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Note

Studies of the epimerization of 2-acetamido-2-deoxy-D-glucopyranose : selective deuteration at C-2 of 2-acetamido-2-deoxyaldoses

MICHAEL HANCHAK AND WALTER KORYTNYK*

Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, New York 14263 (U. S. A.)

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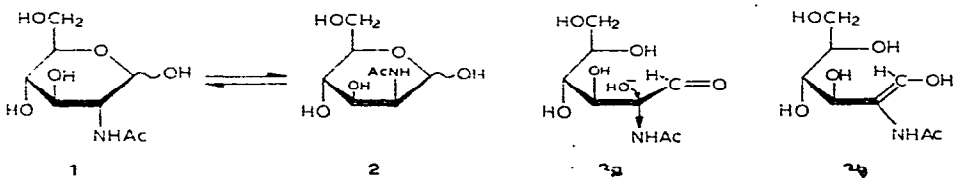
In connection with our studies of the synthesis of D-mannosamine analogs as potential inhibitors of sialic acid biosynthesis[1], we have investigated the alkali-catalyzed epimerization of 2-acetamido-2-deoxy-D-glucose analogs to the corresponding *manno* epimers. Kuhn *et al.*[2,3] and Spivak and Roseman[4] have described the epimerization of 2-acetamido-2-deoxy-D-glucose (**1**), from which, in addition to 2-acetamido-2-deoxy-D-mannose (**2**), unidentified components were isolated[4]. Coxon and Hough[5] extended the epimerization to 2-acetamido-2-deoxy-D-pentoses, and proposed a mechanism involving ring opening to give **3a**, followed by the enolization at H-2 (**3b**), which is accelerated by the inductive effect of the acetamido group. This is followed by ring closure to give a mixture of epimers.



1 2 3a 3b

In order to determine whether this mechanism has validity and whether any additional products of the reaction are present, we used ^{13}C- and ^{1}H-n.m.r. spectroscopy, as well as gas-liquid chromatography. When the isomerization was performed in water solution at pH 11 for 48 h, the ^{13}C-n.m.r. spectrum of the product could be explained by the presence of the anomeric pairs of the two epimers **1** and **2** and, thus, the formation of any by-product in the anomerization reaction is unlikely (Fig. 1a). The ^{13}C-spectra of the anomeric pairs **1** and **2** have been assigned by Bundle *et al.*[6]. When deuterium oxide replaced water for the epimerization reaction, the C-2 resonances in both epimers were found to be replaced by multiplets of low intensity, indicating selective deuteration at these positions (Fig. 1b). The require-

*To whom correspondence should be addressed.

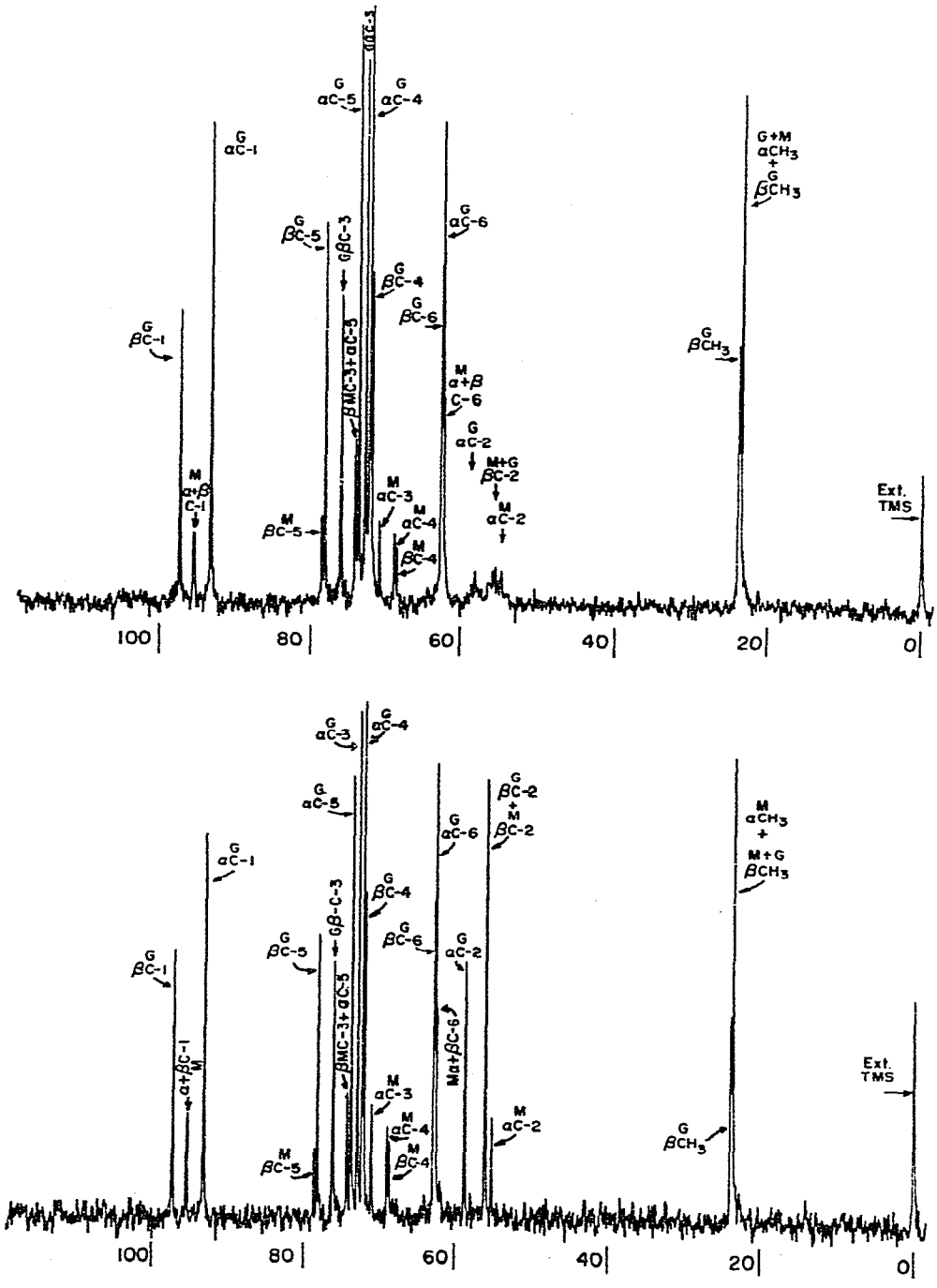


Fig. 1. (a) Lower spectrum: ^{13}C-N.m.r. spectrum of a mixture of 2-acetamido-2-deoxy-D-glucopyranose (**1**) and 2-acetamido-2-deoxy-D-mannopyranose (**2**) obtained by the epimerization of **1** in H_2O. (b) Upper spectrum: Same as spectrum (a), except that the epimerization was performed in D_2O, and shows collapsed C-2 peaks G, 2-acetamido-2-deoxy-D-glucose; M, 2-acetamido-2-deoxy-D-mannose.

ment of ring-opening has been indicated by failure to effect either deuteration or epimerization of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside. Thus, our studies confirm the mechanism proposed[5].

The two epimers were separated by ion-exchange chromatography and crystallization, and the extent of deuteration was determined by the difference between integrals of the unresolved proton peaks of the deuterated and undeuterated compounds. The extent of deuteration of **1** has been estimated as being 80–92%, and that of **2** as 91–95%. *O*-Acetylated derivatives of **1** and **2** increased somewhat the precision of integration on account of improved resolution, and values of 95±1% for **2** have been obtained.

Finally, the 1,3,4,6-tetra-*O*-acetyl-2(*N*-acetylacetamido)-2-deoxy-α-D-glucopyranoses of deuterated and nondeuterated **1** have been prepared by the procedure of Inch *et al.*[7]. In the nondeuterated derivative, the C-2 proton is well separated, and resonates at δ 4.66. In the deuterated compound, no peak could be detected in this region of the spectrum. Accordingly, the deuteration of the C-2 carbon is selective and almost quantitative, thus allowing the preparation of selectively deuterated (or tritiated) 2-acetamido-2-deoxyaldoses.

The extent of the epimerization was followed by ^{13}C-n.m.r. spectroscopy, and was based on the height of the C-5 peaks. This presupposes a similarity of the NOE of the corresponding ^{13}C resonances for the two epimers. The mixture was found to consist of 4 parts of the *gluco* anomer and 1 part of the *manno* anomer. A prepared mixture of the 2-epimers in the same proportions confirmed the validity of this estimate.

The degree of the epimer formation has also been followed by gas–liquid chromatography. The epimerization mixture was converted into the per *O*-(trimethylsilyl) derivatives, which were separated on a Chromsorb G column coated with 1.5% OV-17 silicone oil, and were quantitatively determined by weighing the cut-out peaks[8]. The extent of epimerization was found to be 19% for 2-acetamido-2-deoxy-D-glucose and 80% for 2-acetamido-2-deoxy-D-mannose. Both values are in good agreement with ^{13}C determinations and with previous estimates[4,8]. The convenient and highly regiospecific introduction of deuterium (or tritium) at C-2 of 2-acetamido-2-deoxyaldoses should be useful in biochemical studies, *e.g.* in studies of the mechanism of enzymic epimerization reactions[9].

EXPERIMENTAL

General. — ^{13}C- and ^{1}H-n.m.r. spectra were determined on a Varian XL-100-12A spectrometer operating in the Fourier-transform mode. G.l.c. was performed with a Packard 7400 series gas chromatograph equipped with a glass column (7 mm × 2 m) containing Chromsorb G coated with 1.5% OV-17 silicone oil (Applied Science Laboratories, Inc., State College, PA 16801) and with an H_2 flame-detector.

*2-Acetamido-2-deoxy-*D*-[2-^{2}H]glucopyranose* (**1**) *and 2-acetamido-2-deoxy-*D*-[2-^{2}H]mannopyranose* (**2**). — 2-Acetamido-2-deoxy-D-glucopyranose (2.0 g) was

dissolved in D_2O (10 ml). The pH was adjusted to 11.0 by the addition of 10M NaOD, and the solution was kept at room temperature for 72 h, after which the pH was 10.8. The solution was neutralized with Dowex 50W-8X (H^+) ion-exchange resin, filtered, and washed with H_2O. The solution was evaporated to reduce the volume, and chromatographed on a Dowex 50W-8X (H^+), the fractions being monitored by t.l.c. in 2:1 (v/v) chloroform–methanol; R_F of **1**, 0.37, R_F of **2**, 0.42. Fractions rich in **2** were combined, and the water was removed *in vacuo*. The residue was extracted several times with hot ethanol. The ethanol extracts were combined, and were allowed to crystallize overnight. The filtrate was evaporated, and the residue was crystallized from 1:1 (v/v) ethanol–acetone and from water–acetone. The yield of **2** was 305 mg (15%), m.p. 126–128° (lit.[10]: m.p. 127–128°). An additional amount of **2** was obtained from the earlier fractions, which were evaporated and extracted with ethanol. After being kept for 24 h at room temp., **1** was filtered off. The mother liquor was evaporated and the residue was again extracted with hot ethanol. The extracts contained mainly **2**; recrystallization gave 78 mg of pure **2**, increasing the yield to 18.7%. The combined residues from initial extraction were recrystallized from ethanol to yield 1.46 g (73%). The purity of **1** and **2** was shown by both ^1H- and ^{13}C-n.m.r. spectroscopy, and by gas chromatography.

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