Nitrosation of 2-Amino-2-deoxy-D-galactitol; a Model Experiment for the Study of Reduced Oligosaccharides derived from Mucus Glycoproteins

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Sequential nitrosation, and reduction with sodium cyanoborohydride, of 2-amino-2-deoxy-D-galactitol gave, as major products, 2-deoxy-D-*lyxo*- (and/or -*xylo*-)hexitol (39%) and glycerol (43%). Minor products included a hexitol and diastereoisomeric 2-deoxy-2-hydroxymethyl-D-pentitols (provisionally identified). Reduction of the nitrosation products with sodium borodeuteride gave 2-deoxy-D-hexitols with more deuterium at C-3 than at C-1. Glycerol was also shown to be a product of the nitrosation and reduction of 2-amino-2-deoxy-D-glucitol. The implications of these results for the nitrosation of the 2-amino-2-deoxy-D-galactitol-containing oligosaccharides derived from mucus glycoproteins are discussed.

The regiospecific cleavage, by nitrous acid, of the glycosidic linkages of 2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-galactopyranose moieties in compounds containing these monosaccharide amines is now a well established method of degradation as an aid to structure elucidation.[1] In order to apply such a degradation to the reduced oligosaccharides obtained from mucus glycoproteins it was necessary to know the fate of the terminal 2-acetamido-2-deoxy-D-galactitol moiety which each oligosaccharide possessed. The nitrosation of 2-amino-2-deoxy-D-galactitol has been studied as a model for the behaviour of the alditol moiety of the above oligosaccharides.

Results

Crystalline 2-amino-2-deoxy-D-galactitol was prepared from the *N*-acetyl derivative by *N*-deacetylation with hydrazine hydrate. Nitrosation was carried out by adding glacial acetic acid in portions to an ice-cooled aqueous solution of the amine and sodium nitrite and the reaction was followed by paper electrophoresis. The solution was purged with nitrogen and the nitrosation products were then reduced by the addition of sodium cyanoborohydride. The deionized products were then analysed by g.l.c.–m.s. measurements on the per-*O*-trimethylsilyl ether derivatives. Glycerol, a 2-deoxyhexitol which co-chromatographed with 2-deoxy-D-*lyxo*-hexitol (**1**), and a hexitol were identified. The nitrosation was repeated in the presence of *meso*-inositol as an internal standard and the products were reduced with sodium borodeuteride. The yield of glycerol was found to be 43%, and that of the 2-deoxyhexitol was calculated as 39% using 2-deoxy-D-*lyxo*-hexitol as the standard and assuming that the detector response of any 2-deoxy-D-*xylo*-hexitol (**2**) present was the same as that of the *lyxo* isomer. The mass spectrum of the deuterio-2-deoxyhexitol(s) product was the same as that of authentic 1-deuterio-2-deoxy-D-*lyxo*-hexitol except that the trimethylsilyloxymethyl ion had *m/z* 103 in the former † and 104 in the latter. The glycerol product contained deuterium but the hexitol did not. Two minor products that eluted between glycerol and the 2-deoxyhexitol were provisionally identified as diastereoisomeric 2-(hydroxymethyl)pentitols (**3**); their mass spectra were very similar and the ion of highest mass at *m/z* 347 was attributed to loss of two molecules of trimethylsilanol from the monodeuteriated derivatives.

Nitrosation and reduction of 2-amino-2-deoxy-D-glucitol gave the products reported by Matsushima and Bando,[2] together with glycerol which had not been previously identified.

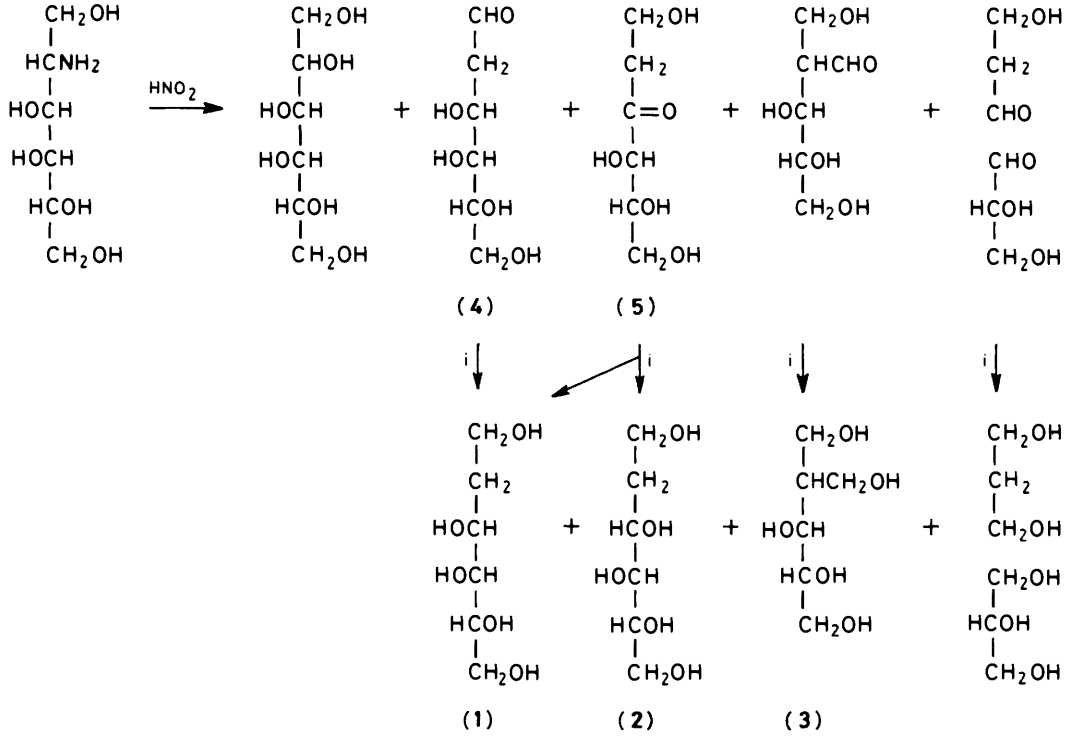
† The *m/z* 103/104 ratio was 5.5:1.

Discussion

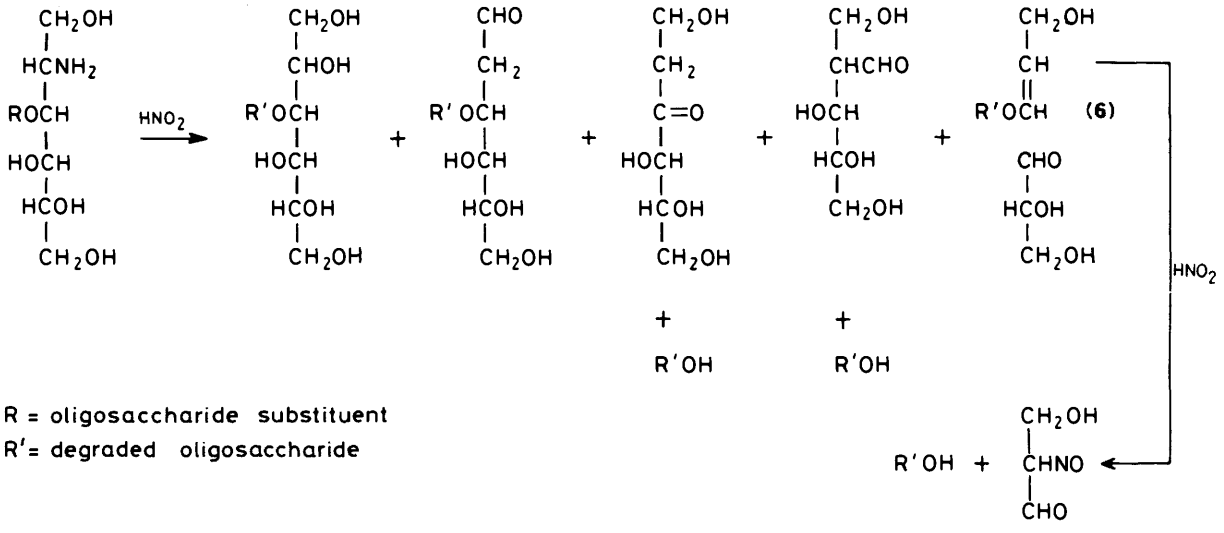
The 2-deoxyhexitol could arise from 2-deoxy-D-*lyxo*-hexose (**4**) or from 2-deoxy-D-*threo*-hex-2-ulose (**5**). When the nitrosation products were reduced with sodium borodeuteride the mass spectrum of the 2-deoxyhexitol derivative contained a strong ion at *m/z* 103 due to $\overset{+}{C}H_2OSiMe_3$. Cleavage of the C-1–C-2 bond in the mass spectral fragmentation was shown to predominate over C-5–C-6 bond cleavage by the spectrum of authentic per-*O*-trimethylsilyl-2-deoxy-1-deuterio-D-*lyxo*-hexitol which gave an *m/z* 104/103 ratio of 4:1. There was therefore more deuterium at C-3 than at C-1 in the product 2-deoxyhexitol, and thus hydride shift from C-3 to C-2 predominated over hydride shift from C-1. In contrast the latter pathway predominated in the nitrosation of 2-amino-2-deoxy-D-glucitol to give 2-deoxy-D-*arabino*-hexose.[2]

The fragmentation which yielded glycerol (after reduction) would be expected to give an equimolar amount of propane-1,3-diol (see Scheme 1) but none was detected. This was attributed to loss of the relatively volatile bis(silyl ether) when the pyridine was removed by evaporation under reduced pressure. When the pyridine solution of the silylated products was analysed by g.l.c. the diol derivative was obscured by the solvent peak. Glycerol was also shown to be a product of the nitrosation of 2-amino-2-deoxy-D-glucitol. A similar fragmentation occurred in the nitrosation of 2-amino-2-deoxy-D-glucose hydrazone to give, after reduction, glycerol.[3] Fragmentation of the carbon skeleton is less common in the nitrosation of cyclic amines. One example is provided by the formation of methyl L-*threo*-pent-4-enopyranoside as a minor product of the nitrosation of methyl 4-amino-4-deoxy-α-D-galactopyranoside.[4] It is noteworthy that in a study of the nitrosation of acyclic 3-amino-alcohols fragmentation of the carbon skeleton occurred only for compounds which possessed an aryl substituent on the amine-bearing carbon.[5] Presumably conjugative stabilisation of the transition state leading to fragmentation is involved.

By analogy with the nitrosation of 2-amino-2-deoxy-D-glucitol,[2] some migration of C-4 from C-3 to C-2 to give, after reduction, 2-(hydroxymethyl)pentitols (**3**) would be expected to occur, and the two minor products provisionally identified as 2-(hydroxymethyl)pentitols had similar relative retention times



Scheme 1. *Reagents:* i, $NaBH_3CN$



Scheme 2.

to the corresponding products formed from the aminodeoxyglucitol.

The *N*-deacetylated reduced oligosaccharides[6] obtained from mucus glycoproteins possess a terminal 2-amino-2-deoxy-D-galactitol moiety which is usually substituted at C-3. The fate of such oligosaccharides upon nitrosation is summarised in Scheme 2. The fragmentation at C-3–C-4 would give an enol ether (**6**) which would be expected [7] to react with excess of nitrous acid as shown. All the pathways except hydride shift from C-1 and solvolysis to give a hexitol would result in the release of a reducing saccharide (R'OH) lacking the terminal alditol. The rest of the oligosaccharide chain would be cleaved [1] at the 2-amino-2-deoxyglucosidic and 2-amino-2-deoxygalactosidic linkages and the reduced products would then be saccharide alditols terminating in 2,5-anhydro-D-mannitol, 2,5-anhydro-D-talitol, and an alditol (usually galactitol), the latter originating from the monosaccharide attached to the terminal 2-amino-2-deoxy-D-galactitol.

However, it is known that the reaction pathways in amine nitrosation are very sensitive to the conformation of the amine starting material.[8] The difference in the relative importance of the pathways followed in the nitrosation of 2-amino-2-deoxy-D-galactitol and its D-gluco analogue presumably reflect differences in the conformational equilibria. Since substituted 2-amino-2-deoxy-D-galactitols may have different preferred conformations from those of the unsubstituted alditol the

proportions of nitrosation products formed from the 2-amino-2-deoxy-D-galactitol moieties of oligosaccharides may differ significantly from those reported above for the free alditol.

The nitrosation of the 3-*O*-β-D-galactopyranosyl derivative of 2-amino-2-deoxy-D-glucitol involved[9] all the pathways reported above except that no cleavage of the C-3–C-4 bond was found, possibly because the products were identified by g.l.c. analysis of the per-*O*-methyl ethers and small molecules such as tri-*O*-methylglycerol may have eluted with the solvent. In a paper which was mainly concerned with the nitrosation of per-*O*-methylated oligosaccharides, Aspinall and co-workers reported the identification of free 2-deoxy-D-*lyxo*-hexitol as one of the products of the nitrosation and reduction of oligosaccharide alditols from hog gastric mucus.[10] This product was incorrectly referred to as the 1-deuterio derivative (formed by reduction with sodium borodeuteride) instead of the 3-deuterio derivative.

The application of nitrosation to the regiospecific degradation of reduced oligosaccharides from gastric mucus glycoproteins will be described elsewhere.

Experimental

Methods.—General methods were as in ref. 11 except that g.l.c. analyses were carried out with a 1 m column of 5% silicone gum rubber on Chromosorb G (80—100 mesh) at 130 °C for 3 min, then under a temperature gradient of 10 °C min^{-1} to 200 °C. For g.l.c.–m.s. measurements this column was coupled to an AEI MS 9 instrument and ionisation was effected by electron impact at 50 eV. The *O*-trimethylsilyl ether derivatives were prepared[12] in dry pyridine which was then removed under reduced pressure and the silyl ethers were dissolved in dry hexane for g.l.c. analysis.

2-*Amino*-2-*deoxy*-D-*galactitol.*—2-Acetamido-2-deoxy-D-galactitol (97 mg; m.p. 176 °C) was heated in hydrazine hydrate (0.5 ml) at 110 °C for 20 h. Hydrazine hydrate was removed under reduced pressure and acetal hydrazine was then removed from the residue by sublimation at 80 °C and 0.1 mmHg. Recrystallisation of the crude product from MeOH–water (8:1) gave the pure *title amine* (32 mg), m.p. 174—178 °C (Found: C, 40.1; H, 8.55; N, 7.65. $C_6H_{15}NO_5$ requires C, 39.8; H, 8.3; N, 7.7%).

Nitrosation and Reduction of 2-*Amino*-2-*deoxy*-D-*galactitol.*—A magnetically stirred solution of 2-amino-2-deoxy-D-galactitol (13.5 mg) and sodium nitrite (52 mg) in water in a stoppered flask was cooled in an ice–water bath and treated with glacial acetic acid (64 μl) in three equal portions during 30 min. The solution was kept in an ice–water bath for a further 1 h and aliquots were analysed at intervals by paper electrophoresis (at pH 5.4) which showed that the amine, detected with ninhydrin, was absent after 1 h.

Excess of nitrous acid was removed by purging the solution with nitrogen for 20 min, and the stirred solution was then cooled in an ice–water bath and treated with sodium cyanoborohydride (22 mg). The solution was allowed to warm to room temperature (1 h) and Amberlite IR-120 (H^+) (3 ml) was added. When evolution of gas had ceased the resin and supernatant were added to a column of more resin (9 ml) which was then eluted with water (120 ml). The eluate was freeze-dried and boric acid was removed from the residue as methyl borate. Aliquots of the dried residue were silylated and analysed by g.l.c.–m.s.

The nitrosation was repeated on the same scale in the presence of *meso*-inositol (10.1 mg) as internal standard. After the purging with nitrogen the pH was adjusted to 6.5 with saturated aqueous borax and sodium borodeuteride (24.3 mg) was added to the stirred ice-cold solution. After 5 min the solution was allowed to warm to room temperature (55 min). Sodium ions were removed as above and the eluate from the cation-exchange resin was passed through a column of Amberlite IRA-400 (acetate form) anion-exchange resin (3 ml). The eluate was freeze-dried and boric acid was removed from the residue as above. Aliquots of the dried residue were trimethylsilylated and analysed by g.l.c.–m.s. which identified glycerol (retention time, R_t, 5.4 min), 2-deoxy-D-*lyxo*-(and/or -*xylo*-) hexitol [(**1**)/(**2**)] (R_t 15.1 min), and hexitol (R_t 19.7 min). Minor products (R_t 12.0, 12.6 min) were provisionally identified as diastereoisomeric 2-deoxy-2-(hydroxymethyl)pentitols. *Meso*-inositol had R_t 26.5 min.

2-*Deoxy*-D-lyxo-*hexitol* (**1**).—A solution of 2-deoxy-D-*lyxo*-hexose (**4**) (90 mg) in water (0.5 ml) was treated with aqueous sodium borohydride (23 mg in 0.5 ml) and kept for 25 min. Removal of sodium ions with Amberlite IR 120 (H^+) resin (1.5 ml) and of boric acid as methyl borate gave solid 2-deoxy-D-*lyxo*-hexitol (**1**) (81 mg). Recrystallisation from aqueous EtOH gave the analytically pure alditol (**1**), m.p. 114—115 °C (lit.,[13] 112—113 °C). The mass spectrum of the per-*O*-trimethylsilyl ether was measured by g.l.c.–m.s.: R_t 15.0 min; *m/z* 333 (1.2%), 321 (1.9), 307 (4.6), 231 (6.7), 219 (23), 217 (9.3), 205 (4.5), 147 (19), 103 (69), and 73 (100). The 1-deuterio derivative was similarly prepared using sodium borodeuteride, and the deuterium-containing ions in the mass spectrum of the per-*O*-trimethylsilyl ether were (*m/z*) 334, 322, 232, 220, and 104.

Acknowledgements

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