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Note

Determination, by gas liquid chromatography, of the anomeric configuration of a 2-acetamido-2-deoxy-D-glucopyranosyl residue linked to a secondary hydroxyl group of another sugar residue

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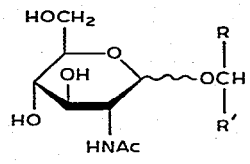
A convenient micromethod for the determination of the anomeric configuration of a 2-acetamido-2-deoxy-D-glucopyranosyl residue bound to the terminal position of an aldose residue has recently been described[1]. The present paper extends this work to provide for the identification of the chirality of the anomeric centre of 2-acetamido-2-deoxy-D-glucopyranosyl residue linked to a secondary hydroxyl group of another sugar residue. It is based on the observation[2] that when 2-*O*-(2-acetamido-2-deoxy-α,β-D-glucopyranosyl)-D-arabinose was successively treated with borohydride, periodate, and borohydride, and the resulting mixture was acetylated and then analyzed by g.l.c.–m.s., two peaks having identical mass spectra and corresponding to that of per-*O*-acetyl-2-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl) glycerols were detected. It was concluded that, if these very easily separable peaks could be identified as the α- and β-D anomers, respectively, a micromethod for the determination of the chirality of the glycosidic bond of a 2-acetamido-2-deoxy-D-glucopyranosyl residue bound to secondary alcohol groups of neutral sugars could be elaborated.

2-*O*-(2-Amino-2-deoxy-α and β-D-glucopyranosyl) glycerol have been synthesized by Hardy[3], and the β-D anomer was obtained later *via* another route by Antonenko *et al.*[4]. By use of the procedure of Antonenko *et al.*[4], but starting from 1,3-di-*O*-benzylglycerol[5] instead of the 1,3-benzylidene derivative, the *N*-acetylated β-D anomer and its crystalline peracetate were prepared in better yield. Co-chromatography of the authentic β-D anomer with the mixture obtained by degradation of the disaccharide mentioned earlier permitted unambiguous identification of the isomers; in the conditions used, the α-D anomer had the shorter retention time (*t* 3.22).

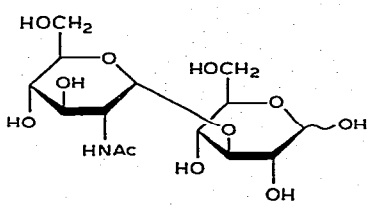
It is known that disaccharides giving rise to hydroxymalonaldehyde derivatives undergo overoxidation. The initial borohydride reduction of the disaccharide mentioned earlier was therefore introduced to obtain 2-*O*-(2-acetamido-2-deoxy-D-glucopyranosyl)glycerol (**2**) *via* the glyceraldehyde derivative **1**, rather than *via* the

hydroxymalonaldehyde derivative **3**. The initial, borohydride reduction-step is, however, not always necessary; it has to be performed or omitted according to the



1 R = CH_2OH, R′ = CHO
2 R = R′ = CH_2OH
3 R = R′ = CHO

structure under investigation. Thus, when 3-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-D-glucose (**4**) and 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose (**5**) were analyzed, the initial reduction-step was omitted. Indeed, oxidation of these disaccharides with one molar equivalent of periodate cleaved the C-1–C-2 linkage and led to the formyl esters **6** and **7**, which are protected against further oxidation. Sequential treatment with borohydride, periodate, and again borohydride then led to the α- and β-D-glycosylglycerol derivatives (**2**). Intermediary formation of the glycosyl(hydroxymalonaldehyde) (**3**) was thus avoided: this would not have been the case had the initial reduction step been performed.



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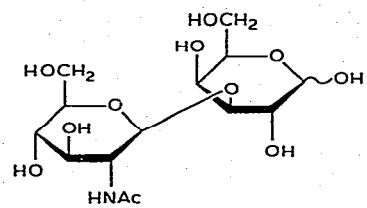

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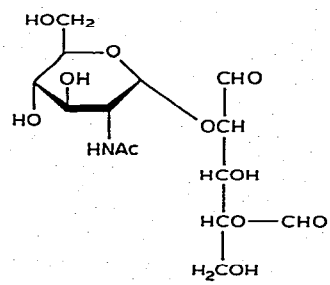

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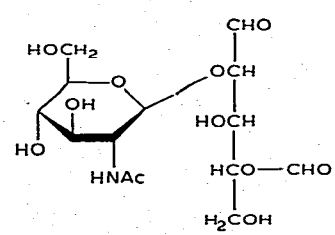

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EXPERIMENTAL

General methods. — Melting points were determined with a Kofler hot-plate and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter. Solvents were evaporated *in vacuo* at a temperature below 40°. P.l.c. was performed on Silica gel (Merck 60 PF_{254}), and g.l.c. with a Varian Aerograph 1800 instrument, equipped with a flame-ionization detector and a stainless-steel column (0.3 × 150 cm) packed with 3% of SE 30 on Varaport 30 (100–120 mesh), and programmed for a temperature rise 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[6].

2-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-1,3-di-O-benzylglycerol (**8**). — *p*-Toluenesulfonic acid (15 mg) and a solution of 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[7] (2.8 g) in anhydrous toluene (30 mL) were added to a solution of 1,3-di-*O*-benzylglycerol[5] (2.2 g) in dry nitromethane (25 mL). The mixture was stirred for 50 min at 110°, cooled, and diluted with chloroform (60 mL). The solution was washed with a saturated solution of sodium hydrogencarbonate and dried (Na_2SO_4). Solvents were removed and the brown residue, dissolved in methanol, was treated with sodium methoxide overnight at room temperature. Solvents were removed and the yellow residue was chromatographed on a column of silica gel (200 g) with 20:3 (v/v) chloroform–methanol to give crystalline (ethanol) **8** (1.9 g), m.p. 145°, $[\alpha]_D^{25}$ –24° (*c* 0.5, chloroform).

Anal. Calc. for $C_{25}H_{33}NO_8$: C, 63.15; H, 7.00; N, 2.94. Found: C, 62.91; H, 6.85; N, 2.77.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3-di-O-acetylglycerol (**9**). — The dibenzyl ether **8** (0.5 g) was hydrogenated in glacial acetic acid solution (15 mL) in the presence of 10% palladium-on-charcoal (0.2 g) overnight at room temperature and atmospheric pressure. After removal of the catalyst and the solvent, the residue was treated with acetic anhydride (2.5 mL) and anhydrous sodium acetate (0.2 g) for 1 h at 100°. After the usual work-up, the crystalline (ethanol) peracetate had m.p. 147–148°, $[\alpha]_D^{25}$ —15.5° (*c* 1, chloroform); g.l.c.: 3.56.

Anal. Calc. for $C_{21}H_{31}NO_{13}$: C, 49.89; H, 6.18; N, 2.77. Found: C, 49.71; H, 6.19; N, 2.61.

Procedure 1. — A solution of 40mM sodium metaperiodate (25 μl, corresponding to 1 μmol of the oxidant) was added to a sample (490 μg, 1 μmol) of the trisaccharide 3,6-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose[9] in water (100 μL), and the mixture was kept for 30 min at room temperature. An aqueous solution (250 μL) of 1% (w/v) sodium borohydride was added; 30 min later, the solution was acidified with dilute acetic acid (1%), and then evaporated to dryness. Methanol (2 mL) was added to and evaporated from the dry residue three times. The residue was taken up in water (100 μL), and the oxidation–reduction procedure was repeated. Acetic anhydride (250 μL) and dry sodium acetate (50 mg) were added to the dry residue, the mixture was kept for 30 min at 100°, acetic anhydride was

removed by co-distillation with toluene, the residual solid was extracted with ethyl acetate (2 × 500 μL), and the extract was evaporated to dryness. The sample was dissolved in ethyl acetate (100 μL), and aliquots (1 μL) were used for analysis by g.l.c. Two peaks corresponding to 2-acetoxyethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[1] (*t*, 2.44) and 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3-di-*O*-acetylglycerol (*t*, 3.56), respectively, were observed and identified by g.l.c.–m.s.

Procedure 2. — An aqueous solution (250 μL) of 1% (w/v) sodium borohydride was added to a sample (320 μg, ~1 μmol) of disaccharide **4** or **5** in water (100 μL), and the mixture was kept for 30 min at room temperature. The solution was then acidified with dilute acetic acid (1%) and evaporated to dryness. Methanol (2 mL) was added to and evaporated from the dry residue three times. The residue was taken up in water (100 μL), a solution of 40mM sodium metaperiodate (75 μL, ~3 μmol) was added, and the mixture was kept for 30 min at room temperature. The solution was then reduced and acetylated as described in Procedure 1. It is possible to conduct the analysis of the disaccharide without performing an initial reduction with sodium borohydride.

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