Carbohydrate Research, 135 (1985) 342–347
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

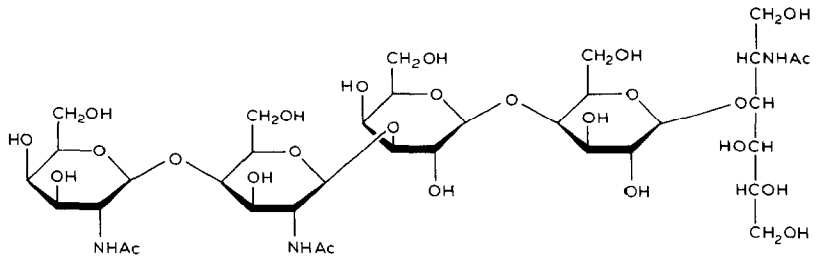
A mechanistic consideration of the hydrazinolysis of 3-*O*-substituted 2-acetamido-2-deoxy-D-hexopyranosyl and -D-hexitol residues in the absence and presence of hydrazinium sulfate

YASUO INOUE AND KEN KITAJIMA
Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113 (Japan)
(Received June 19th, 1984; accepted for publication, August 10th, 1984)

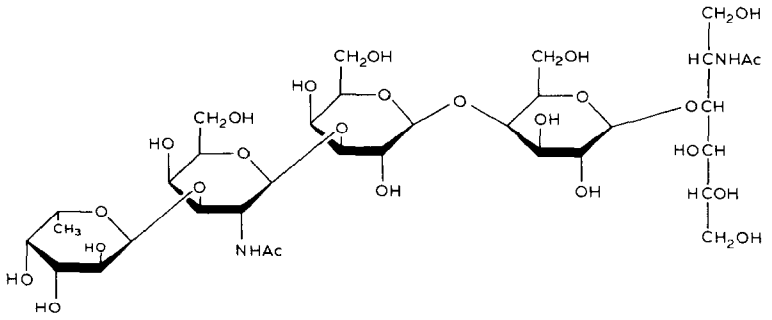
The hydrazinolysis–nitrous-deamination procedure is a useful method for the selective cleavage of 2-acetamido-2-deoxy-D-hexopyranosyl linkages of oligo- and poly-saccharides[1,2]. The facility with which *N*-deacetylation of 2-acetamido-2-deoxy-D-gluco- and/or -galacto-pyranosyl residue(s) occurs depends on the position of substituents attached thereto. Indeed, 3-*O*-substituted 2-acetamido-2-deoxy-D-hexopyranosyl residues are resistant to hydrazinolysis and require vigorous conditions for complete *N*-deacetylation[3,4]. However, no detailed mechanistic consideration of this reaction has been made, and we now report on the hydrazinolysis of the oligosaccharide alditols β-D-GalNAc-(1→4)-β-D-GalNAc-(1→3)-β-D-Gal-(1→4)-β-D-Gal-(1→3)-D-GalNAc-ol (**1**) and α-L-Fuc-(1→3)-β-D-GalNAc-(1→3)-β-D-Gal-(1→4)-β-D-Gal-(1→3)-D-GalNAc-ol (**2**), and the factors affecting the reactivity of the 2-acetamido-2-deoxy-D-galactopyranosyl and 2-acetamido-2-deoxy-D-galactitol residues.

Compounds **1** and **2** were treated with anhydrous hydrazine in the absence of hydrazinium sulfate at 105° for 24 h, and subsequently with sodium nitrite, and the products were subjected to column chromatography on Bio-Gel P-2. The percentage recoveries of the original monosaccharide residues are shown in Table I. Thus, total *N*-deacetylation occurred of the non-reducing terminal 2-acetamido-2-deoxy-D-galactopyranosyl group and the penultimate 4-*O*-substituted 2-acetamido-2-deoxy-D-galactopyranosyl residue of **1**, and *N*-deacetylation of the terminal 2-acetamido-2-deoxy-D-galactitol residue was incomplete. With **2**, neither the penultimate 3-*O*-substituted 2-acetamido-2-deoxy-D-galactopyranosyl residue nor the terminal 3-*O*-substituted 2-acetamido-2-deoxy-D-galactitol residue was completely *N*-deacetylated. 2-Acetamido-2-deoxy-D-galactitol was completely *N*-deacetylated under the same conditions, and quantitative *N*-deacetylation of all the compounds studied occurred on treatment with hydrazine–hydrazinium sulfate

0008-6215/85/$ 03.30



1



2

(data not shown)[5]. The recoveries of the 3-*O*-substituted 2-acetamido-2-deoxy-D-galactitol residue after hydrazinolysis ranged from 30 to 40% and the 3-*O*-substituted 2-acetamido-2-deoxy-D-galactopyranosyl residue seems to be slightly less reactive than the corresponding alditol.

There are many examples of demonstrated or presumed hydrazinolysis reactions in 2-acetamido-2-deoxy-D-hexopyranose-containing oligo- and polysaccharides[6–11]. Examples of incomplete hydrazinolysis have been reported, where substitution at position 3 results in a decrease in the rate of *N*-deacetylation[4]. The suggested participation[12] of HO-3 in the rate-determining step fails to explain why

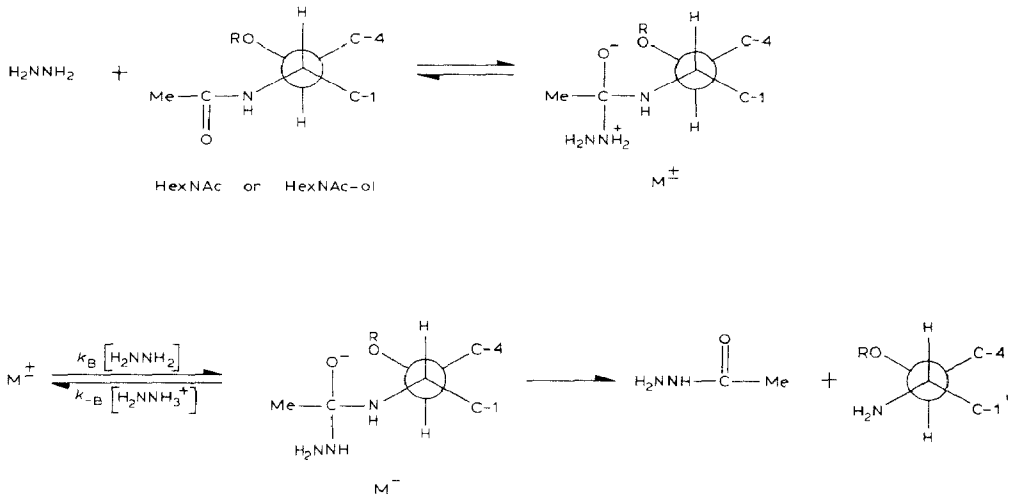
TABLE I

RECOVERIES (%) OF THE CONSTITUENT MONOSACCHARIDE RESIDUES AFTER HYDRAZINOLYSIS AND NITROUS DEAMINATION[a]

Compound	*Galactose*	*Fucose*	*2-Acetamido-2-deoxygalactose*	*2-Acetamido-2-deoxygalactitol*
1	100	—	0	41
2	100	100	50	29

[a]Data are expressed relative to galactose (set at 100%).

a retarding effect of the substitution is also observed with 3-*O*-substituted 2-acetamido-2-deoxy-D-hexitols since these compounds have a hydroxyl group vicinal to the acetamido group.

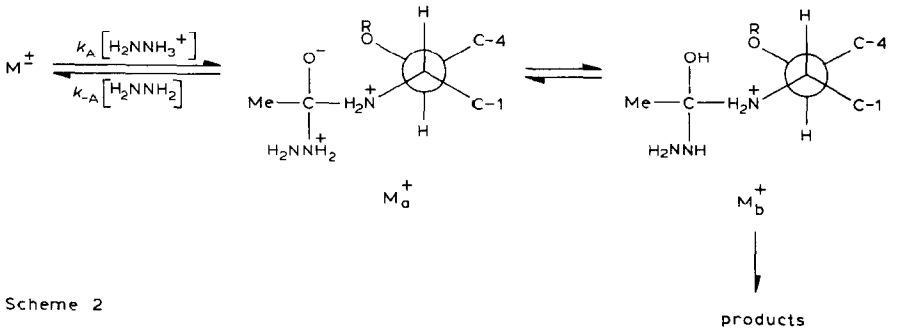


Scheme 1

According to the simplest mechanism of hydrazinolysis of 2-acetamido-2-deoxy-D-hexopyranoses (HexNAc) and 2-acetamido-2-deoxy-D-hexitols (HexNAc-ol) (Scheme 1), the formation of the unstable tetrahedral intermediate ($M^{\pm}$) is rapid and reversible because this zwitterion lacks the resonance stabilisation[13,14] from the lone-pair of electrons on nitrogen. In the hydrazinolysis of HexNAc or HexNAc-ol, which has a relatively poor leaving-group of $pK > 14$ (*e.g.*, $GalNH_2 + H_2O \rightleftarrows GalNH^- + H_3O^+$), in order to avoid expulsion of hydrazine from $M^{\pm}$ and thereby generate the starting material, $M^{\pm}$ must react with a second molecule of hydrazine in a rate-determining removal of a proton to form the anionic intermediate (M^-), which rapidly breaks down to products.

The resistance of 3-*O*-substituted 2-acetamido-2-deoxy-D-hexopyranosyl towards anhydrous hydrazine, previously ascribed[12] to the absence of HO-3 and lack of intramolecular catalysis, is more likely to be due to steric hindrance of the reaction of $M^{\pm}$ with a second molecule of hydrazine. The crowding of OR (R = galactopyranosyl in **1**, and galactopyranosyl or L-fucopyranosyl in **2**) and the neighboring tetrahedral-adduct disturbs the general-base catalysis. Inspection of space-filling CPK models of $M^{\pm}$ indicates a considerable crowding around the reaction centre when HO-3 is substituted by a glycosyl residue.

Hydrazinolysis in the presence of hydrazinium sulfate results in more or less quantitative *N*-deacetylation[15,16], and the simplest mechanism that is consistent with the observation involves general-acid-catalysed proton transfer (Scheme 2) in which rate-determining proton donation to $M^{\pm}$ gives M_a^+, which rapidly breaks down to hydrazide *via* M_b^+. So long as the catalysis is observed, lifetimes of the



Scheme 2

tetrahedral intermediates must be in a certain limited range[17], *i.e.*, not long enough to allow equilibration with respect to proton transfer but not too short to allow their existence. The direct expulsion of a protonated amine from the intermediate M_a^+ will be energetically unfavorable because of the formation of the unstable protonated hydrazide $CH_3CON^+H_2NH_2$ ($pK \sim -3$; *cf.* $pK \sim 3.2$ for $CH_3CONHN^+H_3$). The breakdown of M^+ will then proceed by the energetically more-favorable pathway through M_b^+, in which there is a strong driving force for breakdown from the electron-pairs on the oxygen and nitrogen[17] and also because there is a much better leaving-group than M^-. The catalytic effect of hydrazinium ion on hydrazinolysis of the 3-*O*-substituted 2-acetamido-2-deoxy-D-hexopyranoses or -hexitols thus offsets the retarding effect of steric hindrance. A fuller understanding of the relative importance of these factors must await an investigation of more highly substituted 2-acetamido-2-deoxy-D-hexopyranose derivatives.

The resistance of 3-*O*-substituted 2-acetamido-2-deoxy-D-galactitol to hydrazinolysis in the absence of hydrazinium sulfate has been utilised in the structural analysis of certain oligosaccharide-alditols[6].

EXPERIMENTAL

Materials. — Two pentasaccharide-alditols **1** and **2** were prepared[6,18] from polysialoglycoproteins isolated from the eggs of rainbow trout. Other materials were of analytical grade.

Hydrazinolysis and nitrous-deamination procedure. — The oligosaccharide **1** or **2** (~1 mg) was heated[2] in anhydrous hydrazine (0.3 mL) in a sealed Pyrex-tube at 105° for 24 h. The cooled tube was opened, excess of hydrazine was largely evaporated under nitrogen, and the residue was stored in a vacuum desiccator over conc. sulfuric acid. To a solution of the residue in 0.5M acetic acid (1 mL) was added sodium nitrate (5 mg), and the solution was left for 3 h at room temperature. Ethylamine (5~6 drops) was added to decompose the excess of nitrous acid, the pH of the solution was adjusted to >9 with 0.01M NaOH, and sodium borohydride (5 mg) was added. The solution was kept for 2 h at room temperature and then

acidified with 0.5M acetic acid, and boric acid was removed by co-distillation with methanol.

The product mixture from **1** was eluted from a column (1.6 × 132 cm) of Bio-Gel P-2 with 0.05M pyridinium acetate bufer (pH 5.0) [the fraction volume was 2.5 mL; the flow rate was 15 mL/h]. The eluate was monitored by the phenol–sulfuric acid method[19]. Two partially resolved, major fractions (65–70 and 71–81) and two minor fractions (82–86 and 96–100) were recovered. The first major fraction was homogeneous by t.l.c. [Kieselgel 60 (Merck); ethyl acetate–pyridine–acetic acid–water, 5:5:1:3, 2.5 h] and had the composition $(Gal)_2$GalNAc-ol (yield ~41%). The second major fraction (71–81) was resolved by t.l.c. into at least two galactose-containing components none of which contained GalNAc nor GalNAc-ol. Accordingly, in view of the position of elution and the reaction sequence (hydrazinolysis–nitrous deamination), it can be presumed that these are Gal–Gal–X where X is deaminated galactosaminitol.

Similarly, the product mixture obtained from **2** was fractionated on a column (1.6 × 132 cm) of Bio-Gel P-2 with 0.05M pyridinium acetate buffer (pH 5.0); the fraction volume was 2.5 mL. At least four partially resolved peaks were detected. The first major peak (fractions 61–65) was Fuc–GalNAc–Gal–Gal–X (yield, ~28%), the second major peak (fractions 52–60) was **2** (yield, ~22%), and the trisaccharide eluted as the third peak (fractions 66–69) was Gal–Gal–GalNAc-ol (yield, ~8%). The other minor peaks (fractions 70–78 and 79–87) were not characterised, because the amounts were insufficient for quantification or they lacked GalNAc or GalNAc-ol. The analytical data for the combined fractions are given in Table I.

In the above experiments, Gal, GalN, GalN-ol, and Fuc were analysed[20,21] by g.l.c.

REFERENCES

1 B. Bayard and J. Montreuil, *Colloq. Int. C.N.R.S.*, 221 (1974) 209–218.
2 G. Strecker, A. Pierce-Cretel, B. Fournet, G. Spik, and J. Montreuil, *Anal. Biochem.*, 111 (1981) 17–26.
3 M. Fujinaga and Y. Matsushima, *Bull. Chem. Soc. Jpn.*, 39 (1966) 185–190.
4 B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, *Carbohydr. Res.*, 29 (1973) 451–457.
5 G. O. Aspinall, M. M. Gharia, and C. O. Wong, *Carbohydr. Res.*, 78 (1980) 275–283.
6 M. Iwasaki, S. Inoue, K. Kitajima, H. Nomoto, and Y. Inoue, *Biochemistry*, 23 (1984) 305–310.
7 B. A. Dmitriev, Yu. A. Knirel, and N. K. Kochetkov, *Carbohydr. Res* , 40 (1975) 365–372.
8 J. M. Williams, *Adv. Carbohydr. Chem. Biochem.*, 31 (1975) 73–77.
9 C. L. Reading, E. E. Penhoet, and C. E. Ballou, *J. Biol. Chem.*, 253 (1978) 5600–5612.
10 P. N. Shaklee and H. E. Conrad, *Biochem. J.*, 217 (1984) 187–197.
11 M. Shimamura, T. Endo, Y. Inoue, and S. Inoue, *Biochemistry*, 22 (1983) 959–963.
12 M. S. Saeed and J. M. Williams, *Carbohydr. Res.*, 84 (1980) 83–94.
13 W. P. Jencks, *Catalysis in Chemistry and Enzymology*, McGraw–Hill, New York, 1969, Chap. 10.
14 A. C. Satterthwait and W. P. Jencks, *J Am. Chem. Soc.*, 96 (1974) 7018–7031
15 J. H. Bradbury, *Nature (London)*, 178 (1956) 912–913.
16 Z. Yosizawa, T. Sato, and K. Schmid, *Biochim. Biophys. Acta*, 121 (1966) 417–419.
17 M. I. Page and W. P. Jencks, *J. Am. Chem. Soc.*, 94 (1972) 8828–8838.

18 M. IWASAKI, H. NOMOTO, K. KITAJIMA, S. INOUE, AND Y. INOUE, *Biochem. Int.*, 8 (1984) 573–580.
19 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
20 V. N. REINHOLD, *Methods Enzymol.*, 25B (1972) 244–249.
21 B. KOZULIĆ, B. RIES, AND P. MILDNER, *Anal. Biochem.*, 94 (1979) 36–39.