Carbohydrate Research, 26 (1973) 201–203

Note

2-Acetamido-3,6-anhydro-2-deoxy-D-gulose and -D-idose: products of the alkaline degradation of 2-acetamido-2-deoxy-D-galactose

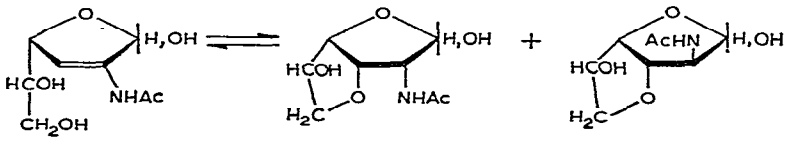
V. A. DEREVITSKAYA, L. M. LIKHOSHERSTOV, V. A. SCHENNIKOV, AND N. K. KOCHETKOV
Institute for Organic Chemistry, Academy of Sciences, Moscow (U. S. S. R.)
(Received May 22nd, 1972; accepted for publication, June 19th, 1972)

Recent studies[1] of the alkaline degradation of 2-acetamido-2-deoxy-D-glucose have shown the formation of 2-acetamido-3,6-anhydro-2-deoxy-D-glucose (**1**) and -D-mannose (**2**).

We have now studied the structure and properties of 3,6-anhydro derivatives formed during the alkaline degradation of 2-acetamido-2-deoxy-D-galactose. 2-Acetamido-3,6-anhydro-2-deoxy-D-gulose (**3**) and -D-idose (**4**) were isolated by chromatography on silica gel after 2-acetamido-2-deoxy-D-galactose had been treated with 50mM sodium carbonate at 70°. Compounds **3** and **4** were contaminated with chromogens[2] from which they were separated during the following conversions. Compound **3** was converted into the crystalline methyl glycoside **5**, the mass spectrum of which was closely similar to that of methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-mannofuranoside[1]. This fact and the high, negative optical rotation (−162°) allow the identification of **5** as a methyl 2-acetamido-3,6-anhydro-2-deoxy-β-D-hexofuranoside. The acetate (**6**) of **5** gave an n.m.r. spectrum ($CDCl_3$) which exhibited a 3-proton singlet at τ 6.64 (OMe) and two 3-proton singlets at 7.92 and 8.01 (Ac). The following chemical shifts and coupling constants were determined for the ring protons by using decoupling experiments: τ 5.18 ($J_{1,2}$ 1.8 Hz, H-1); 5.73 ($J_{2,3}$ 6.2 Hz, H-2); 3.89 ($J_{2,NH}$ 6.2 Hz, NH); 5.32 ($J_{3,4} \simeq 5.2$ Hz, H-3); 5.42 ($J_{4,5} < 1$ Hz, H-4); 4.74 ($J_{5,6'}$ 3.2, $J_{5,6}$ 2.0 Hz, H-5); 5.88 and 6.10 ($J_{6,6'}$ 10.9 Hz, H-6,6'). These data accord with the structure methyl 2-acetamido-5-*O*-acetyl-3,6-anhydro-2-deoxy-β-D-gulofuranoside, and consequently **3** is 2-acetamido-3,6-anhydro-2-deoxy-D-gulose. The 3,6-anhydro derivative **4** was converted into methyl α- and β-glycosides (**7** and **8**), the mass spectra of which correspond to methyl 2-acetamido-3,6-anhydro-2-deoxy-hexofuranoside structures. The *ido* configuration was confirmed by epimerisation of **3** or **4** in alkali, the equilibrium ratio of **3** and **4** being 1.7:1.

A possible pathway for the formation of 3,6-anhydro derivatives **3** and **4** involves an intramolecular attack of HO-6 on the double bond of a chromogen (**9**); this reaction is reversible. 3,6-Anhydro derivatives with *gulo* and *ido* configurations are more stable than those with *gluco* and *manno* configurations. During the alkaline

treatment of 2-acetamido-3,6-anhydro-2-deoxy-D-gulose, less chromogen is formed than during treatment of the *manno* analogue[1]. 2-Acetamido-2-deoxy-D-galactose and -D-talose have not practically been formed.



9 3 4

Under the above conditions, the alkaline degradation of 2-acetamido-2-deoxy-D-galactose was virtually complete, and ~90% of 2-acetamido-2-deoxy-D-glucose was degraded. It should be pointed out that, after acid hydrolysis of the products of alkaline degradation of 2-acetamido-2-deoxyhexoses, a substance (in addition to 3,6-anhydro derivatives, 2-amino-2-deoxyhexoses, and ammonia) was detected with the elution time of 2-amino-3,6-anhydro-2-deoxy-D-glucose. This substance disappears simultaneously with 2-amino-2-deoxyhexose upon alkaline treatment. It is possibly a product of acid hydrolysis of a chromogen (2-acetamido-2,3-dideoxyhex-3-enose) and was not formed during the alkaline degradation of 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose or -D-galactose which yield only the corresponding 3,6-anhydro derivatives.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) on non-activated silica gel was performed with chloroform–methanol, 4:1 (*A*) and 9:1 (*B*). Periodate–cuprate, *p*-dimethylaminobenzaldehyde, and sulphuric acid were used as detection reagents.

N.m.r. spectra were recorded with a DA-60-IL 60 MHz instrument and mass spectra with a Varian MAT CH 6 spectrometer at 70 eV with an ion-source temperature of 65–115°. Analyses were performed on an Amino-acid analyser 6020 A (Czechoslovakia) with a column (30 × 0.8 cm) of Chromex UA-8 and elution at 52° with a standard citrate–hydrochloric acid buffer (pH 5.28, 0.35M Na^+) or with a column (60 × 0.8 cm) with borate buffer at 75° (pH 5.15, 0.05M $Na_2B_4O_7$, 0.1M $Na_3C_6H_5O_7$, 0.05M NaCl, ~15 ml of conc. HCl, water to 1 litre) at 60 ml/h.

*2-Acetamido-3,6-anhydro-2-deoxy-*D*-gulose* (**3**) *and* -D-*idose* (**4**). — 2-Acetamido-2-deoxy-D-galactose (7.3 g) dissolved in 50mM sodium carbonate (730 ml) was heated at 70° for 4 h in a nitrogen atmosphere. The solution was neutralized with Dowex-50(H^+) resin, filtered, and evaporated *in vacuo*. The product mixture was eluted from a column of silica gel by using a gradient of chloroform → chloroform–methanol (9:1). Fractions containing a component with R_F 0.47 and 0.35 (solvent *A*) were collected and evaporated. The products (720 mg of **3** and 680 mg of **4**) were subjected to preparative t.l.c. (solvent *A*) to give **3** (330 mg) and **4** (170 mg) containing ~10% of chromogens.

Methyl 2-acetamido-3,6-anhydro-2-deoxy-β-D-gulofuranoside (**5**), *-α-D-idofurano-side* (**7**), *and -β-D-idofuranoside* (**8**). — A mixture of **3** (100 mg), Dowex-50(H^+) resin (300 mg), and methanol (3 ml) was stirred at room temperature for 5 h, and then filtered and evaporated. The residue was subjected to preparative t.l.c. (solvent *A*). Compound **5** (syrup, 75 mg, 70%), R_F 0.65 (solvent *A*), partly crystallized and had m.p. 91–92°, $[\alpha]_D^{20}$ −162° (*c* 0.5, methanol) (Found: C, 49.54; H, 6.86. $C_9H_{15}NO_5$ calc.: C, 49.77; H, 6.95%). The mass spectrum showed peaks at *m/e* 217 (M^+, 0.41% of base peak), 199 (M^+-H_2O, 0.92%), 186 (M^+-CH_3O, 5.5%), 158 ($M^+-CH_3CONH_2$, 100%), 128 ($M^+-CH_3CONH_2-CH_2O$, 96%).

Compounds **7** and **8** were obtained analogously from **4** as syrups: **7** (30%), R_F 0.5 (solvent *A*), $[\alpha]_D^{20}$ −1° (*c* 0.5, methanol); *m/e* 217 (M^+, 0.3% of base peak), 199 (M^+-H_2O, 2.2%), 186 (M^+-CH_3O, 4.6%), 158 ($M^+-CH_3CONH_2$, 100%), 128 ($M^+-CH_3CONH_2-CH_2O$, 93%); **8** (25%), R_F 0.63 (solvent *A*), $[\alpha]_D^{20}$ −190° (*c* 0.5, methanol); *m/e* 217 (M^+, 1.3% of base peak), 199 (M^+-H_2O, 1.58%), 186 (M^+-CH_3O, 8.4%), 158 ($M^+-CH_3CONH_2$, 100%), 128 ($M^+-CH_3CONH_2-CH_2O$, 94%).

A mixture of **5** (10 mg) and Dowex-50(H^+) resin (40 mg) in water (2 ml) was stirred at 50° for 40–46 h. The solution was filtered and evaporated, and the residue was subjected to preparative t.l.c. (solvent *A*) to give **3** (7.5 mg, 80%), $[\alpha]_D^{20}$ −75° (*c* 0.4, methanol). Likewise, **4**, $[\alpha]_D^{20}$ −80° (*c* 1, methanol), was obtained from **7** and **8**.

Methyl 2-acetamido-5-O-acetyl-3,6-anhydro-2-deoxy-β-D-gulofuranoside (**6**). — Compound **5** (30 mg) was treated with acetic anhydride (0.13 ml) and pyridine (0.17 ml) in the usual manner. The product was subjected to preparative t.l.c. (solvent *B*) to give **6** (30 mg, 84%), m.p. 117–118°, $[\alpha]_D^{20}$ −88° (*c* 0.5, chloroform), R_F 0.77 (solvent *A*) (Found: C, 51.18; H, 6.63. $C_{11}H_{17}NO_6$ calc.: C, 50.96; H, 6.61%).

The alkaline degradation and analysis with an amino-acid analyzer. — Samples (0.5 mg) of 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-3-*O*-methyl-D-galactose or -D-glucose were treated with 0.5 ml of 50mM sodium carbonate at 70° for periods of 3 min to 6 h. 4M Hydrochloric acid (0.5 ml) was added and the mixture was kept for 2 h at 100°. The hydrolyzate was evaporated, and 0.25–0.5 ml of water was added. A sample (0.2 ml) was then examined in the amino-acid analyzer. For the separation of 3,6-anhydro derivatives having the *gluco* and *manno* configuration from each other and from a non-separable mixture of the *gulo* and *ido* derivatives, a citrate buffer was used. The latter compounds could be separated from each other by using a borate buffer. The calculation was made by molar colour yields of hydrolyzates of **1**, **5**, and **8**, which amounted to ~60% of the colour yields of the hydrolyzate of 2-acetamido-2-deoxy-D-glucose (citrate buffer).

The epimerisation and stability of **3** and **4** were studied in a similar manner.

REFERENCES

1 V. A. Derevitskaya, L. M. Likhosherstov, V. A. Schennikov, and N. K. Kochetkov, *Carbohyd. Res.*, 20 (1971) 285.
2 R. Kuhn, G. Kruger, and H. H. Baer, *Chem. Ber.*, 89 (1956) 1473; 90 (1957) 264.