Carbohydrate Research, 72 (1979) 297–300

Note

Determination of the anomeric configuration of 2-acetamido-2-deoxy-D-glucopyranosides*

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(Received January 23rd, 1978; accepted for publication in revised form, August 1st, 1978)

Linkage analysis of polysaccharides available in quantities insufficient for determination by the usual physical methods is a major problem. Although cleavage by enzymes can sometimes be used, the specific enzymes are not always readily accessible. This is the case for glycosides of 2-acetamido-2-deoxy-D-glucose where the 2-amino-2-deoxy-α-D-glucosidases, although known, are not commercially available. In addition, these enzymes do not cleave glycosides having different aglycons to the same extent, nor does the cleavage often go to completion.

It was observed[1] recently that 7-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-L-*glycero*-D-*manno*-heptose, isolated from the lipopolysaccharide of *Bordetella pertussis*, could be transformed into 2-hydroxyethyl 2-acetamido-2-deoxy-D-glucopyranoside by successive treatments with borohydride, periodate, and borohydride, and that the peracetylated derivative was amenable to g.l.c. It was concluded that if conditions could be found under which the anomeric 2-hydroxyethyl 2-amino-2-deoxyglucosides could be separated, a micro-method for the determination of the chirality of the glycosidic bond between 2-acetamido-2-deoxy-D-glucose and the terminal position of any aldose would become available. A preliminary experiment in which 2-acetamido-2-deoxy-D-glucose was heated with ethylene glycol containing gaseous hydrogen chloride gave a mixture which, after acetylation, showed one major and one minor peak in g.l.c. The mass spectra for these components were identical, thus showing that the separation of the anomers was possible.

It has been shown previously that per(trimethylsilyl) derivatives of anomeric pairs of 1-*O*-D-glucopyranosylglycerol[2], 2-*O*-D-glucopyranosyl-D-erythritol[2], and 1-deoxy-3-*O*-L-rhamnopyranosyl-D-erythritol[3] can be separated by g.l.c. (*cf.* Ref. 4). Accordingly, chemical syntheses of 2-hydroxyethyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides were undertaken.

A crystalline product was obtained by condensation of 2-acetamido-2-deoxy-D-

*Dedicated to Professor Edgar Lederer on the occasion of his 70th birthday.

glucose with ethylene glycol in the presence of a strongly acidic ion-exchange resin followed by peracetylation. By its mode of formation, the product was assumed to be the α anomer and this was confirmed by ^{13}C-n.m.r. spectroscopy of the *O*-deacetylated compound.

Condensation of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′:4,5]-2-oxazoline[5] with 2-acetoxyethanol gave a mixture of the α and β isomers in the ratio of 1:3 as judged by g.l.c. The β isomer crystallised after p.l.c. on silica gel, followed by purification on Sephadex LH 20. Its structure was confirmed by ^{13}C-n.m.r. spectroscopy of the *O*-deacetylated compound.

The 2-hydroxyethyl 2-acetamido-2-deoxy-D-glucoside obtained from the disaccharide[1], as described above, was identical with the synthetic α anomer, thus providing definitive proof of its structure.

In addition, these syntheses allowed us to show that the 7-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-D-*glycero*-L-*manno*-heptopyranose obtained by condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphinylamino-α-D-glucopyranosyl bromide[6] with 1,2,3,4,6-penta-*O*-benzoyl-β-D-*glycero*-L-*manno*-heptopyranose[7], followed by removal of protecting groups and *N*-acetylation, was a mixture of α and β anomers.

As the α and β anomers are easily separable by t.l.c., it should be possible to perform linkage analyses using this method, either by performing the final reduction with sodium borotritiide or the acetylation step with ^{14}C-labelled acetic anhydride.

EXPERIMENTAL

Evaporations were carried out *in vacuo* at 40°. Melting points were determined on a Kofler hot-plate and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter. G.l.c. was performed with a Varian Aerograph 1800 instrument equipped with a flame-ionisation detector and a stainless-steel column (0.125 in. × 5 ft) packed with 3% of SE-30 on Varaport 30 (100–120 mesh), using a programme of 4°/min from 220→285°. Retention times (*T*) are given with respect to that of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose[8]. P.l.c. was performed on silica gel (Merck 60 PF_{254}) and t.l.c. on silica gel (F 1500 LS_{254}; Schleicher and Schüll) with benzene–ether–methanol (7:7:1). Migrations (R_X) are given relative to that of the same compound as used for g.l.c. ^{13}C-N.m.r. spectroscopy (external Me_4Si) was performed on the solutions of *O*-deacetylated (Zemplén) products.

*2-Acetoxyethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside* (**1**). — 2-Acetamido-2-deoxy-D-glucopyranose (5 g), freshly distilled ethylene glycol (100 ml), and dry Dowex-50(H^+) resin (7.5 g) were stirred at 70–75° for 66 h. The cooled supernatant was decanted from the resin, which was then washed with ethylene glycol (5 ml). The combined supernatants were concentrated at 80–90°/1 mmHg. The residue was acetylated with acetic anhydride (20 ml) and anhydrous sodium acetate (2 g) for 1 h at 100°. Acetic anhydride was removed by codistillation with

toluene, and a small portion of the residue was purified by p.l.c. and eluted with chloroform. After removal of solvents, **1** crystallised from water; m.p. 56–58°, $[\alpha]_D^{20}$ +90° (*c* 1, ethanol).

Anal. Calc. for $C_{18}H_{27}NO_{11} \cdot H_2O$: C, 47.89; H, 6.43. Found: C, 48.08; H, 6.37.

When dried at 40° *in vacuo* over P_2O_5, **1** gave a colourless, hygroscopic oil, *T* 2.24, R_X 1.0. ^{13}C-N.m.r. data: 179.7 (C=O), 98.5 (C-1α), 73.2 (C-5), 72.4 (C-1'), 71.3 (C-3), 70.1 (C-4), 61.9 (C-2',6), 54.8 (C-2), and 23.1 p.p.m. (Me) (*cf.* Ref. 9).

Anal. Calc. for $C_{18}H_{27}NO_{11}$: C, 49.88; H, 6.24. Found: C, 49.48; H, 6.02.

*2-Acetoxyethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**2**). — Anhydrous toluene (10 ml) was evaporated four times from toluene-*p*-sulphonic acid (15 mg). To the dry residue were added anhydrous nitromethane (25 ml), 2-acetoxyethanol (7.5 ml), and a solution of freshly prepared 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2',1':4,5]-2-oxazoline[5] in anhydrous toluene (25 ml). The mixture was heated at 117° for 1 h, cooled, diluted with chloroform (50 ml), washed with saturated, aqueous sodium hydrogen carbonate (5 ml) and then with water (5 ml), and dried (Na_2SO_4). The solvents were evaporated, and excess of 2-acetoxyethanol was removed at 100°/1 mmHg. A solution of the residue in water was decolourised with charcoal and concentrated, and the residue was dissolved in ethyl acetate. G.l.c. showed the presence of both α and β isomers in the ratio ~1:3. A preliminary purification by p.l.c. removed the α isomer, and the β isomer (400-mg portions) was then eluted from a column (2.5 × 100 cm) of Sephadex LH 20 with chloroform–ethanol (1:1). Crystallization from acetone–hexane gave **2**, m.p. 122–123°, $[\alpha]_D^{20}$ −13° (*c* 1, ethanol), *T* 2.44, R_X 0.78. ^{13}C-N.m.r. data: 176.1 (C=O), 102.5 (C-1β), 77.1 (C-5), 75.1 (C-1'), 72.3 (C-3), 71.15 (C-4), 61.8 (C-2',6), 56.8 (C-2), and 23.4 p.p.m. (Me).

Anal. Calc. for $C_{18}H_{27}NO_{11}$: C, 49.88; H, 6.24; N, 3.23. Found: C, 49.65; H, 6.21; N, 3.71.

*7-*O*-(2-Acetamido-2-deoxy-*D*-glucopyranosyl)-*D*-glycero-*L*-manno-heptopyranose* (**4**). — A mixture of 1,2,3,4,6-penta-*O*-benzoyl-β-D-glycero-L-*manno*-heptopyranose[7] (2 g), ethanol-free, freshly distilled chloroform (60 ml), finely powdered, dry mercuric cyanide (2 g), and molecular sieve (4 Å) was stirred and boiled under reflux for 1 h. Freshly prepared, dry 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphinylamino-α-D-glucopyranosyl bromide[6] (1.8 g) was added to the mixture which was stirred and boiled under reflux for 24 h [the condensation was monitored by t.l.c. (ethyl acetate–hexane, 2:1)]. The mixture was cooled, diluted with chloroform (60 ml), filtered, washed with ice-cold, saturated, aqueous sodium hydrogen carbonate (100 ml) and then with ice–water (2 × 100 ml), dried (Na_2SO_4), and concentrated. The residual syrup (3.5 g) was eluted from a column of silica gel (200 g) with ethyl acetate–hexane (1:2), to give 1,2,3,4,6-penta-*O*-benzoyl-7-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphinylamino-D-glucopyranosyl)-β-D-*glycero*-L-*manno*-heptopyranose (**3**, 2.2 g) as a powder.

Anal. Calc. for $C_{66}H_{60}NO_{22}P$: C, 63.41; H, 4.80; N, 1.12. Found: C, 63.08; H, 4.93; N, 0.96.

A solution of **3** (2 g) in acetic acid (100 ml) was hydrogenated at room temperature and pressure over platinum oxide (1 g). The inorganic phosphate[10] liberated was monitored and reaction was complete after 48 h. The filtered solution was concentrated (to ~20 ml), water (10 ml) was added, and the solution was again concentrated. This operation was repeated twice and the solution was then concentrated to dryness. To a solution of the residue in methanol (100 ml) were added freshly distilled triethylamine (1 ml) and water (1 ml), and the mixture was incubated at 50° for 50 h and then concentrated to dryness. A solution of the residue in water (15 ml) was passed through a column of Dowex-1 X8 (AcO^-) resin (100–200 mesh, 2 ml) and eluted with water (40 ml). The effluents were concentrated to dryness, the residue was dried *in vacuo* over P_2O_5 and then dissolved in anhydrous methanol (50 ml), and acetic anhydride (2.5 ml) was added. The mixture was left for 18 h at room temperature and then concentrated to dryness. The residue was triturated with ethanol to give **4** as a hygroscopic powder. G.l.c. of the borohydride–periodate–borohydride-treated material showed it to consist of a mixture of α and β isomers in the ratio ~1:2.

Compound **4** gave a single spot in t.l.c. (6:2:1 butanone–acetic acid–water) having mobilities of 0.45 and 0.52 with respect to 2-acetamido-2-deoxy-D-glucose and D-*glycero*-L-*manno*-heptose, respectively, when revealed with aniline hydrogen phthalate[11], the reagents for the detection of *N*-acetyl groups[12], and ethanolic H_2SO_4. It reacted positively in the cysteine–H_2SO_4 test[13] for heptoses and, after acidic hydrolysis, gave a positive reaction for hexosamine[14].

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