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SELECTIVE CLEAVAGE OF GLYCOSIDIC LINKAGES: STUDIES WITH THE MODEL COMPOUND BENZYL 2-ACETAMIDO-2-DEOXY-6-*O*-α-D-MANNOPYRANOSYL-α-D-GLUCOPYRANOSIDE

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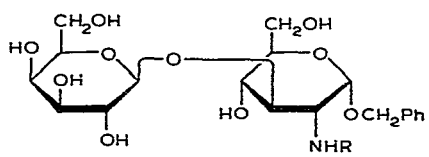
ABSTRACT

N-Deacetylation of benzyl 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranoside (**3**) by alkaline hydrolysis, or hydrazinolysis in the presence of hydrazine sulphate, proceeds quantitatively to yield the amine **4**. The mannosyl glycosidic linkage in **4** can be selectively hydrolysed by acid, whereas the 2-amino-2-deoxyhexosyl glycosidic linkage is selectively cleaved upon treatment with sodium nitrite in dilute acetic acid. Aspects of the selective cleavage of hexosaminoglycans are discussed.

INTRODUCTION

In a recent paper[1], we described a selective cleavage for each glycosidic linkage in benzyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranoside (**1**), based upon *N*-deacetylation, and subsequent acid hydrolysis or deamination with nitrous acid. The bioside **1** was chosen as a model for a repeating unit of 2-amino-2-deoxyhexose-containing polysaccharides, with the aim of elaborating a standard procedure for the selective cleavage of hexosaminoglycans.



1 R = Ac
2 R = H

This paper describes the selective cleavage of benzyl 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranoside (**3**), which, in comparison with model compound **1**, represents a 2-acetamido-2-deoxyglucoside substituted at a different position. The synthesis of **3** was described recently by Shaban and Jeanloz[2].

RESULTS AND DISCUSSION

As for bioside **1**, *N*-deacetylation of **3** was performed with hydrazine or aqueous alkali, because the action of each reagent is unaffected by the presence of unsubstituted hydroxyl groups. The results obtained are presented in Table I.

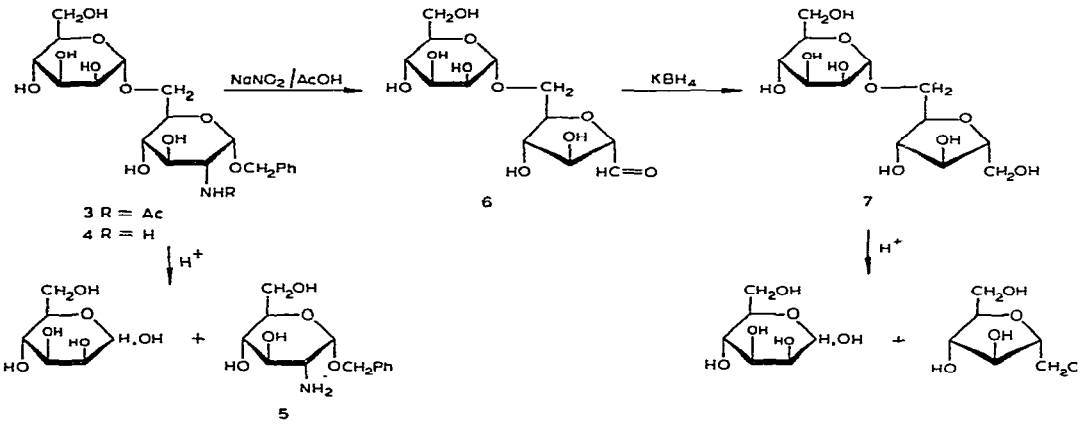
TABLE I

N-DEACETYLATION OF BIOSIDE **3**

Reagent	*Time (h)*	*Yield (%) of bioside 4*[a]	*Recovery (%) of bioside 3*[a]	*Total (%)*
H_2NNH_2	5	40 (41)	58 (55)	98
	10	68 (66)	30 (37)	98
$H_2NNH_2 \cdot H_2O$	5	40 (35)	69 (60)	109
	10	59 (42)	41 (55)	100
$H_4N_2 \cdot H_2SO_4$	5	96 (69)	0 (28)	96
2.5M NaOH	2.5	88 (60)	11 (40)	99
	5	100 (65)	0 (35)	100

[a]The figures in parentheses are the corresponding results[1] for bioside **1**.

N-Deacetylation of **3** with 2.5M sodium hydroxide, or with hydrazine in the presence of hydrazine sulphate, proceeded quantitatively and was not accompanied by destruction of the bioside. The structure of the crystalline, free-amine bioside **4** was proved by reconversion into **3** by acetic anhydride in methanol.



N-Deacetylation proceeded much more readily for **3** than for **1**, so that saponification gave a quantitative yield and hydrazinolysis required less time. These results conform to literature data[3], which indicate that the absence of a 3-substituent from a hexosaminide facilitates *N*-deacetylation. However, the action of anhydrous hydrazine

and hydrazine hydrate on **3** did not effect complete *N*-deacetylation and gave **4** in yields of 68 and 59%, respectively. Evidently, hydrazinolysis in the absence of hydrazine sulphate, as a catalyst, is not an effective method for *N*-deacetylation, even for disaccharides. When used, for example, for *N*-deacetylation of the K-specific polysaccharide from *E. coli*[4] and of the O-specific lipopolysaccharide and lipid A material from *Pseudomonas aeruginosa*[5], the method resulted in incomplete *N*-deacetylation of the former and extensive decomposition of the latter. The results on model biosides suggest that *N*-deacetylation of complex hexosaminoglycans is more advantageously carried out by hydrazine in the presence of hydrazine sulphate, as first recommended by Yosizawa *et al.*[6].

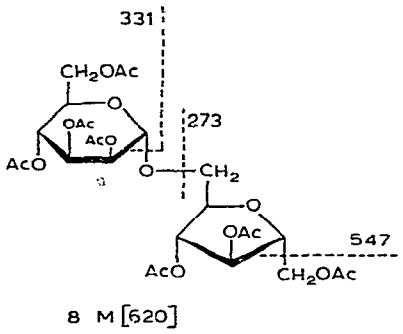
Treatment of the free-amine bioside **4** with M hydrochloric acid at 100° caused complete release of the mannose residue in 1 h, whereas hydrolysis of the free-amine **2** required either a longer period of heating or an increased concentration of acid. The difference in rates of acid hydrolysis of biosides **2** and **4** may be due to the shorter distance (and therefore increased electrostatic shielding) in **2** between the protonated amino group and the glycosidic linkage cleaved. Mannose and benzyl 2-amino-2-deoxy-α-D-glucopyranoside (**5**), formed on hydrolysis of **4**, were identified by paper and thin-layer chromatography.

The data now reported on the selective hydrolysis of biosides **2** and **4** with acid validate the earlier conclusion[1] that the selective cleavage of *N*-deacetylated hexosaminoglycans into disaccharides having terminal, non-reducing 2-amino-2-deoxyhexose residues should be carried out with reaction times shorter than indicated in the literature[7].

Deamination[8] of **4** with sodium nitrite in acetate buffer (pH 3.5), in contrast to bioside **2**, apparently was complete. In addition to the expected product, 2,5-anhydro-6-*O*-α-D-mannopyranosyl-D-mannose (**6**), a minor, unidentified component could be readily detected (t.l.c) after reduction with potassium borohydride and subsequent acetylation. Deamination of **4** with sodium nitrite in dilute acetic acid, under conditions[9] recommended by Dische for the quantitative determination of 2-amino-2-deoxyhexoses, gave **6** as the sole product. Reduction of **6** with potassium borohydride gave 2,5-anhydro-6-*O*-α-D-mannopyranosyl-D-mannitol (**7**), which was isolated as the hepta-acetate **8**. The structure of **8** was proved by mass spectrometry, and the principal fragmentation pathways, shown in formula **8**, confirm the proposed structure and are analogous to those reported[1] for 2,5-anhydro-3-*O*-β-D-galactopyranosyl-D-mannitol. Acid hydrolysis of **7** gave D-mannose and 2,5-anhydro-D-mannitol, which were identified by g.l.c. after conversion into the corresponding alditol acetates.

The deamination of biosides **2** and **4**, under the Dische conditions[9], is unaffected by the positions of substituents in the 2-amino-2-deoxyglucoside residue, and these conditions may therefore be recommended for cleavage of *N*-deacetylated, 2-amino-2-deoxyhexosamine-containing polysaccharides.

As expected for 6-*O*-substituted 2-acetamido-2-deoxyglycosides, biosides **3** and **4** consumed 3 and 4 mol. of periodate, respectively, with concomitant destruction of both monosaccharide residues.



m/e 560 (M—AcOH)

m/e 361 $\xrightarrow{-42}$ 391 $\xrightarrow{-46}$ 273

EXPERIMENTAL

General. — T.l.c. was performed on Silica Gel KSK with conventional detection by conc. sulphuric acid or ninhydrin (for amines). Paper chromatography (p.c.) was effected by the ascending method on Whatman No. 1 paper, using 1-butanol–ethanol–water (3:2:2), and detection with alkaline silver nitrate and ninhydrin. G.l.c. was carried out on a Pye Argon Chromatograph with β-ionization detection, a glass column (140 × 0.4 cm) packed with 3% ECNSS-M on Gas-Chrom Q, and an argon flow-rate of 50 ml/min. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter. Mass spectra were recorded with a Varian CH-6 instrument, with an inlet temperature of 80° and an ionizing potential of 70 eV. Evaporations were performed under diminished pressure with a bath temperature below 40°. Melting points were determined with a Koffler apparatus.

*Hydrazinolysis of benzyl 2-acetamido-2-deoxy-6-*O*-α-*D*-mannopyranosyl-α-*D*-glucopyranoside* (3). — Three portions (5 mg) of **3**, prepared as described by Shaban and Jeanloz[2], were heated severally in sealed tubes with hydrazine (0.2 ml), hydrazine hydrate (0.2 ml), and hydrazine (0.2 ml) in the presence of hydrazine sulphate (2 mg), for 5 and 10 h at 105°. After cooling, hydrazine was removed by addition and distillation of toluene (3 times), and the residue was dried over conc. sulphuric acid. Solutions of the residues in water (1 ml) were applied to columns of Dowex-50 x4 (H^+) resin (1 ml), and eluted first with distilled water (15 ml) and then with 2M ammonium hydroxide (15 ml). The eluates were evaporated, and the residues were thoroughly washed with ether to remove acetylhydrazine, dried, and dissolved in water (15 ml). The amount of unchanged **3** in the neutral eluates, and the amount of **4** in the ammonia eluates, were determined by the phenol–sulphuric acid procedure[10]. The results are presented in Table I. Evaporation of the alkaline eluate, followed by washing with ether, yielded benzyl 2-amino-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranoside (**4**), m.p. 102–104°, $[\alpha]_D^{20}$ +139° (*c* 1.1, methanol) (Found: C, 53.07; H, 6.84, N, 3.64. $C_{19}H_{29}NO_{10}$ calc.: C, 52.90; H, 6.73; N, 3.25%).

Bioside **4** was homogeneous [p.c., and t.l.c. in benzene–methanol (3:7)] and gave a positive reaction with ninhydrin.

When a solution of **4** (8 mg) in methanol (1 ml) was treated with acetic anhydride

(0.2 ml) for 2 h at room temperature and then evaporated, crystallization of the residue from ethyl acetate–hexane gave **3** (7.3 mg), m.p. 117–118°, $[\alpha]_D^{20}$ +166° (*c* 1, methanol).

Alkaline hydrolysis of **3**. — Solutions of **3** (5 mg) in 2.5M sodium hydroxide (0.2 ml) were heated in sealed tubes for 2.5 and 5 h at 90°. After cooling, the solutions were passed through a column of Dowex-50 x4 (H^+) resin (1 ml) and eluted as described above to give neutral and alkaline fractions. The amounts of **3** and **4**, determined as described above, are shown in Table I. The characteristics of **4** were identical with those of the sample described above.

Hydrolysis of **4** *with acid.* — Aliquots (0.5 ml) of a solution of **4** (5 mg) in M hydrochloric acid (3 ml) were heated in sealed tubes at 100°, then cooled, and neutralized with M sodium hydroxide, and the volume of each solution was adjusted to 2 ml. 2-Amino-2-deoxyglucose was determined by a modified Elson–Morgan procedure[11], and mannose by the Park–Johnson method[12]. The results were as follows:

Time (h)	0.25	0.5	1	2
Mannose (%)	60 (33)	84 (55)	97 (79)	97 (97)

The figures in brackets show the corresponding release of galactose from **2**. After 2 h, 1% of 2-amino-2-deoxyglucose had been released.

In a separate run, the hydrolysate was neutralized with Amberlite IRA-400 (HCO_3^-) resin. P.c. and t.l.c. then revealed benzyl 2-amino-2-deoxy-α-D-glucopyranoside[1] and mannose.

Cleavage of **4** *by nitrous acid.* — (*a*) A solution of **4** (15 mg) in water (5 ml) was treated in succession with 33% acetic acid (5 ml) and 5% aqueous sodium nitrite (5 ml). The mixture was stirred for 30 min, then neutralized with Amberlite IRA-400 (HCO_3^-) resin, and filtered. The filtrate was extracted with ether (5 × 3 ml), and the extract was dried (Na_2SO_4) and concentrated to small volume. Benzyl alcohol was identified in the residue by g.l.c. at 120°, directly or as benzyl acetate after acetylation. The aqueous solution remaining after the ether extraction was treated overnight with an excess of potassium borohydride and then neutralized to pH 3 with KU-2(H^+) resin. After evaporation, boric acid was removed from the residue by several distillations of methanol therefrom. The residue was treated overnight with acetic anhydride–pyridine (1:1, 2 ml). The mixture was evaporated and the residue was dried, by distillation of toluene therefrom until the odour of pyridine was not detectable, to give the hepta-acetate **8** as a yellow syrup. A solution of the product in chloroform was passed through a small pad of silica gel, and evaporation then gave a colourless syrup **8** (4.5 mg), $[\alpha]_D^{20}$ +27° (*c* 0.3, chloroform). The material was deacetylated by heating with 2M methanolic hydrogen chloride (2 ml) for 30 min and the product was then hydrolysed with M sulphuric acid (2 ml) for 2 h at 100°. Mannose

and 2,5-anhydro-D-mannitol were subsequently identified by g.l.c. at 180° after reduction and acetylation.

(*b*) A solution of **4** (15 mg) in water (5 ml) was mixed with 0.45M sodium acetate buffer (pH 3.5, 5 ml) and 5% aqueous sodium nitrite (5 ml). The mixture was treated as described above. After acetylation, t.l.c. revealed **8** together with a product (~15%) having lower mobility. The acetate **8** was isolated by chromatography on silica gel and appeared to be identical with a specimen obtained as described above.

Periodate oxidation of **3** *and* **4**. — Solutions of **3** and **4** (3 mg of each) in 15mM sodium periodate (3 ml) were kept in the dark at room temperature. The consumption of periodate, monitored spectrophotometrically at 310 nm, was as follows:

Time (h)		0.1	0.25	0.5	1	2	4	8	22
Oxidant consumed (mol.)	**3**	0.75	1.28	1.66	2.02	2.15	2.50	2.80	2.93
	4	0.71	1.25	1.82	2.41	2.90	3.50	3.83	3.90

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