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CHARACTERIZATION OF 2-AMINO-2,6-DIDEOXY-D-GLUCOSE AS A CONSTITUENT OF THE LIPOPOLYSACCHARIDE ANTIGEN OF *Pseudomonas aeruginosa* IMMUNOTYPE 4*

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ABSTRACT

The title lipopolysaccharide was freed from its lipid A component by mild, acid hydrolysis, to give a polysaccharide fraction that was subsequently hydrolyzed completely to afford a mixture of neutral sugars and amino sugars. The amino sugars were separated, and identified as 2-amino-2-deoxy-D-galactose, 2-amino-2,6-dideoxygalactose as a 2:1 mixture of the D and L enantiomers, and 2-amino-2,6-dideoxy-D-glucose. A reference sample of 2-amino-2,6-dideoxy-D-glucose was synthesized by an improved preparative route. Among the lipopolysaccharide antigens of the seven recognized immunotypes of *Pseudomonas aeruginosa*, 2-amino-2,6-dideoxyglucose is also characterized as a constituent of two others, types 3 and 5.

INTRODUCTION

The preceding report[1] described the detailed analytical characterization of lipopolysaccharide antigens from seven serotype strains of *Pseudomonas aeruginosa* that represent the seven classes (immunotypes) of cross-protective homogeneity in the classification scheme of Fisher *et al.*[2]. A combination of these seven antigens has been employed as a heptavalent vaccine[3] (Pseudogen®) for immunization against infection by *P. aeruginosa*. The analytical data[1] showed in particular that the qualitative and quantitative distribution of amino sugars in each of the seven immunotype antigens displays a characteristic pattern that might constitute the basis of a chemical method for differentiating the immunotype strains of *P. aeruginosa*.

All seven immunotype antigens contain an aminodideoxyhexose component[4], which was unambiguously identified[1] as 2-amino-2,6-dideoxy-DL-galactose (DL-fucosamine) in the type 2 antigen. All but the type 5 antigen contain 2-amino-2,6-dideoxygalactose; a second aminodideoxy sugar is also present in the types 3 and 4 antigens, and it comprises the sole aminodideoxy sugar in the type 5 antigen. The

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two aminodideoxy sugars are separable by g.l.c. of their alditol acetates, but exhibit very similar retention-times[1].

This report describes the isolation of both aminodideoxy sugars from the type 4 antigen, and their characterization on a crystalline basis. The 2-amino-2,6-dideoxygalactose is found to be a 2:1 mixture of the D and L forms, and the second amino sugar is shown to be 2-amino-2,6-dideoxy-D-glucose (D-quinovosamine) by comparison with a synthetic sample of this sugar. The second amino sugar in the type 3 antigen is also characterized as 2-amino-2,6-dideoxyglucose.

DISCUSSION

Examination of the analytical data[1] for the seven lipopolysaccharide antigens (immunotypes 1–7 as classified by Fisher *et al.*[2]) showed that types 3 and 4 both contain two aminodideoxyhexoses, one of which was recognized as 2-amino-2,6-dideoxygalactose from the g.l.c. and mass-spectral behavior of its alditol acetate. The second aminodeoxy sugar in the type 4 antigen amounted to 10% of the total amino sugar fraction (2.7% of the original lipopolysaccharide), whereas, in the type 3 antigen, it constituted only 4% of the amino sugar fraction (0.7% of the original lipopolysaccharide). Accordingly, the type 4 antigen was selected as the richer source for isolation and detailed characterization of the second aminodideoxy sugar*.

Mild hydrolysis of the immunotype 4 lipopolysaccharide with 1% acetic acid at 100° cleaved off the water-insoluble, lipid A portion (13%). Dialysis of the aqueous solution gave a polysaccharide fraction (42%) that was further fractionated on a column of Sephadex G-75 to remove a high-molecular weight component eluted in the void volume and a late-eluted fraction of relatively low molecular weight; the main fraction (29% of the original antigen) is presumed to be a mixture of the "O-specific chain" and "core" polysaccharides[6] of the antigen. More-vigorous hydrolysis of this polysaccharide fraction, with 0.25M sulfuric acid for 16 h at 100°, gave the neutral sugars rhamnose and glucose (6% and 12%, respectively, in the fraction as determined by g.l.c. of the alditol acetates[1,7]), together with a mixture (34%) of two 2-amino-2,6-dideoxyhexoses; the remaining material appeared to be 2-amino-2-deoxygalactose.

The polysaccharide fraction was hydrolyzed on a preparative scale with 2M hydrochloric acid, and amino sugars were separated from neutral sugars by ion-exchange chromatography. The neutral-sugar fraction showed (papergram) only two components, having the respective characteristics of glucose and rhamnose. Three components were present in the amino sugar fraction, having paper-chromatographic characteristics of 2-amino-2,6-dideoxygalactose, 2-amino-2,6-dideoxyglucose, and 2-amino-2-deoxygalactose. 2-Amino-2-deoxyglucose, although present[1] in the intact,

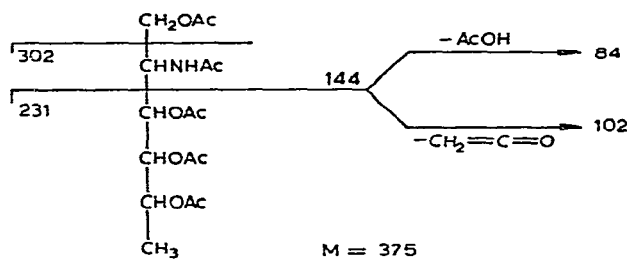
*The type 5 antigen, which is still richer in this component (8.4% of total lipopolysaccharide), was available in only limited amount when this work was conducted. Subsequent investigations[5] have established that 2-amino-2,6-dideoxy-D-glucose is the aminodideoxy sugar component of the type 5 antigen.

type 4 lipopolysaccharide, was not found in this fraction; presumably, it is removed in the lipid A component.

The three amino sugars were separated by preparative, paper chromatography, and the identity of each was established by comparison with authentic reference samples of their hydrochlorides, *N*-acetyl derivatives, and alditol acetates. Full details are recorded in the Experimental section. 2-Amino-2-deoxy-D-galactose was obtained as its crystalline hydrochloride, whose specific rotation established that it was the D enantiomer.

The 2-amino-2,6-dideoxygalactose, whose identity (except for enantiomeric assignment) in the type 4 antigen had already been established[1], was isolated crystalline as its hydrochloride, whose specific rotation (+40°, equil.), in comparison with the equilibrium rotations recorded[8] for the D and L enantiomers respectively (+93° and −95°), indicated that this amino sugar is an approximately 2:1 mixture of 2-amino-2,6-dideoxy-D-galactose and its L enantiomer. It is noteworthy that the diamino sugar from the type 2 antigen has been characterized[1] as a mixture of equal parts of 2-amino-2,6-dideoxy-D- and -L-galactose.

The third amino sugar was not obtained crystalline, but it was identified as 2-amino-2,6-dideoxy-D-glucose hydrochloride from its specific rotation, by comparison with an authentic sample, and by preparation of derivatives. *N*-Acetylation gave crystalline 2-acetamido-2,6-dideoxy-α-D-glucose, showing downward mutarotation and a final rotation closely corresponding to that of an authentic, reference sample; the two samples gave identical X-ray powder diffraction patterns. The derived alditol acetate gave a mass spectrum practically identical to that of the corresponding galactose analogue; the principal fragmentation-pathways observed are summarized in Fig. 1.



m/e 332 (M−Ac)
m/e 316 (M−$AcNH_2$)
m/e 315 (M−AcOH)
m/e 260 (302−CH_2=C=O)
m/e 242 (302−AcOH)

Fig. 1. Principal mass-spectral fragmentations observed with 2-acetamido-1,3,4,5-tetra-*O*-acetyl-2.6-dideoxyhexitols.

Essentially the same procedures as those described for isolation of the aminodideoxy sugars from the type 4 lipopolysaccharide were also performed with the immunotype 3 lipopolysaccharide, but with a smaller sample of material. Full details are recorded in the Experimental section. Three amino sugars were again isolated from the lipid A-free, dialyzed polysaccharide; they were found to be chromatographically indistinguishable (as their hydrochlorides and *N*-acetyl derivatives) from authentic samples of 2-amino-2-deoxygalactose, 2-amino-2,6-dideoxygalactose, and 2-amino-2,6-dideoxyglucose, respectively; the samples obtained were insufficient for polarimetric measurement to establish enantiomeric identity.

To provide reference samples of 2-amino-2,6-dideoxy-D-glucose and its hydrochloride, a sequence of reactions was performed to convert 2-amino-2-deoxy-D-glucose into its 6-deoxy derivative. 2-Acetamido-2-deoxy-D-glucose was converted[9] into a mixture of methyl glycopyranosides, which was benzylidenated with α,α-dimethoxytoluene[10] to give methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α,β-D-glucopyranoside. Treatment of the latter with *N*-bromosuccinimide in carbon tetrachloride[11], and chromatographic purification of the product, afforded methyl 2-acetamido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-α-D-glucopyranoside, which was hydrogenolyzed in the presence of Raney nickel to give crystalline methyl 2-acetamido-4-*O*-benzoyl-2,6-dideoxy-α-D-glucopyranoside. Complete hydrolysis of this product with 2M hydrochloric acid furnished 2-amino-2,6-dideoxy-D-glucose hydrochloride, *N*-acetylation of which gave crystalline 2-acetamido-2,6-dideoxy-α-D-glucose having m.p. and specific rotation in good accord with literature values[12].

The foregoing route is preparatively convenient in scaled-up reactions, as the difficultly separable[9], anomeric glycosides are carried together through the benzylidenation procedure[10] and the reaction with *N*-bromosuccinimide[11]; subsequent hydrogenolysis gives the crystalline, protected aminodideoxy sugar in reproducible net yield with few manipulative steps. The literature procedure of Kuhn *et al.*[12] involves selective monosulfonylation of ethyl 2-acetamido-2-deoxy-β-D-glucopyranoside followed by deoxygenation at C-6 *via* the 6-iodo derivative.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Solutions were evaporated *in vacuo* below 50°. Specific rotations were measured in 1-dm tubes with a Perkin–Elmer Model 141 polarimeter. Mass spectra were recorded with an AEI MS-9 double-focusing, high-resolution spectrometer operating at an ionizing potential of 70 eV and an accelerating potential of 8 kV, and the direct-inlet source was maintained at 150°. G.l.c. was performed on a Hewlett–Packard 5720-A gas chromatograph equipped with a flame-ionization detector, on a glass column (300 × 0.15 cm) packed with 3% of ECNSS-M on Gaschrom Q (Applied Science Laboratories, State College, Pa.) maintained isothermally at 200°, with helium (flowrate 25 ml/min) as the carrier gas. Paper chromatography was performed on Whatman No. 1 paper (analytical) and 3MM (preparative) with 4:1:5 1-butanol–ethanol–water (upper phase, solvent A) and

2:5:5 pyridine–ethyl acetate–water (upper phase, solvent B). Elemental analyses were made by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. The camera diameter was 114.59 mm. Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The strongest lines are numbered (1, strongest).

Characterization of the amino sugars in the lipopolysaccharide antigen of Pseudomonas aeruginosa *immunotype 4*

Degradation of the type 4 lipopolysaccharide. — The type 4 lipopolysaccharide (1.0 g) in 1% aqueous acetic acid (500 ml) was heated for 80 min at 100°, and the resultant mixture was then centrifuged at 6500 *g* for 1 h. The supernatant solution was dialyzed against distilled water for 2 h in a Bio-Fiber 50 beaker (100-ml size; nominal, molecular-weight cut-off, 5000), and the resultant solution was freeze-dried to yield 452.1 mg (45.2%) of type 4 degraded polysaccharide. The precipitate from the centrifugation was dissolved in chloroform, and the solution was dried (magnesium sulfate) and evaporated to give type 4 lipid A (127.3 mg, 12.7%)*.

Gel-permeation chromatography of type 4 degraded polysaccharide. — The polysaccharide from the preceding experiment (452 mg) was dissolved in water (5 ml) and applied to a column (70 × 2.6 cm) of Sephadex G-75, which was eluted with water at a flow-rate of 35 ml/h, with monitoring of the effluent by means of a refractive-index monitor (Pharmacia, Model, 300L). Three peaks were observed, and the corresponding fractions were pooled and freeze-dried. The first component, eluted in the void volume (50–130 ml), appeared to be undegraded lipopolysaccharide (52.5 mg, 11.6%). The second component (130–250 ml) appeared to be a mixture of "O-side chain" and "core" polysaccharides (317.5 mg, 70.2%) that is designated type 4 lipid A-free polysaccharide. The third component (250–320 ml), yield 33 mg (7.3%), appeared to consist of degradation products of low molecular weight, but it possibly contained some "core" material.

Hydrolysis of type 4 lipid A-free polysaccharide, and analysis of sugar components. — *A. Analysis for neutral sugars.* The polysaccharide fraction (5.7 mg), together with D-mannose (3.5 mg, added as an internal standard), were heated in 0.25M sulfuric acid for 16 h at 100°. The solution was made neutral with barium carbonate, and the monosaccharide components were analyzed by g.l.c. of the derived alditol acetates by the procedure already described[1,7]. Neutral sugars detected were rhamnose (5.6%, T_R 0.23) and glucose (12%, T_R 1.00), together with 34% of a 3:2 mixture of components (T_R ~1.51 and 1.59, respectively) corresponding to 2-amino-2,6-dideoxyglucose and 2-amino-2,6-dideoxygalactose. The sugars gave alditol acetates having g.l.c. retention-times and mass spectra identical to those of authentic, reference standards. No components corresponding to dideoxy sugars were observed, but further experiments indicated that 2-amino-2-deoxygalactose was a major constituent

*This value is lower than that (19%) obtained[1] when a longer time of hydrolysis and a stage of extraction by chloroform were used to separate the lipid A component more completely.

of the material; possibly, it was not completely liberated by the hydrolysis procedure used.

The quantitative differences between these sugar analyses and those reported[1] for the lipid A-free polysaccharide of the type 4 antigen presumably result from selective removal of certain constituents in the gel-permeation step employed here.

B. Hydrolysis to obtain the amino sugars. — The polysaccharide fraction (221.5 mg) in 2M hydrochloric acid (100 ml) was heated for 2 h at 100°. The mixture was evaporated at 40° with addition of 1-propanol several times during the evaporation (to remove volatile acid and prevent development of a high concentration of acid). A 10-mg aliquot of the resultant, brown syrup (230 mg) was dissolved in water, and passed through a column (40 × 1.6 cm) of Sephadex G-15 that was then eluted with water. A single, symmetrical peak was observed in the elution region (41–45 ml) anticipated for monosaccharides, together with material eluted later that was presumed to consist of degradation products.

The main batch of hydrolyzate (220 mg) was dissolved in water (5 ml), and applied to a column (10 × 1.5 cm) of Dowex 50W-X8 (H^+) ion-exchange resin. Elution with water (80 ml), and evaporation of the effluent, gave the neutral sugars (65.8 mg, 30%) as a brown, partially crystallized syrup. Paper chromatography of this mixture (solvent A) showed two components, behaving as glucose (R_{Glc} 1.00) and rhamnose (R_{Glc} 2.37). Subsequent elution with 0.5M hydrochloric acid (100 ml), and evaporation of the effluent with use of 1-propanol as before, gave the amino sugars as a mixture (141 mg, 64%).

Resolution of the amino sugars from the hydrolyzate of the type 4 lipid A-free polysaccharide. — Paper chromatography of the amino sugar fraction with solvent A showed three components, all ninhydrin-positive, indistinguishable in chromatographic characteristics from 2-amino-2-deoxygalactose (R_{GlcN} 0.88), 2-amino-2,6-dideoxygalactose (R_{GlcN} 1.60), and 2-amino-2,6-dideoxyglucose (R_{GlcN} 1.87).

The mixture of amino sugars (109.8 mg) was resolved by preparative, paper chromatography (2 sheets, 25 × 40 cm) with solvent A as eluant, by the descending method for 50 h. Guide strips from the sides and center of sheets were sprayed with ninhydrin, and the appropriate bands were excised, eluted with water, and the extracts evaporated, to give the chromatographically homogeneous amino sugars as their hydrochlorides. The fraction having R_{GlcN} 0.88 was obtained as a solid (64.8 mg, 59%) that was identified as 2-amino-2-deoxy-D-galactose hydrochloride by its chromatographic characteristics (including g.l.c. of the derived alditol acetate) and specific rotation: $[\alpha]_D$ +89° (equil., *c* 0.8, water), lit.[13] +47.3 → +91.5° (*c* 1, water).

The fraction having R_{GlcN} 1.60 was obtained as a white, crystalline solid (10.4 mg, 9.5%) having $[\alpha]_D^{20}$ +47 → +40° (30 min; equil., *c* 0.7, water) that was identical in all chromatographic characteristics (papergram in solvent A, and as its *N*-acetyl derivative in solvent B; and g.l.c. of the alditol acetate) with a reference sample of 2-amino-2,6-dideoxy-D-galactose hydrochloride. The observed, final rotation (+40°) of the product, in comparison with literature values for the D

enantiomer[8] (+93°, in water) and L enantiomer[8] (−95° in water), indicated that the product was an approximately 2:1 mixture of the D and L forms.

The fraction having R_{GlcN} 1.87 was isolated as a colorless syrup (13.1 mg, 12%) that had chromatographic characteristics [papergram, solvent A (lit.[8] R_{GlcN} 1.73), and g.l.c. of the derived alditol acetate] identical to those of a reference sample of 2-amino-2,6-dideoxy-D-glucose hydrochloride. Its specific rotation, $[\alpha]_D^{20}$ +46° (*c* 0.8, water), corresponded closely to that recorded[12] ($[\alpha]_D^{20}$ +53° in water) for the authentic, D enantiomer.

N-*Acetylation of the 2-amino-2,6-dideoxyhexoses.* — The 2-amino-2,6-dideoxy-D(and L)-galactose hydrochloride (9.9 mg) from the preceding experiment was dissolved in water (2 ml). To the solution were added acetic anhydride (0.5 ml), methanol (0.5 ml), and Dowex 1-X8 (CO_3^{2-}) resin, and the mixture was stirred for 2 h at 5°. An additional 0.5 ml of acetic anhydride was then added, and the mixture was stirred for a further 2 h at ~25°. After removal of the resin, the solution was evaporated. The residue was dissolved in water (2 ml), and the solution was applied to a column (5 × 1 cm) of Dowex 50W-X8 (H^+) resin. The column was eluted with water (75 ml), and the effluent was evaporated to give *2-acetamido-2,6-dideoxy-D(and L)-galactose* as a colorless syrup; yield 7.9 mg (78%), $[\alpha]_D^{20}$ +22° (*c* 0.8, water); R_{GlcNAc} 1.35 and R_{Gal} 2.38 in solvent B. Comparison with literature data for 2-acetamido-2,6-dideoxy-D-galactose[8] ($[\alpha]_D$ +129 → +92° in water) and the L enantiomer[8] ($[\alpha]_D$ −116 → −82° in water) indicated that the product was an approximately 2:1 mixture of the D and L forms.

N-Acetylation of the 2-amino-2,6-dideoxyglucose (13.1 mg) from the antigen, by the same procedure as just described, gave *2-acetamido-2,6-dideoxy-D-glucose* as a crystalline solid; yield 8.0 mg (59%), $[\alpha]_D^{20}$ +56 → +11° (3 h; equil., *c* 0.8, water); R_{FucNAc} 1.15, R_{GlcNAc} 1.55, and R_{Gal} 2.38 in solvent B (lit.[14] R_{FucNAc} 1.20, R_{Gal} 3.07 in solvent B). Recrystallization from acetone–petroleum ether (b.p. 30–60°) gave the product as white crystals, m.p. 192–194°; X-ray powder diffraction data, 10.27 w, 9.06 vs (2), 5.89 m, 5.15 w, 4.43 vs (3), 4.28 vs (1), 3.67 w, 3.36 m, and 2.90 m.

For this compound, the literature records melting points of 201–206° (from natural sources)[15] and 209–211° (synthetic)[12], and $[\alpha]_D^{20}$ +63 → +15° (equil., *c* 1, water)[12]. The product was identical in all respects to the synthetic material reported here.

2-Acetamido-1,3,4,5-tetra-O-acetyl-2,6-dideoxy-D-glucitol. — A solution of the isolated 2-acetamido-2,6-dideoxy-D-glucose (2.7 mg) in water (5 ml) was treated for 2 h with sodium borohydride (10 mg), and the resultant alditol was isolated, and acetylated with acetic anhydride–pyridine as already described[1], to yield the product (4.1 mg, 83%) as a slightly yellow oil; T_R 1.51 relative to glucitol hexaacetate, 4.20 relative to arabinitol pentaacetate, 0.95 relative to the alditol peracetate of 2-amino-2,6-dideoxygalactose (lit. values[14] 4.15 relative to arabinitol pentaacetate, 0.96 relative to the alditol peracetate of 2-amino-2,6-dideoxygalactose); *m/e* 417 (0.1, M+Ac), 375 (0.2, M^+), 316 (0.5, M−$AcNH_2$), 302 (5), 260 (6), 201 (7), 195 (7), 144 (30, $AcOCH_2$–$\overset{+}{C}H$–NHAc), 102 (24), 84 (45), and 43 (100).

Isolation of the amino sugars in the lipopolysaccharide antigen of Pseudomonas aeruginosa *immunotype 3*

Degradation of the type 3 lipopolysaccharide. — The immunotype 3 antigen (50 mg) in 1% aqueous acetic acid (25 ml) was heated for 90 min at 100°. The mixture was centrifuged, and the supernatant liquor dialyzed, as already described for the type 4 degraded polysaccharide. The polysaccharide fraction (23 mg) was not further fractionated, but was subjected directly to the next step.

Amino sugar components of the type 3 lipid A-free polysaccharide. — The polysaccharide fraction (20 mg) in 2M hydrochloric acid (10 ml) was heated for 5 h at 100°, and the solution was evaporated (with periodical additions of 1-propanol) to give a light-brown syrup (22.4 mg), which was dissolved in water (3 ml) and placed on a column (5 × 1 cm) of Dowex 50W-X8 (H^+) resin. Elution with water (100 ml) gave a neutral sugar fraction (8.2 mg), and elution with M hydrochloric acid (150 ml) gave the amino sugar fraction (10.6 mg).

Paper chromatography (solvent A) showed three ninhydrin-positive components, corresponding to the three components already found in the hydrolyzate from the type 4 amino sugar fraction; they had R_{GlcN} 0.94 (2-amino-2-deoxygalactose), R_{GlcN} 1.53 (2-amino-2,6-dideoxygalactose), and 1.83 (low intensity, 2-amino-2,6-dideoxyglucose .

N-Acetylation of the mixture of amino sugars, as described for the products from the type 4 antigen, gave a mixture of three components (papergram, solvent B; silver nitrate detection) corresponding to 2-acetamido-2-deoxygalactose (R_{GlcNAc} 0.95), 2-acetamido-2,6-dideoxygalactose (R_{GlcNAc} 1.34), and (low intensity), 2-acetamido-2,6-dideoxyglucose (R_{GlcNAc} 1.57).

*Improved synthesis of 2-amino-2,6-dideoxy-*D*-glucose*

*Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α,β-*D*-glucopyranoside.* — A suspension of 2-acetamido-2-deoxy-D-glucose (10.0 g, 45 mmol) and dry Amberlite IR-120 (H^+) resin (10 g) in dry methanol (300 ml) was boiled for 3 h under reflux. The resin was removed, and washed with methanol, and the combined solutions were evaporated, to give 9.0 g (85%) of syrupy *methyl 2-acetamido-2-deoxy-α,β-*D*-glucopyranoside*, $[\alpha]_D^{20}$ +70° (*c* 0.6, water) (lit.[9] for the α anomer, $[\alpha]_D$ +131° in water; for the β anomer[9], $[\alpha]_D$ −47° in water).

The foregoing glycoside mixture (160 g, 68 mmol) in *N,N*-dimethylformamide (110 ml) together with α,α-dimethoxytoluene (24 g) and *p*-toluenesulfonic acid (200 mg) was heated for 2 h at 60° under vacuum (water aspirator)[10]. The mixture was then poured into ice–water containing sodium hydrogencarbonate (15 g), and the precipitate formed was filtered off and washed with water. The solid was dissolved in chloroform, and the solution was dried (sodium sulfate), and evaporated to a solid that was recrystallized from methanol to give the title compound; yield 13.0 g (56%), $[\alpha]_D^{20}$ +3° (*c* 0.3, chloroform) (lit.[16] for the α anomer, $[\alpha]_D$ +19° in chloroform; for the β anomer[16], $[\alpha]_D$ −40° in chloroform).

*Methyl 2-acetamido-4-O-benzoyl-6-bromo-2,6-dideoxy-α-*D*-glucopyranoside.* —

The foregoing mixture of glycosides (9.0 g, 28 mmol) and barium carbonate (4 g) were suspended in dry carbon tetrachloride (500 ml), *N*-bromosuccinimide (5.4 g, 31 mmol) in carbon tetrachloride (30 ml) was added, and the mixture was boiled for 3 h under reflux. The mixture was filtered, the filter was washed with hot carbon tetrachloride, and the combined filtrates were evaporated. The product was purified on a column (80 × 2 cm) of silica gel-60 (Merck No. 7734) with 10:1 chloroform–methanol as eluant, to give the pure α anomer as a chromatographically homogeneous syrup; yield 4.7 g (44%), $[\alpha]_D^{22}$ +29° (*c* 0.5, chloroform) (lit.[11] $[\alpha]_D$ +29.4° in chloroform).

Methyl 2-acetamido-4-O-benzoyl-2,6-dideoxy-α-D-glucopyranoside. — A solution of the preceding bromo glycoside (4.5 g, 11 mmol) in ethyl acetate (50 ml), together with freshly prepared Raney nickel (2 g) and triethylamine (5 ml), was shaken for 4 h at ~25° under hydrogen at a pressure of 60 lb. in.$^{-2}$. The mixture was filtered, the filtrate extracted with water, the dried (sodium sulfate) organic phase evaporated, and the product crystallized from chloroform–petroleum ether (b.p. 30–60°); yield 2.5 g (69%), m.p. 100–102° (lit.[11] m.p. 92–94° without recrystallization), $[\alpha]_D^{20}$ +24° (*c* 0.2, chloroform); n.m.r. (100 MHz, chloroform-*d*): δ 8.10 and 5.25–7.60 (5-proton m, aryl protons), 6.15 d ($J_{2,NH}$ 8 Hz, NH), 4.97 t ($J_{3,4} \approx J_{4,5} = 9$ Hz, H-4), 4.72 d ($J_{1,2}$ 4 Hz, H-1), 4.4–3.6 m (H-2,3,5), 3.40 s (OCH_3), 1.97 s (Ac), and 1.21 d ($J_{5,6}$ 6 Hz, H-6); *m/e* 291 (1), 245 (16), 219 (12), 201 (3), 163 (4), 141 (8), 122 (6), 114 (10), 105 (100), 101 (48), 85 (28), 83 (43), 77 (25), 59 (35), and 43 (25).

2-Amino-2,6-dideoxy-D-glucopyranose hydrochloride. — The foregoing glycoside (2.2 g, 7 mmol) in 2M hydrochloric acid (100 ml) was boiled for 1.5 h under reflux with stirring. The solution was evaporated, with periodical additions of 1-propanol to prevent development of excess acidity. The residue was dissolved in water (10 ml), and transferred to a column (25 × 2 cm) of Dowex 50W-X8 (H^+) resin, which was washed initially with water (100 ml) and then with 0.5M hydrochloric acid (200 ml). The acid effluent was evaporated to dryness, to give colorless, syrupy, amino sugar hydrochloride; yield 0.8 g (59%); $[\alpha]_D^{20}$ +52.5° (*c* 0.5, water); homogeneous by paper chromatography in 4:1:4 1-butanol–ethanol–water (upper phase).

This compound has been reported[12] as having m.p. 163–170°, $[\alpha]_D$ +88 → +53° (*c* 0.5, water).

2-Acetamido-2,6-dideoxy-D-glucose — The preceding amino sugar hydrochloride (0.30 g, 1.5 mmol) in water (10 m) was *N*-acetylated essentially as described for the material derived from the natural product. The solid product, yield 0.25 g (81%), was recrystallized from acetone; m.p. 205–208°, $[\alpha]_D$ +65 (2 min)→ +15° (*c* 0.3, water) (lit.[12] m.p. 209–211°, $[\alpha]_D$ +63 → +15° in water); X-ray powder diffraction data: 10.39 m, 9.06 vs (2), 5.89 s, 5.08 m, 4.59 w, 4.43 vs (3), 4.29 vs (1), 3.70 m, 3.35 s, 3.07 w, 3.02 w, and 2.90 m.

Anal. Calc. for $C_8H_{15}NO_5$: C, 46.82; H, 7.37; N, 6.83. Found: C, 47.05; H, 7.65; N, 6.99.

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