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Preliminary communication

Degradation during the hydrazinolysis of 2-acetamido-1-*N*-acyl-2-deoxy-β-D-glucopyranosylamines

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Hydrazinolysis is a convenient reaction for the isolation of the asparagine-bound carbohydrate moieties of glycoproteins[1]. It also effects the *N*-deacetylation of *N*-acetylhexosamine residues, and the resulting glycan amine can be degraded regiospecifically by the nitrosation procedure[2]. Excessively vigorous conditions can cause degradation during the hydrazinolysis and two sets of conditions are currently used, namely heating an anhydrous hydrazine solution in a sealed tube at 100° for 30 h (Bayard–Montreuil conditions[3]), and at 105° for 10 h in the presence of hydrazinium sulphate as a catalyst (Kochetkov conditions[4]).

Previous work[5] has established that the major product of the hydrazinolysis of 2-acetamido-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine, under Kochetkov conditions, was 2-amino-2-deoxy-D-glucose hydrazone. We now report the identification of a degradation product that is formed in this reaction.

As part of a study[6] of the behaviour of saccharides under the conditions of hydrazinolysis, it was found that 2-amino-2-deoxy-D-glucose hydrochloride was converted, *via* 2-amino-2-deoxy-D-glucose hydrazone, into 1-deoxy-D-fructose hydrazone under Bayard–Montreuil conditions. The 1-deoxy-D-fructose hydrazone was identified by ^{1}H (Me singlet at δ 1.84) and ^{13}C (Me at 13.8 p.p.m.) n.m.r. measurements, by comparison with the hydrazone prepared from authentic 1-deoxy-D-fructose[7], and by sequential nitrosation and reduction with sodium borodeuteride, to give a mixture of 1-deoxy-D-[2-^{2}H]glucitol and -D-[2-^{2}H] mannitol which were identified by g.l.c.-m.s. measurements on the peracetates (*m/z* 88, MeCDOAc$^+$).

The product of hydrazinolysis of 2-acetamido-1-*N*-acetyl-2-deoxy-β-D-glucopyranosylamine under Kochetkov conditions contained 22% of 1-deoxy-D-fructose hydrazone, estimated from the p.m.r. spectrum. However, under Bayard–Montreuil conditions, amide cleavage was very inefficient; starting material (35%) and 1-*N*-acetyl-2-amino-2-deoxy-β-D-glucopyranosylamine (35%) were detected in the product by n.m.r. spectroscopy. This result was surprising in view of the recent report[1] that a reaction time of only 8–12 h is sufficient to effect the complete release of the asparagine-bound oligosaccharides in glycoproteins and glycopeptides. We considered that the hydrazinolysis of glycoproteins and

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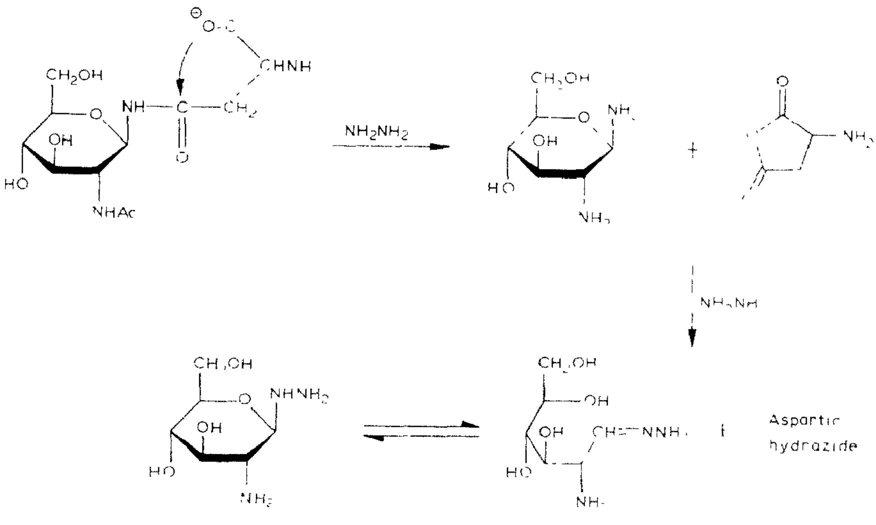
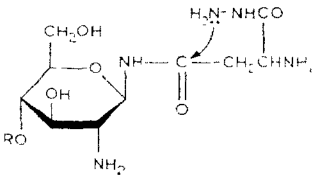
glycopeptides might involve a neighbouring-group reaction, and this was confirmed by the hydrazinolysis of 2-acetamido-1-*N*-(L-β-aspartyl)-2-deoxy-β-D-glucopyranosylamine (GlcNAc-Asn). Under Kochetkov conditions, 1-deoxy-D-fructose hydrazone was formed in ~44% yield; under Bayard Montreuil conditions, amide cleavage was complete and the yield of 1-deoxy-D-fructose hydrazone was lower (~14%), presumably because its precursor, 2-amino-2-deoxy-D-glucose hydrazone, was released more slowly than under Kochetkov conditions. The more efficient amide-cleavage in GlcNAc-Asn than in 2-acetamido-1-*N*-acetyl-β-D-glucopyranosylamine must be due to an intramolecular nucleophilic catalysis, as depicted in Scheme 1.



Scheme 1

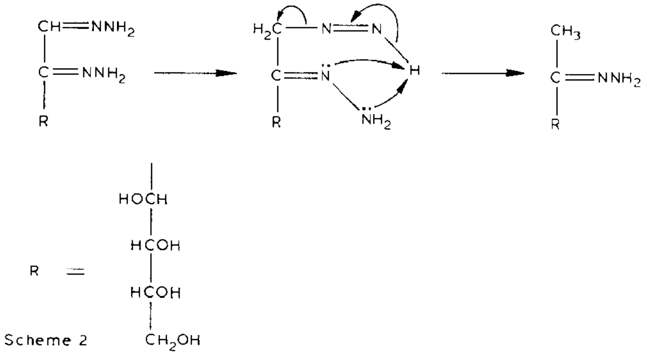
In the hydrazinolysis of glycoproteins and glycopeptides in which asparagine is linked through its amino group to another amino acid, the hydrazide **1** is a possible intermediate in the reaction. Participation by the hydrazide nitrogen atom as shown (**1**) is then possible.



1

The formation of 1-deoxy-D-fructose hydrazone from 2-amino-2-deoxy-D-glucose hydrazone can be envisaged as proceeding *via* the 1,2-bishydrazone, whose formation is analogous to the formation of phenylosazone derivatives. A Wolff–Kishner type reaction at

C-1, possibly facilitated by an intramolecular process involving a six- or seven-membered ring transition-state as depicted in Scheme 2, would then give 1-deoxy-D-fructose hydrazone. A similar reaction at C-2 would give 2-deoxy-D-*arabino*-hexose hydrazone, and it is interesting to note that a 2-deoxy-D-*arabino*-hexitol derivative was recently identified[8] as a minor product of the sequential hydrazinolysis, nitrosation and reduction of glycopeptides.



Scheme 2

Our studies of these reactions continue, and the significance of the results in the context of structural studies of glycoproteins will be discussed elsewhere.

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