives of metasaccharinolactones were undertaken.

It is known that, when D-glucono-1,4-lactone is treated with pyridine under conditions of acetylation[7] (acetic anhydride) or benzoylation[8] (benzoyl chloride), the 3-hydroxyl group is eliminated. For the former reaction, the product is 2,4,6-tri-*O*-acetyl-3-deoxy-D-*erythro*-hex-2-enono-1,5-lactone[7] (**7**), and for the latter reaction, the corresponding tribenzoate[8] is formed. For the tribenzoate, it has been clearly shown[8] that catalytic hydrogenation converts it nearly stereospecifically into **10** (having the D-*arabino* configuration). The known[8] compound **10** was thus prepared, and, a similar reduction was applied to **7**, converting it into **8**. When **10** was

deacylated by treatment with methanol containing a catalytic amount of sodium methoxide, and then acetylated under the conditions described for processing of the original mixture, only **4** and an acyclic methyl ester **9** were isolated. The probable structure is assigned as **9**, on the basis of physical data. Identical results were obtained when **8** was treated similarly. These experiments establish that **3** and **4** are, in fact, tri-*O*-acetylated metasaccharinolactones, that **4** has the D-*arabino* configuration, and that **3**, therefore, has the D-*ribo* configuration. It is also noteworthy that these findings show **8** to have the same configuration at C-2 as the known **10**, as both materials gave identical products after deprotection and subsequent acetylation.

The production of 2,4-dihydroxybutanoic acid upon the treatment of carbohydrates with alkali has been observed many times in the past[6]. It has been demonstrated that this acid arises via complex fragmentation and recombination reactions during alkaline degradation, and its isolation in this study as the optically inactive (probably racemic) 2-*O*-acetylated-1,4-lactone (**2**) is not unexpected. The structure of **2** was determined by analysis of the physical data. The mass spectrum showed **2** to be $C_6H_8O_4$, and the n.m.r. spectrum clearly showed that **2** was a mono-*O*-acetylated lactone that had two methylene ($CH_2$) groups, one of which was linked to an esterified oxygen atom. The appearance of a one-proton doublet of doublets at $\delta$ 5.46 (assignable to H-2) confirmed that C-2 is *O*-acetylated and thus establishes the structure of **2** as shown.

Compound **5** was found to be a disubstituted pyrazine identical with an authentic sample that had been prepared and characterized in a prior report[1].

The isolation of compounds **2**–**4** from the reaction of **1** with alkali shows that **1** undergoes alkaline-degradation reactions similar to those of its oxygenated analogs. *O*-Deacetylated **3** and **4** were the only saccharinolactones isolated in the reaction; if the others (isosaccharinic and saccharinic acids) were produced, they were in traces only. These findings are similar to those of Harris[6], who found that metasaccharinic acids are the major saccharinic acids produced when D-glucose is degraded in alkali. The formation of compounds **3** and **4** (metasaccharinic acid 1,4-lactones) implies the intermediacy of 3-deoxyglycos-2-ulose (**11**) in the degradation pathway, as it has been shown that **11** is probably a reaction intermediate, and is transformed in basic solution via a benzilic acid type of rearrangement to give the metasaccharinic acids[9,10], epimeric at C-2. Pyrazine heterocycles are probably formed by way of cyclization of two different molecules in their acyclic forms through a condensation reaction involving the aldehyde and amino groups, followed by dehydration and aromatization to give[1] **5**.

## EXPERIMENTAL

*Materials and methods.* — Thin-layer and column chromatography were performed with silica gel. Gas-chromatographic analyses were carried out with a Perkin–Elmer model Sigma-3 instrument and a 10 ft × 1/8 in. stainless-steel column packed with 2% diethylene glycol adipate on Chromosorb W. Peaks were detected by a flame-

ionization detector. The column oven was programmed from 180–240° at 20°/min. Melting points were determined with a Thomas–Hoover Uni-melt device and are uncorrected. Optical rotations were measured with a Schmidt–Haentsch polarimeter. Infrared spectra were recorded with a Perkin–Elmer 247-B spectrometer. $^1$H-N.m.r. spectra were recorded with a Varian T-60 instrument for solutions in chloroform-*d*, with tetramethylsilane as the internal standard. Where necessary, proton assignments were confirmed by spin-decoupling experiments. Mass spectra were determined with a CEC model 2031 spectrometer by direct insertion. Chromatographically homogeneous oils were purified for analysis by distillation.

*Alkaline degradation of* **1**. — For analytical purposes, compound **1** (100 mg) was dissolved in sodium hydroxide solution (1 mL) and heated in an oil bath. At the appropriate time-intervals, the solution was cooled, acidified with 3M hydrochloric acid, evaporated to dryness *in vacuo*, dissolved in deuterium oxide (0.2 mL), and examined by $^1$H-n.m.r. spectroscopy. The reaction progress was determined by observation of the diminution of the signals for the anomeric protons of **1** ($\delta$ 4.9–5.5) and the appearance of absorbances in the region $\delta$ 1.2–2.2. The neutralized solutions were evaporated, acetylated with acetic anhydride–pyridine (1:2 v/v, 30 min, 100°) and examined by g.l.c. In all instances where reaction occurred and was complete, three major peaks were observed, having retention times of 2.4, 9.4, and 9.9 min (compounds **2**–**4** respectively) with relative areas of 15, 34, and 45, respectively.

*Preparative-scale reaction.* — Compound **1** (1.0 g, 4.6 mmol) in sodium hydroxide solution (10 mL, 16M) was heated for 10 min at 100°. After acidification, solvent removal and acetylation, the crude product was purified by column chromatography, with 1:1 ethyl acetate–hexane as eluent, giving compound **2** (59.3 mg, 8.8%) $T_R$ 2.4 min, an oil; $[\alpha]_D^{25}$ 0° (*c* 1.0, chloroform); $\nu_{max}$ 1750 cm$^{-1}$ (C=O); *m/e* 144 ($M^+$), 102 ($M^+ - CH_2CO$); n.m.r. $\delta$ 5.46 (1 H, dd, *J* 9.3 and 8.8 Hz, H-2), 4.60–4.10 (2 H, m, H-4,4′), 3.04–2.20 (2 H, m, H-3,3′), and 2.18 (3 H, s, Ac). [Found: *m/e* 144.042; $C_6H_8O_4$($M^+$) requires: *m/e* 144.041].

Further elution gave 2,5,6-tri-*O*-acetyl-3-deoxy-D-*ribo*-hexono-1,4-lactone, (**3**), (151 mg, 11.2%); $T_R$ 9.4 min, an oil; $[\alpha]_D^{20}$ +14.0° (*c* 1.0, chloroform); $\nu_{max}$ 1750 cm$^{-1}$ (C=O); *m/e* 288 ($M^+$); n.m.r. $\delta$ 5.42 (1 H, dd, $H_{2,3}$ 7.9, $J_{2,3'}$ 8.9 Hz) 5.24 (1 H, m, H-5), 4.82 (1 H, m, H-4), 4.25 (1 H, dd, $J_{5,6}$ 4.2, $J_{6,6'}$ 12.2 Hz, H-6), 4.18 (1 H, dd, $J_{5,6'}$ 5.2 Hz, H-6), 3.0–2.3 (2 H, m, H-2,3), 2.19, 2.12, and 2.10 (9 H, 3s, 3 Ac).

*Anal.* Calc. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.90; H, 5.61.

Further elution gave 3-deoxy-2,4,6-tri-*O*-acetyl-D-*arabino*-hexono-1,4-lactone (**4**, 272 mg, 20.4%); $T_R$ 9.9 min, an oil; $[\alpha]_D^{20}$ +37° (*c* 1.0, chloroform); $\nu_{max}$ 1750 cm$^{-1}$ (C=O); *m/e* same as compound **3**; n.m.r. $\delta$ 5.52 (1 H, dd, $J_{2,3}$ 8.7, $J_{2,3'}$ 10 Hz, H-2), 5.32 (1 H, m, H-5), 4.64 (1 H, m, H-4), 4.35 (1 H, dd, $J_{5,6}$ 2.8, $J_{6,6'}$ 12.8 Hz, H-6), 4.18 (1 H, dd, $J_{5,6'}$ 5.5 Hz, H-6′), 3.1–2.3 (2 H, m, H-3,3), 2.17, 2.09, and 2.05 (9 H, 3s, 3Ac).

*Anal.* Calc. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.85, H, 5.60.

Further elution gave compound **5** (11 mg, trace) as an oil, identical by i.r., u.v., and n.m.r. spectra to an authentic sample[1].

*Synthesis of* **4**. — D-Glucono-1,5-lactone (**6**, 1.0 g, 5.6 mmol) was acetylated with pyridine–acetic anhydride (4 mL, 2:1 v/v, 1 h, 23°). Chromatographic purification of the crude mixture of products with 3:2 ethyl acetate–hexane as eluent gave 2,4,6-tri-*O*-acetyl-3-deoxy-D-*erythro*-hex-2-enono-1,5-lactone (**7**, 720 mg, 45%); n.m.r. parameters were identical to those previously reported[7]. Further elution gave the contaminating pyrone (0.15 g, 12%), which has also been described previously.

Compound **7** (0.5 g, 1.7 mmol) was hydrogenated in the presence of 50 mg of 5% palladium-on-charcoal at 22 lb.in for 4 h in ethyl acetate (20 mL) and gave 0.59 (99%) of 2,4,6-tri-*O*-acetyl-3-deoxy-D-*arabino*-hexono-1,5-lactone (**8**), an oil; $[\alpha]_D^{20}$ +16° (*c* 1.0, chloroform); $\nu_{max}$ 1750 $cm^{-1}$ (C=O); *m/e* 288 ($M^+$); n.m.r.: $\delta$ 5.58 (1 H, dd, $J_{2,3}$ 9, $J_{2,3'}$ 10.8 Hz, H-2), 5.22 (1 H, m, H-2), 4.67 (1 H, m, H-5), ~4.4 (2 H, m, H-6,6'), 2.6–2.0 (2 H, m, H-3,3'), 2.21, 2.17, and 2.16 (9 H, 3s, 3 Ac).

*Anal.* Calc. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.59. Found: C, 49.91; H, 5.80.

The 1,5-lactone (**8**, 100 mg, 0.35 mmol) was *O*-deacetylated (catalytic sodium methoxide in methanol), treated with sodium hydroxide solution (pH 10.0, for 1 h at 100°), acidified, and acetylated as already described, giving only compound **4** 84 mg, 84%).

Compound **6** (4.0 g, 22 mmol) was converted into 2,4,6-tri-*O*-benzoyl-3-deoxy-D-*arabino*-hexono-1,5-lactone (**10**) by the method of de Lederkremer *et al.*[8]; yield 9.5 g (89%), m.p. 159–160° (lit. m.p. 158–160°). This material was *O*-debenzoylated with sodium methoxide in methanol to give a crisp foam. Acetylation of a part of this material (200 mg) gave, after chromatographic purification with 3:2 ethyl acetate–hexane as eluent, methyl 2,4,5,6-tetra-*O*-acetyl-3-deoxy-D-*arabino*-hexonate (**9**, 38.2 mg, 10%), an oil, $[\alpha]_D^{20}$ +86° (*c*, 1.0, chloroform); $\nu_{max}$ 1750 $cm^{-1}$ (C=O); *m/e* 331 ($M^+$ < Me); n.m.r. $\delta$ 5.4–4.8 (3 H, m, H-2,4,5), 4.4–4.1 (2 H, m, H-6,6'), 3.78 (3 H, s, $CO_2Me$), 2.35–1.90 (14 H, m, H-3,3', 4 Ac).

*Anal.* Calc. for $C_{14}H_{19}O_9$ ($M^+$ < OMe), *m/e* 331.103; Found: *m/e* 331.103.

Further elution gave compound **4** (191 mg, 65%). Compound **4** was produced exclusively when the foam was treated with sodium hydroxide solution before acetylation; yield 87%.

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