THE SYNTHESIS OF D-*glycero*-D-*manno*-HEPTOSE

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ABSTRACT

The condensation of D-altrose with nitromethane in alkaline methanol gave the corresponding epimeric 1-deoxy-1-nitroheptitols from which 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol was obtained in 46% yield. The 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol was converted to D-*glycero*-D-*manno*-heptose in 61% yield.

DISCUSSION

D-*glycero*-D-*manno*-Heptose is a component of an extracellular polysaccharide synthesized by *Azotobacter indicum* (1, 2), whose mode of biosynthesis has been studied in this laboratory (3). In the course of these studies it became necessary to synthesize the heptose in order to devise a method for its stepwise degradation to determine the ^{14}C content at the individual carbon atoms in its chain, and to prepare D-*glycero*-D-*manno*-heptose-1-^{14}C.

D-*glycero*-D-*manno*-Heptose was first synthesised in 39% yield by the application of the Fisher cyanohydrin synthesis (4) to D-altrose. Sowden (5, 6) has shown that the sugar series may be ascended by reactions involving the condensation of nitromethane in alkaline media with the aldehyde function of a glycose to give a mixture of epimeric 1-deoxy-1-nitroglycitols which on treatment with acid (Nef reaction (7)) yield the corresponding glycoses. This procedure, which avoids the use of hydrogen cyanide and the reduction of aldonolactones to glycoses encountered in the use of the classical cyanohydrin method for the ascent of the sugar series (8), thus has several practical advantages.

In the present work it was found that D-altrose could be condensed with nitromethane in alkaline methanol solution to give the theoretical yield of the mixed crystalline salts of the epimeric 1-deoxy-1-nitroheptitols from which 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol was obtained crystalline in 46% overall yield. Treatment of the sodium salt of the 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol with 6 *N* sulphuric acid gave a product containing mainly D-*glycero*-D-*manno*-heptose which was contaminated with some unchanged starting material, D-altrose, and some D-*glycero*-D-*gluco*-heptose. Fractionation of this mixture by cellulose column chromatography (9) gave pure D-*glycero*-D-*manno*-heptose (60% yield), which remained a syrup. It is considered that the procedure offers a convenient route for the synthesis of D-*glycero*-D-*manno*-heptose-1-^{14}C using nitromethane-^{14}C.

Several derivatives of D-*glycero*-D-*manno*-heptose were prepared to investigate the possibility of using them for selective degradation studies directed to the determination of the distribution of ^{14}C in the heptose found in the extracellular polysaccharide produced by *Azotobacter indicum* grown in the presence of ^{14}C-labelled substrates. A degradation scheme for D-*glycero*-D-*manno*-heptose has been described in a recent paper (3).

Attempts to prepare D-mannose from the mixed anomeric methyl D-*glycero*-D-*manno*-heptopyranosides by selective scission of the exocyclic C_6–C_7 glycol system with periodate followed by reduction of the aldehyde group produced at C_6, in a way analogous to that used for the synthesis of D-gulose from D-*glycero*-D-*gulo*-heptose (10), gave a low yield

of D-mannose, which was isolated and characterized as its crystalline *p*-nitrophenylhydrazone (17% yield).

The D-*glycero*-D-*manno*-heptose gave a crystalline diethyldithioacetal derivative which on Raney nickel desulphurization gave 1-deoxy-D-*glycero*-D-*manno*-heptitol. The deoxyglycitol on periodate oxidation consumed the expected amount of periodate and liberated the theoretical amounts of formaldehyde and formic acid.

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 filter paper by the descending method (11) using the following solvent systems (v/v): (A) ethyl acetate – acetic acid – formic acid – water (18:3:1:4); (B) butan-1-ol – ethanol – water (3:1:1); (C) butan-1-ol – pyridine – water (10:3:3).

The sugars were located on the paper chromatograms using the *p*-anisidine hydrochloride spray reagent (12) and the alkaline silver nitrate spray reagent (13).

Condensation of D-*Altrose with Nitromethane*

To a stirred solution of D-altrose (30 g) (prepared by the method of Rosenfeld, Richtmyer, and Hudson (4)) in dry methanol (180 ml) and nitromethane (180 ml) was added a cold solution of sodium (6.3 g) in dry methanol (140 ml), and the reaction mixture was stirred for 7 hours at room temperature. The mixture was kept at −5° overnight and the precipitated sodium salts of the 1-deoxy-1-nitroheptitols (44 g) were collected by filtration, washed with cold methanol and ether, and then dried *in vacuo*.

The sodium salts of the 1-deoxy-1-nitroheptitols (44 g) were dissolved in water (300 ml), the solution was passed down a column of IR 120(H^+) ion-exchange resin to remove sodium ions, and the effluent was concentrated to a syrup. The syrup was dissolved in ethanol (60 ml) and was kept at 5° for 12 hours, when the resulting crystalline product (18.5 g) was collected by filtration, washed with ethanol, and then dried *in vacuo*; m.p. 105–108°.

Hydrolysis of a small portion of the product with sulphuric acid and paper chromatographic examination of the hydrolyzate indicated it to be mainly 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol contaminated with 1-deoxy-1-nitro-D-*glycero*-D-*gluco*-heptitol. A portion (1 g) of the product was recrystallized five times from methanol to give pure crystalline 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol (0.51 g), which had m.p. 109–110° and $[\alpha]_D^{20}$ −6.8° (*c*, 3.8, 75% ethanol). Anal. Calc. for $C_7H_{15}O_8N$: C, 34.85; H, 6.3; N, 5.8%. Found: C, 35.0; H, 6.8; N, 5.5%.

D-glycero-D-manno-*Heptose*

The crude 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol (9 g), dissolved in *N* sodium hydroxide solution (90 ml), was added dropwise with stirring to 6 *N* sulphuric acid (110 ml) cooled externally with ice. After 30 minutes the solution was diluted with water (200 ml) and was adjusted to pH 3 by the addition of saturated aqueous barium hydroxide solution, whereupon 12% aqueous barium acetate solution (100 ml) was added to complete the removal of sulphate ions. The precipitated barium sulphate was removed by filtration, the filtrate was passed down columns of IR 120(H^+) and Duolite A4 (OH^-) ion-exchange resins, and the deionized solution was concentrated to a syrup (7.7 g). Paper chromatographic examination of the syrup showed it to be composed of D-*glycero*-D-*manno*-heptose contaminated with small amounts of unreacted 1-deoxy-1-nitro-D-*glycero*-D-*manno*-heptitol, altrose, and D-*glycero*-D-*gluco*-heptose. The syrup (7 g) was fractionated chromatographically on a cellulose column (4.5×72 cm) using initially butan-1-ol three-quarters saturated with water as the mobile phase, which was changed to butan-1-ol one-half saturated with water after the D-altrose fraction had been eluted from the column. Using a flow rate of 16 ml of solvent per hour, the following fractions were collected in the order indicated: 1-deoxy-1-nitroheptitol (0.60 g, 8.6%); D-altrose (0.832 g, 12%); D-*glycero*-D-*manno*-heptose (4.23 g, 60.5%); mixed D-*glycero*-D-*manno*-heptose and D-*glycero*-D-*gluco* heptose (0.175 g, 2.5%); and D-*glycero*-D-*gluco*-heptose (1.05 g, 15%).

The D-*glycero*-D-*manno*-heptose, which had $[\alpha]_D^{25}$ +21° (equilib. value; *c*, 3.6 methanol), remained a syrup and on paper chromatography in solvent systems A, B, and C it was indistinguishable from an authentic sample; $R_{glucose}$ 0.90 in solvent A and 0.82 in solvent B.

D-glycero-D-manno-*Heptose p-Nitrophenylhydrazone*

The syrupy D-*glycero*-D-*manno*-heptose (0.2 g) in methanol (3 ml) was heated under reflux for 2 hours with *p*-nitrophenylhydrazine (0.2 g), and the mixture was concentrated and taken up in ethyl acetate (3 ml). On cooling, D-*glycero*-D-*manno*-heptose *p*-nitrophenylhydrazone (0.25 g, 63%), having m.p. 171–174°, was collected and after two recrystallizations from ethanol or aqueous acetone gave crystals (0.15 g) which had m.p. 176–177°. Anal. Calc. for $C_{13}H_{19}O_8N_3$: C, 45.2; H, 5.55; N, 12.2%. Found: C, 45.1; H, 5.7; N, 12.1%.

Hexa-O-acetyl-D-glycero-D-manno-*heptose*

D-*glycero*-D-*manno*-Heptose (1.9 g) was dissolved in acetic anhydride (30 ml) containing sulphuric acid (0.3 ml) and after 48 hours the mixture was poured onto ice; the acetate was extracted in the usual way to

give the derivative from methanol solution as crystals (2.5 g, 59%) having m.p. 135–136°. After two recrystallizations from chloroform – light petroleum (b.p. 40–60°) mixture the crystalline product (2.15 g) had m.p. 139–140° (lit. value m.p. 138–139° (4)) and $[\alpha]_D^{24}$ +65° (*c*, 2.25 chloroform).

D-glycero-D-manno-*Heptonic Acid*

A stirred solution of D-*glycero*-D-*manno*-heptose (0.5 g) in methanol (30 ml) containing iodine (1.4 g) was kept at 40° while 4% methanolic potassium hydroxide solution (25 ml) was added dropwise over 15 minutes. After cooling the mixture to 0° the precipitated potassium D-*glycero*-D-*manno*-heptonate (0.40 g) was collected, washed with methanol, and dried *in vacuo*. D-*glycero*-D-*manno*-Heptono-γ-lactone (0.2 g), prepared from the potassium salt, in water (2 ml), was heated with phenylhydrazine (0.2 ml) for 3 hours and on cooling of the mixture the phenylhydrazide derivative (0.15 g) was obtained. The D-*glycero*-D-*manno*-heptonic phenylhydrazide after two recrystallizations from methanol had m.p. 187–188° and $[\alpha]_D^{25}$ −6° (*c*, 3 water).

Methyl-D-glycero-D-manno-*Heptopyranosides*

D-*glycero*-D-*manno*-Heptose (0.75 g) was heated under reflux with 2% w/v methanolic hydrogen chloride solution (60 ml) for 48 hours. The isolated glycosides (0.75 g), $[\alpha]_D^{25}$ +47° (*c*, 1.7 methanol), failed to crystallize and paper chromatographic examination showed that no unchanged heptose was present.

One mole part of periodic acid (7.4 ml, 0.3 mole) was added slowly to a stirred solution of the methyl D-*glycero*-D-*manno*-heptosides (0.5 g) in water (10 ml) and after 5 hours the iodic acid was removed by passage of the solution through a column of Duolite A4 (OH^-) ion-exchange resin. The eluate was concentrated (ca. 10 ml) and sodium borohydride (0.5 g) was added. After 12 hours at 0°, IR 120 (H^+) resin was added and the solution was then concentrated to a syrup, from which the boric acid was removed by repeated distillation with methanol. The hydrolyzed (dil. H_2SO_4) product when examined by paper chromatography showed the presence of mannose, a trace of D-*glycero*-D-*manno*-heptose, and a non-reducing component tentatively identified as a pentitol. The concentrated hydrolyzate (0.29 g) in methanol (5 ml) was heated for 1 hour with *p*-nitrophenylhydrazine (0.3 g) to give crystalline D-mannose *p*-nitrophenylhydrazone (0.115 g, 17%) having m.p. and mixed m.p. 200–201°.

D-glycero-D-manno-*Heptose Diethyldithioacetal*

D-*glycero*-D-*manno*-Heptose (0.5 g) dissolved in ice-cold hydrochloric acid (0.6 ml) was shaken with ethanethiol (0.6 ml) at 0° for 30 minutes and then kept at −5° overnight. The resulting white crystals were collected and were combined with the crystals obtained on concentration of the neutralized ($PbCO_3$) reaction mixture. The product was recrystallized from ethanol to give D-*glycero*-D-*manno*-heptose diethyldithioacetal (0.48 g, 65%) which had m.p. 155–156° and $[\alpha]_D^{25}$ +29.6° (*c*, 2.1 water). Anal. Calc. for $C_{11}H_{24}O_6S_2$: C, 41.77; H, 7.65; S, 20.2%. Found: C, 41.73; H, 7.51; S, 20.6%.

1-Deoxy-D-glycero-D-manno-*heptitol*

D-*glycero*-D-*manno*-Heptose diethyldithioacetal (0.36 g) in aqueous methanol (10 ml) was desulphurized by refluxing the solution for 6 hours in the presence of Raney nickel (3 g). Concentration of the methanol solution gave 1-deoxy-D-*glycero*-D-*manno*-heptitol as a syrup (0.20 g) which had $[\alpha]_D^{25}$ 0° (*c*, 3.6 methanol).

The periodate oxidation of an aqueous solution of the 1-deoxy-D-*glycero*-D-*manno*-heptitol at room temperature was complete in 1 hour, giving the following final results: 5.10 mole periodate consumed, 3.90 mole formic acid, and 0.93 mole formaldehyde released per mole heptitol.

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