## Note

# The acid hydrolysis of 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine

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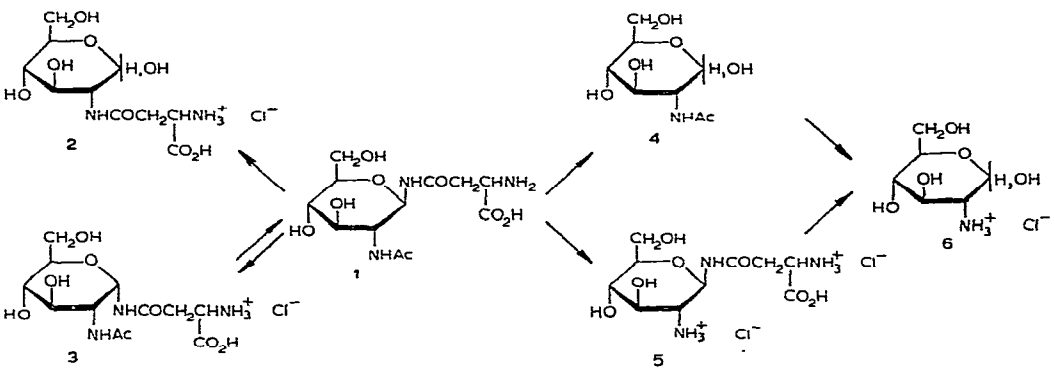
2-Acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (**1**) was reported to rearrange in M hydrochloric acid (100°, 30–45 min) to give[1,2] the α-D anomer **3** and 2-amino-2-*N*-(4-L-aspartyl)-2-deoxy-D-glucose (**2**). In a kinetic study[3], the rate of hydrolysis of **1** (2M HCl at 100°) was approximately twice the rate at which L-aspartic acid was released during the early stages of the reaction. Two reaction pathways were proposed, one proceeding via 2-acetamido-2-deoxy-D-glucose (**4**) and the other via 2-amino-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (**5**). Neither **2** nor **3** was detected, and the evidence for the presence of **5** was inconclusive.



In the present study, the hydrolysis of **1** was examined by t. l.c. but the presence of **2** could not be confirmed by this method. In M hydrochloric acid, **4**, 2-amino2-deoxy-D-glucose hydrochloride (**6**), and L-aspartic acid were detected during the first 2 h. After this time, **4** was absent but glycopeptide material did not completely disappear until after 24 h to give **6** and L-aspartic acid as the final products. Both **4** and 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (**1**) were hydrolysed to give **6** within 2 h. Asparagine was not detected in any of the above hydrolyses.

The composition of hydrolysates of **1** in M hydrochloric acid were then determined on an amino acid analyzer after 40 min, 2 h, 4 h, and 24 h, using a gradient buffer system (pH 3.25 → 5) (Table I). Authentic samples of **1**, **2**, **5**, **6**, and L-aspartic

acid were separated readily (elution times of 67, 78, 116, 260, and 273 min, respectively). Two unidentified but minor components, eluted later than **5** at 300 and 310 min, appeared both in the 40-min and 2-h hydrolysates. Only one of these components remained in the 4-h hydrolysate. As before, **6** and L-aspartic acid were the only detectable products after 24 h. Only a trace (1.4%) of the rearranged product **2** was detected in the 40-min hydrolysate.

TABLE I

COMPOSITION OF HYDROLYSATES (NMOLES/ML)

| *Time of hydrolysis* | **1** | **2** | L-*Aspartic acid* | **6** | **5** |
|---|---|---|---|---|---|
| 40 min | 78 | 3.4 | 48 | 24 | 52 |
| 2 h | 36 | — | 79 | 53 | 58 |
| 4 h | — | — | 94 | 75 | 24 |
| 24 h | — | — | 204 | 228 | — |

The results clearly implicate 2-amino-1-*N*-(4-L-aspartyl)-2-deoxy-*β*-D-glucopyranosylamine (**5**) as an intermediate in the hydrolysis of **1**, and show that a substantial proportion is hydrolysed via this compound. As has been suggested[3], **5** would be expected to hydrolyse more slowly than the starting material (**1**), because of the field effect of the $^{+}NH_3$ group. Whilst the hydrolyses proceed mainly by two pathways involving **4** and **5**, the presence of the two unidentified components suggests additional, but minor, pathways. A small proportion of the hydrolysis could proceed via the rearranged product **2**.

## EXPERIMENTAL

2-Acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-*β*-D-glucopyranosylamine (**1**) was an authentic sample. 2-Amino-2-*N*-(4-L-aspartyl)-2-deoxy-D-glucose (**2**) was synthesized by the method of Marks and Neuberger[4], except that *O*-deacetylation was carried out[5] with ammonia in methanol at 0°; **2** had physical constants in agreement with literature values. 2-Amino-1-*N*-(4-L-aspartyl)-2-deoxy-*β*-D-glucopyranosylamine (**5**) was synthesized as described previously[5].

*Acid hydrolysis.* — Samples (10 mg) of 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-*β*-D-glucopyranosylamine were heated in 0.01, 0.1, and M hydrochloric acid at 100°, and the reactions were followed at regular intervals by t.l.c. on silica gel (butyl alcohol–acetic acid–water, 10:20:5). 2-Acetamido-2-deoxy-D-glucose was detected with *p*-anisidine hydrochloride, and the glycopeptides **1** and **2**, 2-amino-2-deoxy-D-glucose hydrochloride, and L-aspartic acid were detected with ninhydrin.

A Technicon machine was used for amino acid analysis with a gradient buffer system (Autograd mixing chamber, 1 × 75 ml of citrate buffer pH 3.25, 3 × 75 ml of citrate buffer pH 5), and with ninhydrin as the detecting reagent. The buffer solutions and the ninhydrin reagent were prepared according to standard procedures. The

elution times of standards were determined with 1-ml samples of solutions containing 0.1 μmole/ml, and the peak areas corresponding to this concentration were calculated. For the acid hydrolysis, **1** (8.8 mg) was treated with boiling M hydrochloric acid (100 ml), and 1-ml samples (0.25 μmole) were taken after 40 min, 2 h, 4 h, and 24 h, and analysed on the amino acid analyser. Concentrations of the components were determined by comparison of peak areas with those calculated for the standards.

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