Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

THE SYNTHESIS OF 2,3,4,6,7-PENTA-*O*-METHYL-D-*glycero*-L-*manno*-HEPTOSE AND 2,4,6,7-TETRA-*O*-METHYL-D-*glycero*-L-*manno*-HEPTOSE*

PREM PAL SINGH† AND G. A. ADAMS
Biochemistry Laboratory, National Research Council of Canada, Ottawa (Canada)

(Received June 23rd, 1969; in revised form, August 7th, 1969)

ABSTRACT

2,3,4,6,7-Penta-*O*-methyl-D-*glycero*-L-*manno*-heptose was synthesized by methylation of D-*glycero*-L-*manno*-heptose followed by hydrolysis. 2,4,6,7-Tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose was synthesized by using the following sequence of reactions: 2-*O*-benzyl-D-galactose (**7**) → 3-*O*-benzyl-1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol (**8**) → 3-*O*-benzyl-D-*glycero*-L-*manno*-heptose (**9**) → methyl 3-*O*-benzyl-2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptoside (**10**) → methyl 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptoside (**11**) → 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose (**12**).

INTRODUCTION

Studies in this laboratory on the core structure of Gram-negative lipopolysaccharides by the methylation technique required 2,3,4,6,7-penta-*O*-methyl-D-*glycero*-L-*manno*-heptose and 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose as reference compounds. Earlier studies by Risse *et al.*[1] on a partial hydrolysis product from the lipopolysaccharides of *Salmonella minnesota* indicated a heptosyl→heptosyl →3-deoxyoctulosonate structure, although the methylated heptoses could not be characterized positively. Periodate oxidation of the heptose residues in the lipopolysaccharide with cleavage between C-6 and C-7, followed by reduction, yielded residues of a mannose; methylation of the periodate-oxidized and reduced structure established[2] the linkage between the sugar residues as (1→3).

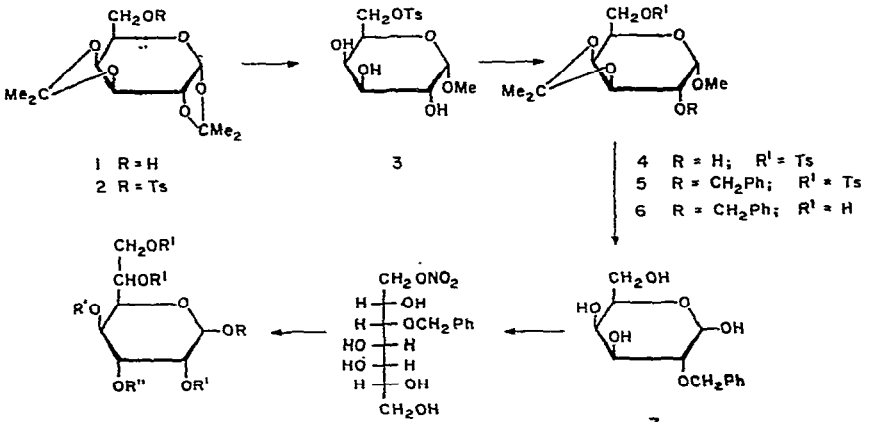
Methylation studies in this laboratory[3] on the lipopolysaccharide of *Neisseria perflava* also established that the heptose residues were (1→3)-linked. It seemed desirable, however, to be able to identify the original methylated heptose residues in these lipopolysaccharides by comparison with authentic 2,3,4,6,7-penta-*O*-methyl-D-*glycero*-L-*manno*-heptose and 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose. With this objective in mind these methyl ethers have been synthesized and their properties are described.

*Issued as NRCC No. 11071.
†National Research Council Postdoctorate Fellow 1967–1969.

Although L-*glycero*-D-*manno*-heptose is the naturally occurring heptose in lipopolysaccharides, it was more expedient in the present work to prepare the enantiomorph, D-*glycero*-L-*manno*-heptose[4]. During the progress of this work a synthesis of L-*glycero*-D-*manno*-heptose has been described[5].

RESULTS AND DISCUSSION

2,3,4,6,7-Penta-*O*-methyl-D-*glycero*-L-*manno*-heptose was obtained by methylation of D-*glycero*-L-*manno*-heptose followed by hydrolysis. The methylated sugar yielded a crystalline aniline derivative. 2,4,6,7-Tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose (**12**) was obtained by methylation of 3-*O*-benzyl-D-*glycero*-L-*manno*-heptose (**9**) followed by debenzylation and hydrolysis. The complete sequence of reactions leading to 3-*O*-benzyl-D-*glycero*-L-*manno*-heptose is shown in the following scheme.



Application of the nitromethane synthesis to 2-*O*-benzyl-D-galactose (**7**) produced, surprisingly, only 3-*O*-benzyl-1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol. This result was probably the outcome of steric hindrance caused by the bulky benzyl group at C-2. The low yield of 3-*O*-benzyl-1-deoxy-1-nitro-D-*glycero*-L-*manno*-heptitol could not be explained. The D-*glycero*-L-*manno* configuration of the heptose sugar was shown by debenzylation to D-*glycero*-L-*manno*-heptose; the latter was identified as its phenylhydrazone and by its conversion into D-*glycero*-L-*manno*-heptitol hepta-acetate. Neither 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose nor its aniline or the di-*O*-*p*-nitrobenzoyl derivatives could be crystallized.

The methyl glycosides of 2,3,4,6,7-penta-*O*-methyl-D-*glycero*-L-*manno*-heptose and 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose had retention times on g.l.c. identical with those of the two methylated heptosides obtained by methylation and methanolysis of a heptose oligosaccharide[1] that had been prepared from a lipopoly-

saccharide of *S. minnesota* and kindly supplied by Dr. Otto Lüderitz. The retention time of the synthetic methyl 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptoside was also the same as that of the partially methylated heptoside isolated from a methylated lipopolysaccharide of *N. perflava* prepared in this laboratory[3].

EXPERIMENTAL

General methods. — All evaporations were carried out under diminished pressure below 40°. Melting points were determined on a Fisher–Johns hot stage and are corrected. Optical rotations, determined on a Perkin–Elmer 141 polarimeter, are equilibrium values, unless otherwise stated. Methoxyl determinations were made according to Steyermark[6]. Paper chromatography was carried out on Whatman No. 1 paper in the following solvent systems: Solvent *A*: butanone saturated with water, Solvent *B*: pyridine–ethyl acetate–water (2:5:5, v/v). R_F, R_G, and R_{Gal} values are with reference to solvent front, 2,3,4,6-tetra-*O*-methyl-D-glucose and D-galactose, respectively. G.l.c. was carried out on columns (120 × 0.5 cm) having the following packings: (*A*) 10% (w/w) neopentyl glycol sebacate on Chromosorb W (100–120 mesh) at 193°, (*B*) 0.3% (w/w) ECNSS-M on Chromosorb Q (100–120 mesh) at 203°. The gas pressure was 30 lb.in^{-2}. T_G and T_{Gal} are retention times with reference to methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucoside and D-glucitol hexaacetate, respectively. N.m.r. spectra were taken in chloroform-*d* with tetramethylsilane as the internal reference with a Varian A-60A n.m.r. spectrometer. I.r. spectra were recorded with a Perkin–Elmer Infracord spectrometer. Hydrolysis was carried out with 0.5M sulfuric acid for 4 h in a boiling water-bath. The hydrolyzate was neutralized by passing over Duolite A-4 (OH^-) resin and the eluates were concentrated.

2,3,4,6,7-Penta-O-*methyl*-D-glycero-L-manno-*heptose.* — D-*glycero*-L-*manno*-Heptose[4] (1 g) was methylated by Srivastava's method[7] with methyl sulfoxide, silver oxide, and methyl iodide, and then by one Purdie methylation[8]. The glassy syrup obtained (1.1 g) was completely methylated, as shown by the absence of OH absorption in its i.r. spectrum. [OMe: found, 62.42; calc. 63.26]. G.l.c. on column *A* showed two peaks having T_G values of 2.71 (major) and 3.21 (minor). Acid hydrolysis of the fully methylated D-*glycero*-L-*manno*-heptose (0.75 g) yielded 2,3,4,6,7-penta-*O*-methyl-D-*glycero*-L-*manno*-heptose (0.62 g), [OMe: found, 54.9; calc. 55.35], $[\alpha]_D$ −35.4° (*c* 1.3, methanol). The sugar could not be crystallized, but on paper chromatography it migrated as a single spot having an R_F value of 0.84 and an R_G value of 1.04 (solvent *A*).

The syrup (0.22 g) was refluxed for 6 h with aniline (0.085 g) in absolute ethanol (10 ml). The solvent was then removed by evaporation and the aniline derivative was crystallized from ethyl acetate. Recrystallization gave 2,3,4,6,7-penta-*O*-methyl-*N*-phenyl-D-*glycero*-L-*manno*-heptosylamine, m.p. 150–151°, $[\alpha]_D$ +142.3° (*c* 0.2, acetone).

Anal. Calc. for $C_{18}H_{29}NO_6$: C, 60.67; H, 8.41; N, 3.94. Found: C, 60.67; H, 8.61; N, 3.83.

1,2:3,4-Di-O-isopropylidene-6-O-p-tolylsulfonyl-α-D-galactose (**2**). — D-Galactose (120 g) was converted into 1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**1**) by treatment with zinc chloride and concentrated sulfuric acid in acetone[9]. Compound **1** (155.5 g) was *p*-toluenesulfonated with *p*-toluenesulfonyl chloride in an acetone–pyridine mixture[9]. The 1,2:3,4-di-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-D-galactose (**2**) (150 g) had m.p. 88–89° (lit.[10] m.p. 89–91°).

Methyl 6-O-p-tolylsulfonyl-α-D-galactopyranoside (**3**). — Compound **2** (145.0 g) was refluxed with 2% methanolic hydrogen chloride for 30 min[11]. The solution on cooling gave crystals of methyl 6-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (**3**). The mother liquor on further concentration gave an additional yield of crystals. The two crops on recrystallization gave pure **3** (65.0 g), m.p. 169–170°, $[\alpha]_D$ +104° (*c* 1, pyridine) (lit.[11] m.p. 170°, $[\alpha]_D$ +103.5°).

Methyl 3,4-O-isopropylidene-6-O-p-tolylsulfonyl-α-D-galactopyranoside (**4**). — Methyl 6-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (**2**, 62.0 g) was suspended in acetone (3 liters) and was shaken for 48 h with anhydrous copper(II) sulfate (320 g) and concentrated sulfuric acid (8.0 ml). The suspension was filtered and the filtrate was neutralized with ammonia, and the suspension was filtered again. The filtrate was concentrated to half its volume and poured into water. The crystalline product that formed was filtered off and dissolved in ether, and the insoluble material was removed by filtration. The filtrate on concentration yielded pure **4** (31.0 g) having m.p. 129–130°, $[\alpha]_D$ +76.7° (*c* 1, methanol); [lit.[12] m.p. 129–130°, $[\alpha]_D$ +73.7° (methanol)].

Methyl 2-O-benzyl-3,4-O-isopropylidene-6-O-p-tolylsulfonyl-α-D-galactopyranoside (**5**). — Compound **4** (30.0 g) was dissolved in dry *N,N*-dimethylformamide (10 liters). The solution was cooled in ice and to it was added silver oxide (250 g) and benzyl bromide (100 g). The mixture after being stirred overnight was poured into chloroform (2 liters). The precipitated silver salts were removed by filtration and the filtrate was concentrated to a thick syrup. The syrup was dissolved in hot ether and the solution on cooling afforded crystals which on recrystallization gave **5** (35.2 g), m.p. 118–119°, $[\alpha]_D$ +78.5° (*c* 1, methanol). The i.r. spectrum showed absence of OH absorption. The n.m.r. spectrum was consistent with the assigned structure.

Anal. Calc. for $C_{24}H_{30}O_8S$: C, 60.25; H, 6.27; S, 6.69. Found: C, 60.17; H, 6.35; S, 6.62.

Methyl 2-O-benzyl-3,4-O-isopropylidene-α-D-galactopyranoside (**6**). — Compound **5** (32.0 g) was dissolved in 80% aqueous methanol and treated with 4% sodium amalgam[13]. After stirring for 14 h, the mixture was neutralized with carbon dioxide. The solution was decanted from the mercury and concentrated to dryness. The resulting solid was extracted twice with hot absolute ether and the extracts, after filtration, were combined and concentrated. Crystallization took place to give **6** (23.0 g), m.p. 105–106°, $[\alpha]_D$ +96.0° (*c* 1, chloroform). The n.m.r. spectrum was consistent with the assigned structure.

Anal. Calc. for $C_{17}H_{24}O_6$: C, 62.96; H, 7.40. Found: C, 63.08; H, 7.46.

2-O-Benzyl-D-galactose (**7**). — Compound **6** (20.0 g) on acid hydrolysis and concentration gave crystals which, on recrystallization from water, yielded pure 2-*O*-benzyl-D-galactose (**7**, 13.5 g), m.p. 70°, $[\alpha]_D$ +72.5 (5 min) → +58.9° (*c* 1, water). Paper chromatography in solvent *B* showed only one spot, R_F 0.77; R_{Gal} 3.43.

3-O-Benzyl-1-deoxy-1-nitro-D-glycero-L-manno-*heptitol* (**8**). — Compound **7** (7.5 g) was suspended in a mixture of methanol (20 ml) and nitromethane (25 ml) and to the suspension was added a cold solution of sodium (2.0 g) in methanol (60 ml). The reaction mixture was stirred overnight and then cooled to −20°. The precipitate was filtered off, washed with cold methanol, dissolved in water, and passed over Rexyn-101 (H^+) resin to remove sodium ions. The effluent on concentration gave crystals which, on recrystallization from water, gave compound **8** (0.51 g); m.p. 160–16[illegible] $[\alpha]_D$ +9.6° (*c* 0.5, water).

An[illegible]. Calc. for $C_{14}H_{21}NO_8$: N, 4.23. Found: N, 4.30.

3-O-Benzyl-D-glycero-L-manno-*heptose* (**9**). — Compound **8** (0.5 g) in M sodium [illegible]ydroxide (2.5 ml) was added dropwise to a solution of sulfuric acid (0.3 ml) in wate[illegible] 0.4 ml)[14]. After stirring for 15 min, the solution was deionized by passing over Re[illegible]yn-101 (H^+) and Duolite A-4 (OH^-) resins. The effluent was evaporated to drynes[illegible]o give 3-*O*-benzyl-D-*glycero*-L-*manno*-heptose (**9**, 0.35 g); $[\alpha]_D$ −5.34° (*c* 1.8, meth[illegible]ol). The sugar moved as one spot on paper in solvent *B*, having R_F and R_{Gal} valu[illegible] of 0.7 and 3.05, respectively

The 3-*O*-[illegible]-heptose (**9**, 0.03 g) was refluxed with 2.5% methanolic hydrogen ch[illegible] [illegible] ml) for 4 h and the acid was neutralized with Ag_2CO_3 and the precipitate was removed by filtration. The filtrate was taken to dryness and debenzylated[15] with sodium (0.03 g) and absolute ethanol for 4 h at 70°. The reaction mixture was then dissolved in water and deonized by passage through Rexyn-101 (H^+) resin. The effluent was concentrated and hydrolyzed with acid. The hydrolyzate moved on paper chromatography in solvent *B* at the same rate as authentic D-*glycero*-L-*manno*-heptose and yielded a crystalline phenylhydrazone, m.p. 190–191°, undepressed when mixed with an authentic sample.

The hydrazone was decomposed with benzaldehyde[16] and the free sugar was reduced with sodium borohydride. The product was acetylated[17] with acetic anhydride for 3 h at 121°. The acetate showed a single peak (T_{Gal} 2.36) on g.l.c. (column *B*), having the same retention time as the authentic D-*glycero*-L-*manno*-heptitol acetate and was distinguishable from the D-*glycero*-L-*gluco*-heptitol acetate, which had a retention time of T_{Gal} 2.7.

2,4,6,7-Tetra-O-*methyl*-D-glycero-L-manno-*heptose* (**10**). — Compound **9** (0.30 g) was methylated by the methods described earlier. The i.r. spectrum of the methylated product (**10**, 0.31 g) showed complete absence of OH absorption. (*Anal.* Found: OMe, 41.52; calc. 41.89); $[\alpha]_D$ +21.3° (*c* 1, methanol). Product **10** was debenzylated[15] to give methyl 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptoside (**11**, 0.22 g) (Found: OMe, 55.0; calc. 55.35). G.l.c. on column *A* showed one major peak (T_G 4.4) and a minor peak (T_G 5.0). Hydrolysis followed by reglycosidation showed essentially a single peak at T_G 4.4.

Compound **11** was hydrolyzed to give 2,4,6,7-tetra-*O*-methyl-D-*glycero*-L-*manno*-heptose (**12**, 0.19 g), $[\alpha]_D$ −28.3° (*c* 1.5, methanol). Paper-chromatographic examination in solvent *A* showed one spot having R_F 0.67 and R_G 0.82. Attempts to crystallize the syrup were unsuccessful. Neither the aniline nor the di-*p*-nitrobenzoyl derivatives could be crystallized.

ACKNOWLEDGMENTS

The authors thank Mr. A. E. Castagne for the microanalyses.

REFERENCES

1 H. J. RISSE, W. DRÖGE, E. RUSCHMANN, O. LÜDERITZ, O. WESTPHAL, AND J. SCHLOSSHARDT, *European J. Biochem.*, 1 (1967) 216.
2 W. DRÖGE, O. LÜDERITZ, AND O. WESTPHAL, *Z. Physiol. Chem.*, 348 (1967) 603.
3 G. A. ADAMS, M. KATES, D. H. SHAW, AND M. YAGUCHI, *Can. J. Biochem.*, 46 (1968) 1175.
4 J. C. SOWDEN AND D. R. STROBACH, *J. Amer. Chem. Soc.*, 82 (1960) 954.
5 M. TEUBER, R. D. BEVILL, AND M. J. OSBORN, *Biochemistry*, 7 (1968) 3303.
6 A. STEYERMARK, *Quantitative Organic Microanalysis*, The Blakiston Company, New York, 1951, p. 230.
7 H. C. SRIVASTAVA, S. N. HARSHE, AND P. P. SINGH, *Indian J. Chem.*, 1 (1963) 304.
8 T. PURDIE AND J. C. IRVINE, *J. Chem. Soc.*, (1903) 1021.
9 R. S. TIPSON, *Methods Carbohyd. Chem.*, 2 (1963) 246.
10 H. SCHMIDT AND P. KARRER, *Helv. Chim. Acta*, 32 (1949) 1371.
11 D. J. BELL AND S. WILLIAMSON, *J. Chem. Soc.*, (1938) 1196.
12 H. OHLE AND H. THIEL, *Ber.*, 66B (1933) 525.
13 R. S. TIPSON, *Methods Carbohyd. Chem.*, 2 (1963) 250.
14 J. U. NEF, *Ann.*, 280 (1894) 263.
15 K. FREUDENBERG AND E. PLANKENHORN, *Ann.*, 536 (1938) 257.
16 M. BERGMANN, H. SCHOLTE, AND W. LECHINSKY, *Ber.*, 55 (1922) 158.
17 P. ALBERSHEIM, D. J. NEVIS, P. D. ENGLISH, AND A. KARR, *Carbohyd. Res.*, 5 (1967) 340.