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Note

Preparation of [2-^{2}H]-2-acetamido-2-deoxy-D-glucose by epimerization of 2-acetamido-2-deoxy-D-mannose in basic deuterium oxide; and proposal of a unifying type of mechanism for the 2-epimerization of 2-acetamido-2-deoxyhexoses

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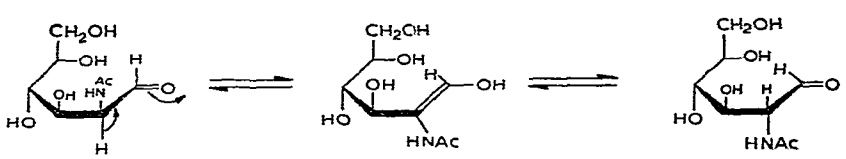
Although it has been known for some time that GlcNAc† and ManNAc are reversibly epimerized in the presence of base[1,2], the mechanism of this epimerization has not been elucidated. For the purpose of studying the epimerization mechanism and attempting to synthesize [2-^{2}H]GlcNAc, we conducted the epimerization of ManNAc to GlcNAc in the presence of D_2O by the method of Spivak and Roseman[2]. The product (GlcNAc) was recovered from the reaction mixture by crystallization, and further purified by acetylation and crystallization. The n.m.r. spectrum of the acetylated GlcNAc is shown in Fig. 1a, along with a spectrum of acetylated, unlabeled GlcNAc (Fig. 1b). These spectra clearly show that deuterium had been incorporated specifically at C-2 of GlcNAc, as evidenced by the collapse of the H-1 doublet, at δ 6.18, to a singlet, and the disappearance of the H-2 quartet at δ 4.45.

To explain the specific labeling by deuterium at C-2, we propose the mechanism shown in Scheme 1. Spivak and Roseman[2] observed the formation of at least three unknown components during the epimerization of GlcNAc to ManNAc. The major, unknown component, isolated by paper chromatography, yielded GlcNAc and



Scheme 1. Mechanism of epimerization of ManNAc⇄ GlcNAc.

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†Abbreviations: GlcNAc, 2-acetamido-2-deoxy-D-glucose; ManNAc, 2-acetamido-2-deoxy-D-mannose; UDP, uridine 5′-diphosphate.

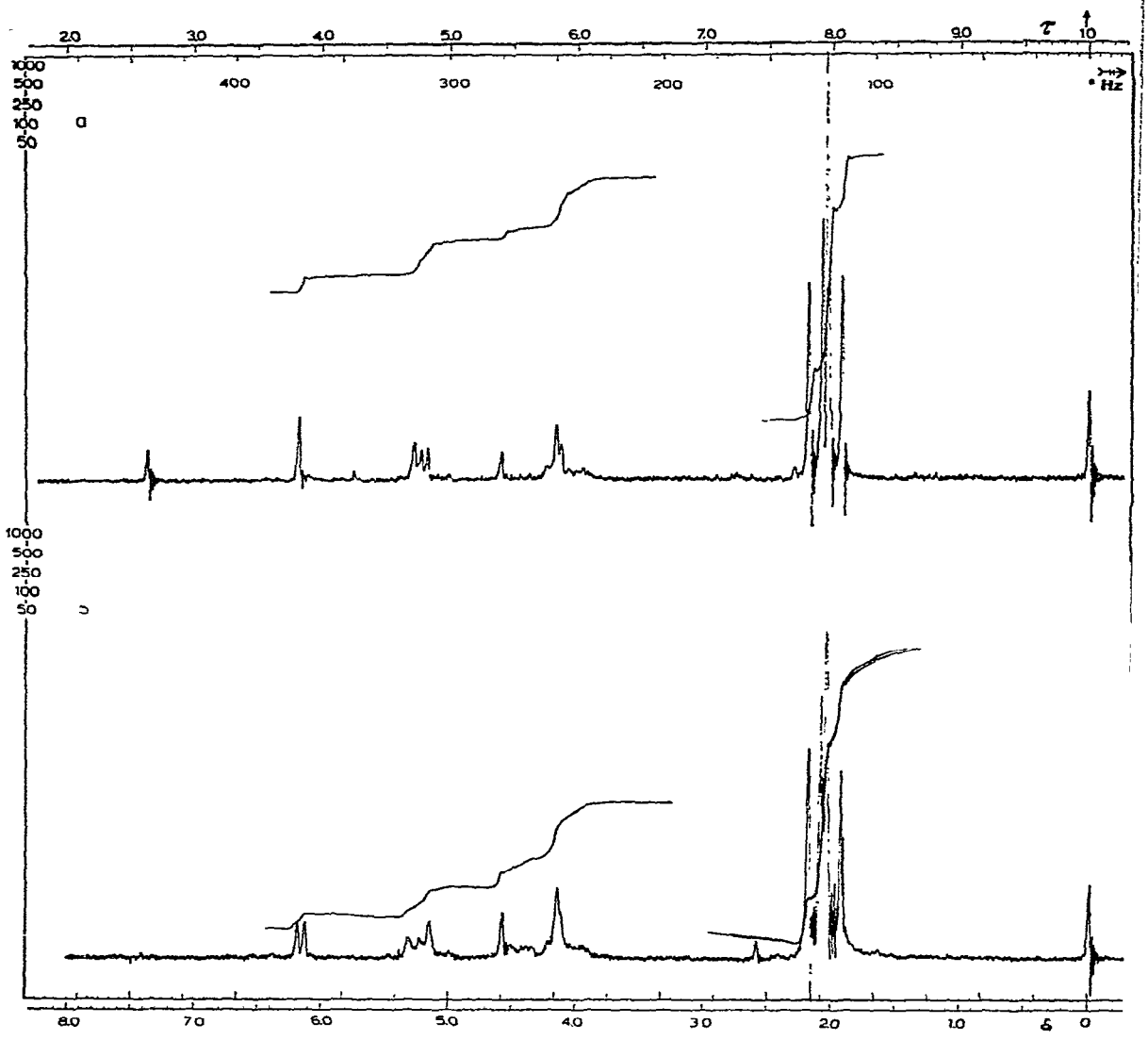
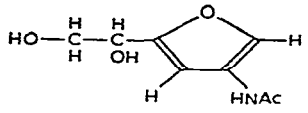


Fig. 1. N.m.r. spectra of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose: (a) prepared from the GlcNAc obtained from the epimerization of ManNAc in basic D_2O; (b) prepared from unlabeled GlcNAc. [The samples were dissolved in $CDCl_3$ and overlayered with D_2O overnight in order to eliminate the NH signals. The sharp peak at δ 4.6 is caused by HOD.]

ManNAc when subjected to the conditions of the epimerization reaction. We did not detect (by the same methods) any components other than GlcNAc and ManNAc in our reaction mixtures, perhaps because of the shorter reaction time (12 h *vs.* 48 h). Later, Spivak-Hardesty[3] suggested that the major, unknown component might be chromogen III, and that it is an intermediate in the epimerization reaction. However, it is difficult to visualize any reasonable mechanism whereby chromogen III can be



Chromogen III

converted into GlcNAc and ManNAc by a base-catalyzed mechanism. Furthermore, were it an intermediate in the epimerization reaction, the epimerization of ManNAc to GlcNAc in D_2O would have resulted in deuterium labeling at C-4 (in addition to C-2), and the n.m.r. spectrum of GlcNAc so labeled would contain a singlet in the region of δ 5.2, instead of a multiplet; clearly, this is not the case. Thus, the mechanism shown in Scheme 1 is adequate to explain the reversible epimerization of ManNAc and GlcNAc.

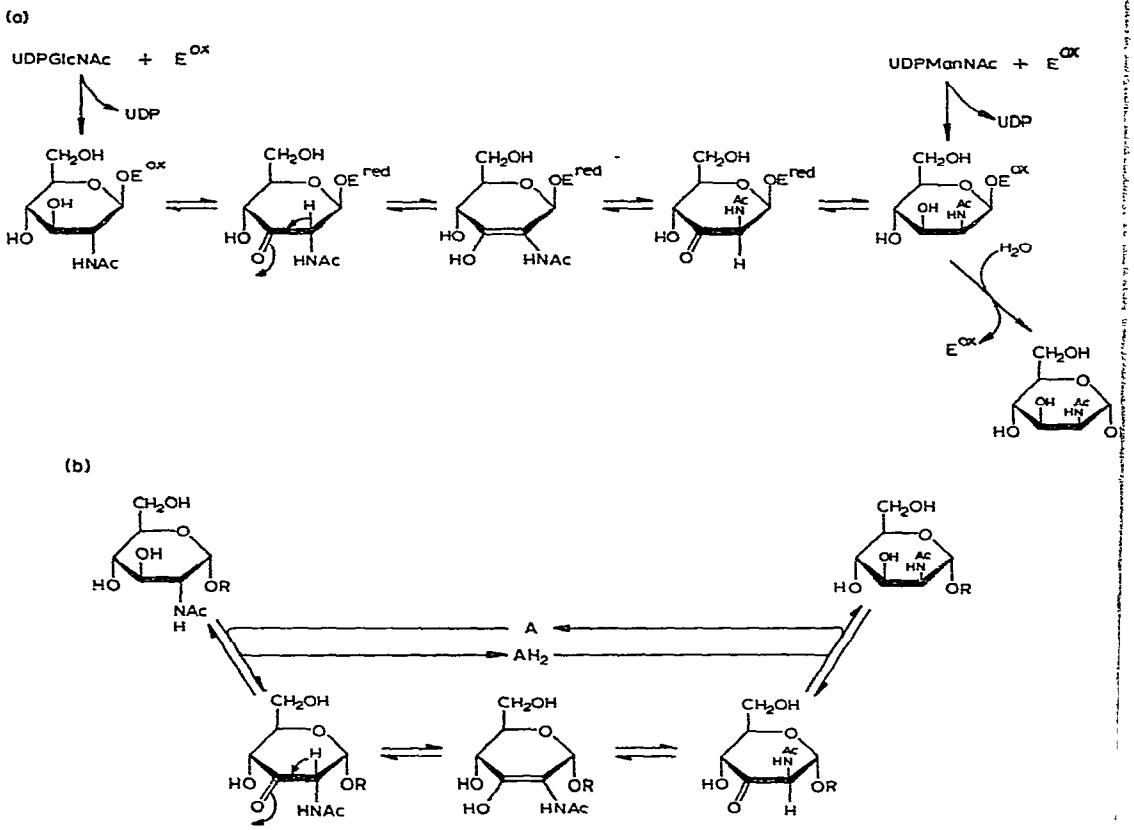
Biologically, GlcNAc and ManNAc are reversibly epimerized by a 2-epimerase found in hog kidney[4], and GlcNAc 6-phosphate and ManNAc 6-phosphate are reversibly epimerized by a 2-epimerase found in bacteria[5]. In addition, UDPGlcNAc is acted upon enzymically by* UDPGlcNAc 2-epimerase (hydrolyzing)[6,7], to yield ManNAc plus UDP, and by UDPGlcNAc 2-epimerase[8] to give UDPManNAc. The mechanisms for these enzyme-catalyzed reactions are not known, but Ghosh and Roseman[5] suggested an enolamine intermediate in the epimerization of GlcNAc and GlcNAc 6-phosphate, and two mechanisms have been proposed for UDPGlcNAc 2-epimerase (hydrolyzing)[9,10].

In 1970, Salo and Fletcher[9] proposed that UDPGlcNAc 2-epimerase (hydrolyzing) catalyzes its reaction by the mechanism shown in Scheme 2a. We now propose that UDPGlcNAc 2-epimerase catalyzes its reaction by an analogous mechanism (see Scheme 2b). We also propose, in support of the earlier suggestion of Ghosh and Roseman[5], that GlcNAc 2-epimerase from hog kidney and GlcNAc 6-phosphate 2-epimerase from bacteria catalyze their respective reactions by the mechanism shown in Scheme 1. Thus, all the known reactions involving the 2-epimerization of 2-acetamido sugars can be accounted for through a unifying type of mechanism involving a tautomerization reaction between the proton on the carbon atom being epimerized and a neighboring carbonyl group.

EXPERIMENTAL

Epimerization of ManNAc to GlcNAc in deuterium oxide. — The method used was similar to that of Spivak and Roseman[2]. ManNAc (1 g; Pfanstiehl) was equilibrated overnight with D_2O (99.7% deuterium; from New England Nuclear), and the solution then lyophilized; this was repeated three times, in order to exchange all of the

*In light of a recent paper by Kawamura *et al.*[8], it seems appropriate and consistent to call the enzyme that catalyzes the reaction UDPGlcNAc⇆UDPManNAc by the name UDPGlcNAc 2-epimerase; and call the enzyme that catalyzes the reaction UDPGlcNAc→UDP+ManNAc by the name UDPGlcNAc 2-epimerase (hydrolyzing).



Scheme 2. Proposed mechanism for the epimerization reactions catalyzed by UDPGlcNAc 2-epimerases. [(a) UDPGlcNAc 2-epimerase (hydrolyzing); (b) UDPGlcNAc 2-epimerase. The letter E signifies the enzyme, namely, 2-epimerase (hydrolyzing); and the letter R denotes uridine 5′-pyrophosphoryl.]

hydroxyl protons. The deuterated ManNAc was then dissolved in D_2O (3.5 ml), and M NaOD (prepared by treating sodium with D_2O) was added to a pD of 11.4. A n.m.r. spectrum was recorded immediately and every 4 h, in order to monitor the deuterium incorporation. Initially, two doublets, at δ 5.11 and 5.23, were observed, corresponding to H-1 of the β and α anomers of ManNAc, respectively. After 8 h, these doublets had collapsed to singlets at δ 5.35 (H-1 of GlcNAc, α anomer) and at δ 4.8 (H-1 of GlcNAc; β anomer hidden by the HOD peak, but observable on cooling, because of a downfield, chemical shift of the HOD peak). Small singlets at δ 5.1 and 5.2 for H-1 of ManNAc (β and α anomers) were also observed.

After 12 h, the reaction was stopped by addition of acetic acid. The reaction mixture was de-ionized by passage through a column of Dowex 50-W (H^+) ion-exchange resin, and evaporated to a syrup. The resulting GlcNAc was crystallized from ethanol–acetone (yield: 1st crop, 0.56 g; chromatography[1] showed only a trace of ManNAc).

Acetylation of GlcNAc to 2-acetamido-1,3,4,6 tetra-O-acetyl-2-deoxy-α-D-glucopyranose. — In order to ascertain if deuterium had been incorporated into the GlcNAc, and, if so, the position of labeling, the GlcNAc was acetylated with pyridine–acetic anhydride. Crystalline 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose was obtained (m.p. 135–137°, yield 0.85 g). The n.m.r. spectrum obtained for this compound is shown in Fig. 1a. The product contained a slight proportion of β anomer, as evidenced by the singlet at δ 5.75.

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