

## Deamination of Methyl 2-Amino-2-deoxy- $\alpha$ - and - $\beta$ -D-glucofuranosides

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Deamination of methyl 2-amino-2-deoxy- $\alpha$ -D-glucofuranoside yields a mixture (3:1) of 2,5-anhydro-D-mannose and methyl 2-deoxy-2-C-formyl-D-*arabino*-pentofuranoside. The latter substance is readily epimerized to the D-*ribo*-isomer. Analogous results are obtained in the  $\beta$ -series.

The deamination of 2-amino-2-deoxy-D-glucose was first investigated by Ledderhose in 1880,<sup>1</sup> although it was not until later that the product was identified as 2,5-anhydro-D-mannose. This reaction and the deamination of 2-amino-2-deoxy-D-glucofuranosides have subsequently been studied by several groups, and have recently been reviewed by Defaye.<sup>2</sup> The deamination reaction has also been used for the controlled degradation of polysaccharides (*e.g.* Ref. 3) and glycoproteins<sup>4</sup> containing 2-amino-2-deoxy-D-glucofuranose residues. With the aim of using this reaction for the same purpose, we have reinvestigated the deamination of methyl 2-amino-2-deoxy- $\alpha$ - and - $\beta$ -D-glucofuranoside.

### RESULTS AND DISCUSSION

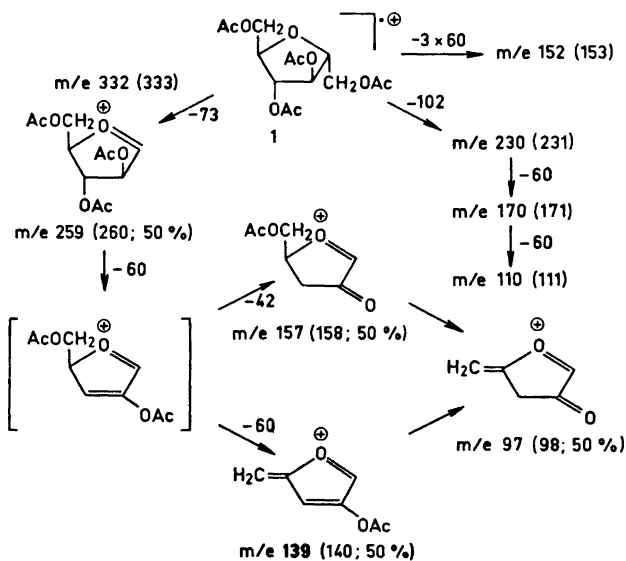
The deamination was performed with sodium nitrite at pH 3.5 and the product was reduced with sodium borohydride or sodium borodeuteride. The reduced product was acetylated before the analysis by GLC-MS. The results are shown in Table 1. The 2,5-anhydro-D-mannitol tetra-acetate (1) gave the expected MS and NMR-spectrum. The origins of the major fragments in the MS are indicated in Scheme 1. (Figures in brackets refer to the compound monodeuteriated at C-1.)

On deamination, followed by reduction and acetylation, the  $\alpha$ - and  $\beta$ -glycoside each yielded two components which were faster, on GLC, than 1. The MS of these four components were almost identical. The fragmentation

Table 1. Products formed by deamination and reduction of methyl 2-amino-2-deoxy- $\alpha$ - and - $\beta$ -D-glucopyranosides.

Products	<i>T</i> -value <sup>a</sup>		Yield (mol %)	
	OV-225	ECNSS-M	Me- $\alpha$ -GNH <sub>2</sub> <sup>b</sup>	Me- $\beta$ -GNH <sub>2</sub>
2,5-Anhydro-D-mannitol	1.51	1.95	73	84
Methyl 2-deoxy-2- <i>C</i> -hydroxymethyl- $\alpha$ -D- <i>ribo</i> -pentofuranoside	0.69	0.79	27	
Methyl 2-deoxy-2- <i>C</i> -hydroxymethyl- $\alpha$ -D- <i>arabino</i> -pentofuranoside	0.69	0.85		
Methyl 2-deoxy-2- <i>C</i> -hydroxymethyl- $\beta$ -D- <i>ribo</i> -pentofuranoside	0.74	0.90		16
Methyl 2-deoxy-2- <i>C</i> -hydroxymethyl- $\beta$ -D- <i>arabino</i> -pentofuranoside	0.76	0.99		

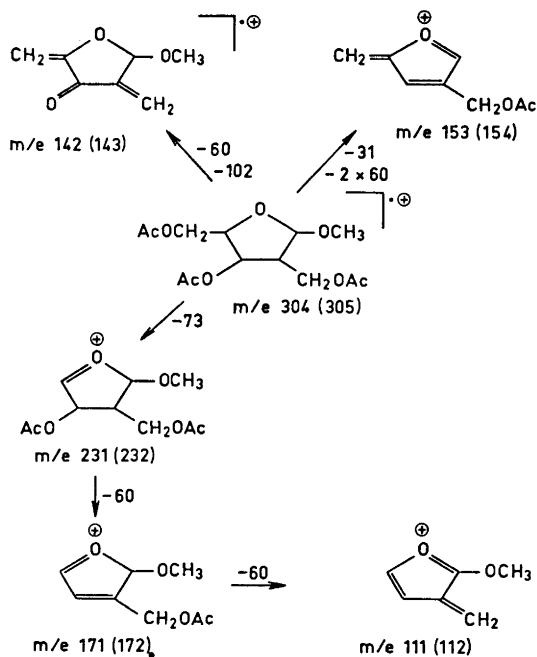
<sup>a</sup> *T*-values for the corresponding sodium borohydride-reduced acetates relative to L-fucitol pentaacetate. <sup>b</sup> Me- $\alpha$ -GNH<sub>2</sub> = Methyl 2-amino-2-deoxy- $\alpha$ -D-glucopyranoside.



Scheme 1.

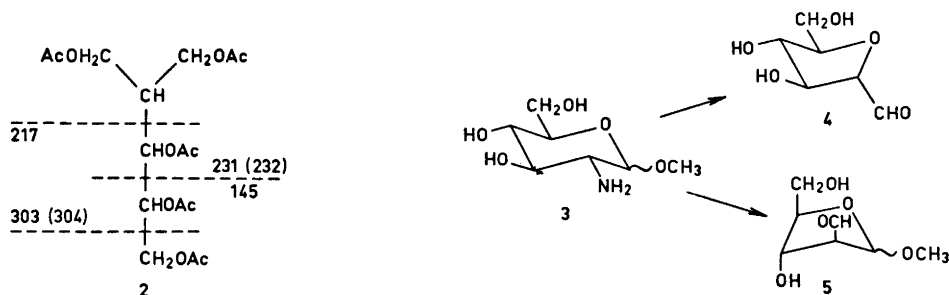
pattern is identical with that expected for an acetylated methyl 2-deoxy-2-*C*-hydroxymethyl-pentofuranoside (Scheme 2). (Figures in brackets refer to the compound monodeuteriated at the 2-*C*-acetoxyethylgroup.)

The mixture of the two isomeric methyl  $\alpha$ -2-deoxy-2-*C*-hydroxymethyl-pentofuranoside acetates was isolated by chromatography on silicic acid. The



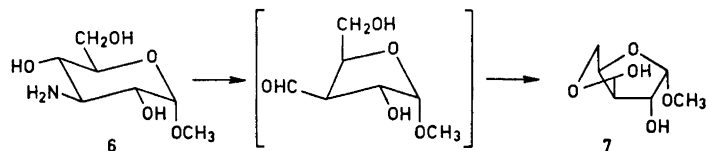
Scheme 2.

NMR spectrum of this mixture showed the presence of acetoxy protons, methoxy protons, and other protons in the relative proportions 9:3:8 in agreement with the postulated structures. Part of the isolated mixture was hydrolysed, reduced with sodium borohydride or sodium borodeuteride and acetylated. GLC revealed only one alditol-acetate (ECNSS-M or OV 225). The MS demonstrated that it was a fully acetylated 2-deoxy-2-C-hydroxymethyl-pentitol (2). The primary fragmentation of the compound is indicated in formula 2.

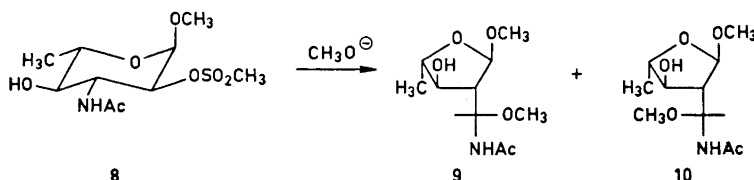


The formation of these products is analogous to the deamination of amino-cyclohexane derivatives, wherein a stereospecific rearrangement resulting from the attack on the diazonium ion by the group *trans* and antiparallel to this is observed.<sup>5</sup> For the methyl 2-amino-2-deoxy-D-glucopyranosides (3), attack by the ring oxygen gives 2,5-anhydro-D-mannose (4), whereas attack by the carbon atom, C-4, with retention of the configuration, gives methyl 2-deoxy-2-C-formyl-D-*arabino*-furanoside (5).

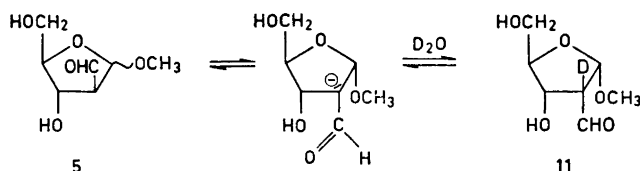
The latter reaction is similar to the deamination of methyl 3-amino-3-deoxy- $\alpha$ -D-glucopyranoside (6) yielding methyl 3-deoxy-3-C-formyl- $\alpha$ -D-*xylo*-furanoside which cyclises to the cyclic hemiacetal 7.<sup>6</sup>



When methyl 3-acetamido-3,6-dideoxy-2-*O*-methanesulphonyl- $\alpha$ -L-glucopyranoside (8) was heated with methoxide, a similar ring contraction, yielding compounds 9 and 10, was observed.<sup>7</sup>



For both the  $\alpha$ - and the  $\beta$ -anomer of 3 the component 5 after reduction consisted of 2 isomers. When the deamination and the reduction of either the  $\alpha$ - or  $\beta$ -isomer were performed in  $D_2O$  using sodium borodeuteride as reducing agent, MS showed that the minor slower moving isomer contained two deuterium atoms. It therefore seems probable that the slower moving isomer is formed by epimerization of the *D-arabino*-isomer into the *D-ribo*-isomer ( $5 \rightarrow 11$ ). This epimerization probably occurs during the borodeuteride reduction alkaline conditions, but does not reach equilibrium, as only small amounts of deuterium are incorporated in 5. The total amount of the two isomers was virtually constant but their relative proportions varied in different experiments. The minor component accounted for between 10 and 40 % of the total amount.



The formation of *1* (73 %) and *3* (27 %) (including epimerized component) by deamination of methyl 2-amino-2-deoxy- $\alpha$ -D-glucopyranoside accounted for all the starting material. The corresponding figures for the  $\beta$ -series were 84 % of *1* and 16 % of *3* (including epimerized component).

When the deamination reaction is applied to the controlled degradation of a polysaccharide containing 2-amino-2-deoxy-D-gluco-pyranose residues, the reaction will presumably follow the same two routes as observed for the low-molecular weight glycosides. The 2-amino-2-deoxy-D-glucopyranosidic linkages will, however, only be cleaved by the major pathway. This should not diminish the value of the method as the linkages of the 2-deoxy-2-*C*-formyl-furanose residues, or the corresponding reduced residues, are labile to acid hydrolysis and will be cleaved under conditions which leave ordinary glycosidic linkages virtually unaffected.

### EXPERIMENTAL

*General methods.* Concentrations were performed under diminished pressure, at bath temperatures not exceeding 40°. For GLC, a Perkin-Elmer 900 instrument, equipped with flame-ionisation detectors, was used. The separations were performed on glass columns (180  $\times$  0.15 cm) packed with (a) 3 % OV 225 on Gas Chrom Q (100–200 mesh), (b) 3 % ECNSS-M on Gas Chrom Q (100–200 mesh) or (c) 3 % UC W-98 on Chromosorb Q (80–100 mesh) at 190°C. For quantitative evaluations of the GLC, a Hewlett Packard 3370 B integrator was used. For GLC-MS, a Perkin-Elmer 270 combined GLC-MS instrument fitted with the appropriate column was used. Mass spectra were recorded at an ionisation potential of 70 eV, an ionisation current of 80  $\mu$ A and an ion source temperature of 80°C. NMR was recorded on a Varian A 60 A using tetramethylsilane as reference.

*Deacetylation of methyl 2-acetamido-2-deoxy- $\alpha$ -D-glucopyranoside.* Methyl 2-acetamido-2-deoxy- $\alpha$ -D-glucopyranoside (78.0 mg) and L-fucitol (75.0 mg, internal standard) were added to a solution of Ba(OH)<sub>2</sub>·6H<sub>2</sub>O (1.0 g) in deaerated water (10 ml). The resulting mixture was heated in a sealed steel ampoule for 4 h at 135°C under nitrogen. The reaction mixture was then cooled and neutralised with carbon dioxide. After filtration, the water solution was concentrated to dryness. Part of the product ( $\approx$  5 mg) was dissolved in water (5 ml) and passed through a column of Dowex 50 (H<sup>+</sup>). The eluate was concentrated to dryness, acetylated and analysed by GLC (column C). This analysis showed only fucitol pentaacetate. Another part of the deacetylated product was re-acetylated and analysed by GLC (column C). This analysis demonstrated that all methyl 2-acetamido-2-deoxy-D-glucopyranoside appeared on reacetylation.

*Treatment of methyl 2-amino-2-deoxy- $\alpha$  and - $\beta$ -D-glucopyranoside with nitrous acid.* Methyl 2-acetamido-2-deoxy- $\alpha$ - or - $\beta$ -D-glucopyranoside ( $\approx$  10 mg, accurately weighed) and L-fucitol ( $\approx$  10 mg, accurately weighed) was deacetylated as above. The deacetylated product was dissolved in a sodium acetate buffer (1 ml, 8 M; pH 3.5) and mixed with an equal volume of sodium nitrite in water (2 % w/v). The solution was kept at room temperature overnight. To the reaction mixture was then added an excess of sodium borohydride or borodeuteride and after 18 h the solution was adjusted to pH 3.0 by the addition of acetic acid. The mixture was then concentrated to dryness and boric acid was removed by codistillations with methanol. The reaction product was acetylated and analysed by GLC-MS (columns A and B). In a separate experiment, the deamination and reduction were carried out in D<sub>2</sub>O. The results of the GLC analyses are given in Table 1. The mass spectra of the two fastest components (Table 1) were virtually identical and displayed peaks at *m/e* 43(100), 54(10) [55(11)], 69(6) [69(5)], 71(11) [72(6)], 82(5), 87(9) [87(5)], 98(5), 99(12) [100(6)], 100(13) [101(6)], 111(35) [111(9)], 112(38)], 142(12) [143(5)], 153(16) [153(4), 154(11)], 171(4) [172(5)], 231(4) [232(4)]. The values for the components which had been reduced with sodium borodeuteride are given in angular brackets. The mass spectra for the two fastest components obtained when the deamination and reduction were carried out in D<sub>2</sub>O were identical except for a shift of the peak at *m/e* 232(233) by

one mass unit. The mass spectrum for the slowest components (Table 1) had peaks at  $m/e$  43(100), 44(3), 69(4) [69(3), 70(2)], 85(2) [85(2), 86(2)], 97(23) [97(10), 98(11)], 110(22) [111(18)], 115(3) [115(2), 116(1)], 139(23) [139(7), 140(8)], 152(8), 153(3) [153(6), 154(2)], 157(3) [157(1), 158(1)], 170(1) [171(1)], 259(3) [259(1), 260(1)].

*Isolation of the reaction products.* Methyl 2-acetamido-2-deoxy- $\alpha$ -D-glucopyranoside (1.6 g) was *N*-deacetylated and treated with nitrous acid as described above. The crude acetylated product was fractionated on a Silica Gel column (50  $\times$  5 cm) using light petroleum-ethyl acetate (2:1 w/v) as irrigant. The separation was monitored by TLC. Two fractions were obtained; I,  $R_F$ =0.37 (330 mg) and II,  $R_F$ =0.31 (700 mg). GLC-MS analysis showed that fraction I was a mixture of two peracetylated methyl 2-deoxy-2-*C*-hydroxymethyl-pentofuranosides and that II was peracetylated 2,5-anhydrohexitol. The NMR spectrum of fraction I showed peaks at  $\tau$  7.90, 6 H, singlet, *O*-acetyl;  $\tau$  6.58, 3 H, singlet, *O*-methyl;  $\tau$  5.1-5.9, 5 H, multiplet, 2 H-1, H-2, H-3 and H-4;  $\alpha$  4.67-5.16, 2 H, multiplet, 2 H-6. The NMR spectrum of fraction II had protons at  $\tau$  7.88, 12 H, singlet, *O*-acetyl;  $\tau$  5.70, 6 H, broad singlet, 2 H-1, H-2, H-3, H-4 and H-5;  $\tau$  4.77, 2 H, broad singlet, 2 H-6.

Part of fraction I (5 mg) was hydrolysed with aqueous sulphuric acid (3 ml) at 100° for 1 h. The hydrolysate was neutralised with barium carbonate, filtered and reduced with sodium borohydride or sodium borodeuteride. The reduced product was acetylated and analysed by GLC-MS. The GLC analysis showed only one component with the *T*-value 4.75 on column A and 6.1 on column B. The mass spectrum had peaks at  $m/e$  43(100), 103(7), 129(16) [130(16)], 145(7), 154(2) [155(2)], 171(3) [172(3)], 189(2) [190(2)], 201(4) [202(4)], 215(1), 217(0.5), 231(5) [232(5)]. Values for peaks after reduction of the products with sodium borodeuteride are given in angular brackets, after corresponding figures for the non-deuterated analogue.

*Added in proof.* Dmitriev, B.A., Knirel, Yu.A. and Kochetkov, N.K. [*Carbohydr. Res.* **29** (1973) 451, **30** (1973) 45] have investigated the deamination of two disaccharide glycosides containing 2-amino-2-deoxy-D-glucose residues. In addition to the 2,5-anhydro-mannose derivatives, they also, for each disaccharide, obtain smaller amounts of an unidentified component. This, in the light of the present study, should most probably be the 2-deoxy-2-*C*-formyl-D-*arabino*-pentofuranose derivative.

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