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Epimerization of *N*-acetyl-D-galactosamine to *N*-acetyl-D-talosamine

It was reported recently that *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) was epimerized in aqueous alkaline solution to *N*-acetyl-D-mannosamine (2-acetamido-2-deoxy-D-mannose)[1,2]. The present paper reports that *N*-acetyl-D-galactosamine (2-acetamido-2-deoxy-D-galactose) is also epimerized under similar conditions to *N*-acetyl-D-talosamine (2-acetamido-2-deoxy-D-talose). This aminosugar was isolated from the hydrolysates of chondroitin-sulfuric acid[3]. Chemical synthesis of D-talosamine was achieved by the cyanohydrin technique from D-lyxosylamine[4,5] and amination followed by configurational transformation of D-galactose[6]. Paper-chromatographic evidence of the epimerization of *N*-acetyl-D-galactosamine was reported[7] while our work was in progress, and conclusive evidence has now been obtained by the isolation and characterization of the converted aminosugar.

In the studies on the epimerization of *N*-acetyl-D-glucosamine[8], it was shown that *N*-acetyl-D-mannosamine formed was more soluble in cold ethanol than *N*-acetyl-D-glucosamine and that the former aminosugar formed more readily the phenylhydrazone than did the latter. *N*-Acetyl-D-talosamine was similarly separated from *N*-acetyl-D-galactosamine by using the different solubility in alcohol, but it was obtained only as a hygroscopic material. KUHN AND FISCHER[4] also reported *N*-acetyl-D-talosamine as a hygroscopic substance. It was characterized as the crystalline phenylhydrazone which was formed more readily than from *N*-acetyl-D-galactosamine. Hydrolysis of the *N*-acetyl-D-talosamine with dil. HCl produced the known D-talosamine hydrochloride[4].

The epimerization was carried out at pH 10–11. Paper-chromatographic examination indicated that this pH was the best for the formation of *N*-acetyl-D-talosamine. At pH 8–9, there was little formation of *N*-acetyl-D-talosamine, and *N*-acetyl-D-galactosamine remained largely unchanged, while at pH 12–14 an additional substance was formed. It migrated more rapidly than the two *N*-acetylhexosamines.

N-Acetyl-D-galactosamine[9] (15 g) was treated with 40 ml of dil. NaOH (pH 11) for 3 days at room temperature. After passing through a column of Amberlite IR-120 (H^+ form) and a column of Amberlite IR-4B (OH^- form), the reaction solution was concentrated to a syrup which was dissolved in a mixture of 15 ml each of methanol and ethanol. Addition of ethyl acetate to a slight turbidity and keeping the mixture in a refrigerator separated 12 g of the unchanged *N*-acetyl-D-galactosamine. After concentration of the filtrate a syrup remained which contained almost pure *N*-acetyl-D-talosamine according to paper chromatography; it was precipitated as a hygroscopic powder from ethanol with ether. $[\alpha]_D = -4°$ (*c*, 1.0 % in water).

The syrupy *N*-acetyl-D-talosamine (1 g) was dissolved in 1 ml of water and to this were added 0.5 g of phenylhydrazine and 0.25 g of acetic acid. After keeping the mixture at room temperature for 6 h a crystalline matter separated which was collected, after standing overnight, by filtration; the yield was 1.5 g. It was dissolved in 20 ml of water, decolorized with charcoal, concentrated to about 5 ml and kept in a refrigerator for crystallization. The recrystallization was repeated once more and the final crystals were washed with a little cold methanol and dried; the yield was 350 mg; m.p. 196°, $[\alpha]_D = -16° \rightarrow +50°$ (*c*, 1.0 % in water). [Calc. for $C_{14}H_{21}N_3O_5$: C, 54.01; H, 6.80; N, 13.50. Found: C, 53.90; H, 7.02; N, 13.48 %.]

Under similar reaction conditions, *N*-acetyl-D-galactosamine phenylhydrazone was not obtained, but refluxing 2.2 g of *N*-acetyl-D-galactosamine, 1.25 g of phenylhydrazine and 1 g of acetic acid in 20 ml of methanol for 1 h, evaporation off the solvent, treatment with ether and recrystallization from hot ethanol gave the phenylhydrazone in a yield of 1.5 g; m.p. 160–162°, $[\alpha]_D = +59°$ (*c*, 1.0 % in water). [Calc. for $C_{14}H_{21}N_3O_5$: C, 54.01; H, 6.80; N, 13.50. Found: C, 54.16; H, 6.94; N, 13.52 %.]

The sirupy *N*-acetyl-D-talosamine prepared from *N*-acetyl-D-galactosamine (15 g) was hydrolyzed in 0.5 N HCl for 6 h on a boiling-water bath[3] and the hydrolysate evaporated to a small volume. It was applied to a column (20 × 700 mm) of Dowex 50W X8 (H^+ form) and eluted with 0.33 N HCl. The fractions which were positive to the ELSON–MORGAN reaction[10] were combined and evaporated to dryness. The residue was dissolved in 10 ml of water and again applied to a column (10 × 400 mm) of Dowex 50W X8 (H^+ form) and eluted similarly. The fractions positive to the ELSON–MORGAN reaction were again combined, concentrated and ethanol and ethyl acetate were added to the residue until a turbidity was obtained. After keeping in a refrigerator, D-talosamine hydrochloride was obtained in crystalline form in a yield of 460 mg. It was repeatedly recrystallized from a mixture of ethanol and methanol by careful addition of ethyl acetate. The final product (yield 44 mg) gave a single spot in paper-chromatographic assay[11]. It had m.p. 151–152° (decomp.) and $[\alpha]_D = -5.0°$ (*c*, 1.0 % in water). [Calc. for $C_6H_{13}NO_5 \cdot HCl$: C, 33.42; H, 6.54; N, 6.50. Found: C, 33.84; H, 6.62; N, 6.13 %.]

KUHN AND FISCHER[4] reported for this compound m.p. 151–153° (decomp.) and $[\alpha]_D = +3.4° \rightarrow -5.7°$. The infrared spectrum of our product was identical with that of D-talosamine hydrochloride synthesized and supplied by Dr. KUHN. It was noted that an absorption at 1485 cm^{-1} in the spectrum of D-talosamine hydrochloride was characteristic of this aminosugar, being absent from that of the hydrochlorides of D-galactosamine, D-glucosamine and D-mannosamine.

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Research Laboratory, Kaken-yaku-kako Co. Ltd., Kyoto (Japan)

SHOJI FUJII
HIDEKO KUSHIDA

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