Carbohydrate Research, 63 (1978) 127–130

STUDIES ON THE EPIMERIZATION OF 2-ACETAMIDO-2-DEOXYHEXOSES: PREPARATION OF 2-ACETAMIDO-2-DEOXY-D-[2-^{3}H]-GLUCOSE AND -MANNOSE

MOHAMMED A. E. SALLAM*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907 (U.S.A.)

(Received August 29th, 1977; accepted for publication, September 15th, 1977)

ABSTRACT

Epimerization of either 2-acetamido-2-deoxy-D-glucose (**1**) or 2-acetamido-2-deoxy-D-mannose (**2**) in basic tritium oxide gave 2-acetamido-2-deoxy-D-[2-^{3}H]-glucose (**3**) and 2-acetamido-2-deoxy-D-[2-^{3}H]mannose (**4**). In both cases, compound **3** was isolated in higher proportion and higher specific activity than **4**. The mechanism of the epimerization of **1** and **2** is discussed.

INTRODUCTION

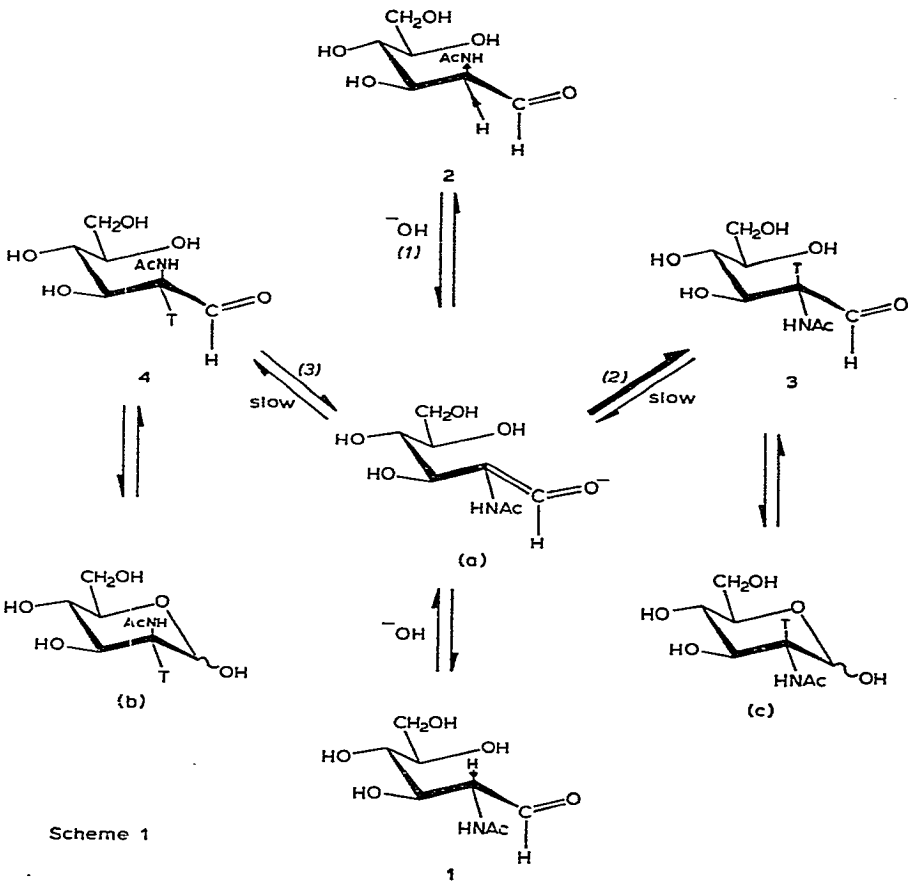
The isomerization of 2-acetamido-2-deoxyaldoses in dilute, aqueous base[1–5] by the Lobry de Bruyn–Alberda van Ekenstein procedure[6] is not complicated by the formation of 2-ketoses or 2-ketimines, in contrast to that of aldoses or 2-amino-2-deoxyaldoses. Epimerization of 2-acetamido-2-deoxy-D-glucose (**1**) in dilute, aqueous base[1,2,7,8] yielded two acetamido epimers and some unidentified components[2]. Studies[9] of the epimerization of **1** and its epimer 2-acetamido-2-deoxy-D-mannose[10] (**2**) in basic deuterium oxide showed a selective deuteration at C-2 of the 2-acetamido-2-deoxyhexose. The extent of the epimerization was monitored by n.m.r.[10] or ^{13}C-n.m.r. spectroscopy, and by gas–liquid chromatography[9].

Our studies on the biosynthesis of the mitomycin antibiotics required a supply of 2-amino-2-deoxy-D-[2-^{3}H]glucose hydrochloride for feeding experiments; therefore, the epimerization of **2** in basic tritium oxide was explored as a route to 2-acetamido-2-deoxy-D-[2-^{3}H]glucose (**3**). In addition, the epimerization of **1** in basic tritium oxide was studied, and the mechanism of the epimerization was investigated by the use of the radioactive isotope tritium.

Epimerization of either **1** or **2** in basic tritium oxide gave a mixture of **3** and 2-acetamido-2-deoxy-D-[2-^{3}H]mannose (**4**), with a preponderance of **3** in the reaction products. Liquid scintillation counting showed greater enrichment of **3** than of **4** with tritium. Radiochromatogram scanning of the reaction products of the epimeriza-

*On leave of absence from the Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.

tion of both **1** and **2** did not show any radioactive components other than **3** and **4**. Identical results had been found[9,10] by epimerization studies in basic deuterium oxide; these results could be explained by the presence of the acyclic, enolate intermediate[4,5,10] (a), whose production is facilitated by the inductive effect of the acetamido group, as shown in Scheme 1.



Scheme 1

The higher proportion of **3** compared to **4** in the epimerization of either **1** or **2** in basic tritium oxide could be explained as follows. (*i*) A fast, forward step in the reversible process (*2*) which results in the production of the conformationally stable $^4C_1$(D) conformer (c), having all bulky substituents in the equatorial position; and (*ii*) a slow, forward step in the reversible process (*3*), which leads to the relatively unfavorable $^4C_1$(D) conformer (b) having the D-*manno* configuration. When an aqueous solution of **2** was kept for 6 days at room temperature, a second spot, corresponding to **1**, appeared, whereas an aqueous solution of **1** remained unaffected; this observation further supports the relative instability of the isomer having the D-*manno* configuration.

The greater specific activity of **3** compared to **4** in the epimerization of either

1 or **2** could be explained as follows. (*i*) A slow, backward step involving the slow rupture of the C-2–T bond in **3** due to the heavy tritium atom, whereas this would be more facile in the nonlabeled compound formed from the acyclic, enolate intermediate (a). (*ii*) A slow, forward step in the reversible process (*3*), leading to the relatively unfavorable $^4C_1$(D) conformer (b). (*iii*) Dilution of the reaction mixture from which **4** was finally isolated, this dilution decreasing the specific activity of **4**.

EXPERIMENTAL

General. — Melting points are uncorrected. Evaporations were performed under diminished pressure below 50°. Paper chromatography was conducted on borated paper, with 6:4:3 1-butanol–pyridine–water as the solvent[2]. Chromatography scanning was performed with a Packard Model 7201 Radiochromatogram scanner, and liquid scintillation counting, with a Beckman LS-250 liquid scintillation spectrometer. Infrared spectra were recorded with a Beckman IR-33 instrument.

*Epimerization of 2-acetamido-2-deoxy-*D*-mannose* (**2**) *in basic tritium oxide.* — 2-Acetamido-2-deoxy-D-mannose hydrate (200 mg) was dissolved in water (1 mL) containing tritium oxide (13 mCi). The pH was adjusted to 11.4 by the addition of M NaOH, and the solution was kept for 48 h at room temperature. The mixture was made neutral by passing it through a column of Dowex 50-W (H^+) ion-exchange resin (20–40 mesh), and the effluent was evaporated to a syrup that was, several times, dissolved in water and evaporated, to remove any exchangeable tritium. Then ethanol was added; compound **3** crystallized, and was filtered off, washed with acetone, and dried; yield (first crop) 95 mg, m.p. 207–210°. Paper chromatography[2] showed only one component, and paper chromatography scanning revealed only one peak, at the R_F of **1** (R_F 0.14). Liquid scintillation counting showed a specific activity of 0.23 mCi/mmol.

The acetone washings and the mother liquor were combined and evaporated to a syrup. Paper chromatography and paper chromatography scanning showed only two components (**3** and **4**), which were separated by fractional recrystallization from ethanol. An additional 10 mg of **3** was isolated. Compound **4** was isolated from the mother in colorless cubes, 16 mg, m.p. 122–125°. Paper chromatography and paper chromatography scanning of **4** revealed only one component, at the R_F of **2** (R_F 0.06). Liquid scintillation counting showed a specific activity of 0.10 mCi/mmol.

*2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-*D*-[2-^3H]glucopyranose.* — In order to ascertain if tritium had been incorporated, **3** (22.7 mg) was acetylated with acetic anhydride and pyridine[11]. Crystalline 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-[2-^{3}H]glucopyranose was isolated, and crystallized from methanol–ether–hexane, m.p. 133–135° (lit.[11] m.p. of the unlabeled compound 135–136°); ν_{max}^{KBr} 3422 (NH), 1735 (OAc), 1665, and 1510 cm^{-1} (NHAc). Liquid scintillation counting showed a specific activity of 0.27 mCi/mmol.

*2-Amino-2-deoxy-*D*-[2-^3H]glucose hydrochloride.* — 2-Acetamido-2-deoxy-D-

[2-^{3}H]glucose (53 mg) was hydrolyzed by the method of Spivak and Roseman[2], and crystalline 2-amino-2-deoxy-D-[2-^{3}H]glucose hydrochloride (47 mg) was obtained. The i.r. spectrum showed the absence of the amide band at 1620 cm^{-1}, and was identical to the i.r. spectrum of authentic 2-amino-2-deoxy-D-glucose hydrochloride. T.l.c. with 5:5:1:3 pyridine–ethyl acetate–water–acetic acid as the solvent and ninhydrin as the spray reagent showed one spot, R_F 0.62, and t.l.c. scanning showed only one peak, at the R_F value of 2-amino-2-deoxy-D-glucose hydrochloride. Liquid scintillation counting showed a specific activity of 0.21 mCi/mmol.

Epimerization of 2-acetamido-2-deoxy-D-glucose (**1**) *in basic tritium oxide.* — 2-Acetamido-2-deoxy-D-glucose (**1**; 200 mg) was epimerized in basic tritium oxide as before. Compound **3** crystallized first from the reaction mixture (yield 149 mg), and compound **4** was finally separated from the mother liquor (yield 17 mg). Paper chromatography and paper chromatography scanning indicated that the products were pure. Liquid scintillation counting showed the specific activities of **3** and **4** in the ratio 5:2.

Epimerization of 2-acetamido-2-deoxy-D-mannose (**2**) *in neutral solution.* — A solution of **2** (5 mg) in water (0.5 mL) was kept at room temperature, and the reaction was monitored by paper chromatography[2]. After 6 days, two spots, corresponding to **1** and **2**, were visible, whereas a solution of **1** showed only one spot on similar treatment.

ACKNOWLEDGMENTS

The author thanks Dr. U. Hornemann for his keen interest and for making available the facilities for this work, which was supported by research grant CA 14378 from the National Cancer Institute, DHEW, and Professor H. S. El Khadem, Michigan Technological University, for valuable discussions.

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