Synthesis of 4-*O*-α-D-Mannopyranosyl-L-rhamnopyranose

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4-*O*-α-D-Mannopyranosyl-L-rhamnopyranose has been synthesized in 55% yield by condensation of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside using mercuric cyanide in acetonitrile (Helferich reaction). The disaccharide has m.p. 143–145°, $[\alpha]_D$ +53.0° → 60.3° (*c*, 1.0 water), and was characterized as the crystalline peracetates of the disaccharide (m.p. 149.5–150.5°, $[\alpha]_D$ −4.4° (*c*, 4.3 chloroform)) and of the derived alditol (m.p. 84–85°, $[\alpha]_D$ −4.4° (*c*, 1.9 chloroform)).

4-*O*-α-D-Mannopyrannosyle-L-rhamnose a été synthétisé avec un rendement de 55% par réaction du α-D-tetrabenzoylbromomannose avec le méthyl 2,3-*O*-isopropylidène-α-L-rhamnopyrannoside en utilisant le cyanure de mercure dans l'acétonitrile (réaction de Helferich). Le disaccharide est cristallin (p.f. 143–145°, $[\alpha]_D$ +53.0° → 60.3° (*c*, 1.0 eau)) et était caractérizé par les peracétates cristallins du disaccharide (p.f. 149.5–150.5°, $[\alpha]_D$ −5.5° (*c*, 4.3 chloroform)) et de l'alditol correspondant (p.f. 84–85°, $[\alpha]_D$ −4.4° (*c*, 1.9 chloroform)).

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The disaccharide unit 4-*O*-D-mannopyranosyl-L-rhamnopyranose occurs with the glycosidic linkage in both the α-D and β-D anomeric forms in the lipopolysaccharides of different *Salmonella* species. The two anomeric disaccharides isolated from natural sources were originally incorrectly identified owing to the lack of authentic standards (1). Synthetic disaccharides are also of use in determining the immunodominant site in antigenic lipopolysaccharides. We now report the synthesis of 4-*O*-α-D-mannopyranosyl-L-rhamnopyranose, a constituent of *Salmonella* types B and D_1 (1).

The general synthetic scheme was devised using the β-D-gluco analog (2) which, as stated there, served as a model for the D-manno compound. Since Gorin and Perlin have noted (3) that 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**3**) gives higher yields and greater stereoselectivity than the more common acetyl derivative, **3** was condensed with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**) in mercuric cyanide and acetonitrile to give methyl 2,3-*O*-isopropylidene-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (**4**) as a syrup. Debenzoylation and subsequent acetylation of **4** gave, in 76% yield based on **1**, crystalline methyl 2,3-*O*-isopropylidene-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (**5**), m.p. 119–120°. Hydrolysis with trifluoroacetic acid (2,4) of the isopropylidene group of **5** produced syrupy methyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (**6**) which, upon acetolysis (2,5), gave crystalline 1,2,3-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranose (**10**) in 78% yield, m.p. 149.5–150.5°. Deacetylation of **10** with sodium methoxide gave crystalline 4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranose (**11**), m.p. 143–145°. Reduction of **11** with sodium borohydride and subsequent acetylation gave crystalline 4-*O*-α-D-mannopyranosyl-L-rhamnitol octaacetate (**13**), m.p. 84–85°.

The α-D configuration of the mannopyranosyl moiety was confirmed by treatment of **11** with crude α-mannosidase prepared from jack bean meal (6). Using this preparation the disaccharide (**11**) and methyl α-D-mannopyranoside were cleaved but methyl β-D-mannopyranoside was not.

The presence of the 1 → 4 linkage was confirmed by periodate oxidation and methylation studies. Deacetylation of **6** with sodium methoxide gave syrupy methyl 4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranoside (**7**). Oxidation of **7** with sodium periodate (7) showed it consumed 3 mol of sodium periodate; subsequent reduction with sodium borohydride, methanolysis, concentration, and acetylation with acetic anhydride gave 1-deoxy-D-erythritol triacetate and glycerol triacetate. Methylation (8) of **7** followed by hydrolysis with trifluoroacetic acid, reduction with sodium borohydride, and acetylation with acetic anhydride gave 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-rhamnitol and 1,5-di-*O*-acetyl-2,3,4,

6-tetra-*O*-methyl-D-mannitol as identified by g.l.c. and mass spectrometry (9).

Experimental

General experimental specifications were the same as for the β-D-gluco analog (2).

Methyl 2,3-O-Isopropylidene-α-L-rhamnopyranoside (1)

Prepared as in (2) except that the Amberlite IR-120 H^+ resin should be soaked in dry methanol and not acetone to avoid the formation of acetone polymers.

Methyl 2,3-O-Isopropylidene-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (4)

1,2,3,4,6-Penta-*O*-benzoyl-α-D-mannopyranose (**2**) (10) was converted to 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**3**) (11) 15 g of which, dissolved in acetonitrile (20 ml, distilled over CaH_2), was added with stirring during 2 h to a solution of methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**1**) (4.0 g, 18.3 mmol) and dry mercuric cyanide (4.0 g) in acetonitrile (10 ml). After stirring for 3 h more the acetonitrile was removed from the reaction mixture under diminished pressure and the remaining syrup was dissolved in chloroform (100 ml). The chloroform solution was washed with 1 *N* potassium bromide (2 × 100 ml), water (100 ml), saturated sodium bicarbonate (100 ml), and water (2 × 100 ml). The chloroform was removed under diminished pressure leaving an impure amorphous solid (17.3 g) which showed a major component (~80%) on t.l.c. with R_f (solvent A) 0.71.

Methyl 2,3-O-Isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (5)

Compound **4** (impure amorphous solid, 17.3 g) was debenzoylated to yield a syrup (R_f (t.l.c. solvent B) 0.31) which on acetylation gave the acetate (**5**) which was crystallized from ethanol (25 ml); yield 7.6 g, 13.9 mmol, 76%. Recrystallization from ethanol gave pure **5**, m.p. 119–120°; $[\alpha]_D$ +30.7° (*c* 2.2, chloroform); R_f (t.l.c. solvent A) 0.53; p.m.r. ($CDCl_3$): τ 5.01 (1H, doublet, $J_{1',2'}$ = 1.7 Hz, H-1'), 5.14 (1H singlet, H-1), 6.63 (3H singlet, C-1, OCH_3), 7.84, 7.90, 7.97, 8.01 (3H singlets, OAc's), 8.48, 8.66 (3H singlets, isopropylidene CH_3's), 8.72 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Anal. Calcd. for $C_{24}H_{36}O_{14}$: C, 52.55; H, 6.62. Found: C, 52.38; H, 6.49.

Methyl 4-O-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (6)

Compound **6** (1.83 g) was obtained as a syrup (t.l.c., R_f (solvent A) 0.17, (solvent B) 0.59) by deacetalation (2, 4) of **5** (2.0 g); p.m.r. ($CDCl_3$): τ 5.04 (1H doublet, $J_{1',2'}$ = 1.7 Hz, H-1') 5.31 (1H singlet, H-1), 6.62 (3H singlet, C-1, OCH_3), 7.85, 7.90, 7.95, 7.99 (3H singlets, OAc's), 8.67 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Methyl 4-O-α-D-Mannopyranosyl-α-L-rhamnopyranoside (7)

Deacetylation of **6** (1.83 g) gave **7**, R_f (solvent B) 0.03; yield 1.17 g; $[\alpha]_D$ +13° (*c* 2.2, water); $R_{glucose}$ (solvent C) 2.9; p.m.r. (D_2O, external TMS): τ 5.01 (1H doublet, $J_{1',2'}$ = 1.8 Hz, H-1'), 5.29 (1H doublet, $J_{1,2}$ = 1.5 Hz, H-1), 6.60 (3H singlet, C-1, OCH_3), 8.67 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Periodate Oxidation of Compound 7

Reaction of the methyl glycoside (7) (0.320 g, 0.94 mmol) with sodium metaperiodate (0.05 *M*, 120 ml) at 5° in the dark showed a rapid uptake of 2 mol with the consumption of periodate becoming constant (3.0 mol) in 30 h (7). Reduction of the polyaldehyde and methanolysis of the polyalcohol gave 1-deoxy-D-erythritol and glycerol (2).

Methylation of Compound 7

The methyl glycoside (**7**) (0.27 g) was methylated (2, 8) and the product (**8**) (0.2 g) gave one spot on t.l.c. (solvent A) with R_f 0.14; $[\alpha]_D$ +10° (*c* 1.0, chloroform); p.m.r. ($CDCl_3$): τ 4.93 (1H doublet, $J_{1',2'}$ = 1.6 Hz, H-1'), 5.27 (1H doublet, $J_{1,2}$ = 1.6 Hz, H-1), 6.46–6.63 (21H, OCH_3's), 8.72 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Hydrolysis of **8** (0.2 g, 2 *M* TFA, 20 ml, reflux, 15 h) gave 2,3,4,6-tetra-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-L-rhamnose as shown by paper chromatography (solvent D) and by g.l.c.–m.s. (2, 9) of the alditol acetates prepared according to Albersheim and co-workers (12).

Methyl 2,3-Di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (9)

Methyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (**6**) (0.1 g) was acetylated with pyridine (4 ml) and acetic anhydride (4 ml); yield 0.11 g. A portion of the syrupy product was purified by preparative t.l.c. (13) for analytical purposes, $[\alpha]_D$ +4° (*c* 1.3, chloroform); R_f (t.l.c., solvent A) 0.41; p.m.r. ($CDCl_3$): τ 5.01 (1H doublet, $J_{1',2'}$ = 1.6 Hz, H-1'), 5.40 (1H doublet, $J_{1,2}$ = 1.5 Hz, H-1), 6.61 (3H singlet, C-1, OCH_3), 7.84–8.01 (18H, OAc's), 8.60 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranose (10)

Methyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (**6**) (1.0 g) in acetic anhydride (5 ml) was shaken with concentrated sulfuric acid 2% (v/v) in acetic anhydride (10 ml) for 3 h (5). The reaction mixture was diluted with chloroform (100 ml) and the chloroform solution was washed with water (2 × 100 ml), saturated sodium bicarbonate (2 × 100 ml), and again with water (2 × 100 ml). Any remaining acetic anhydride or acetic acid was removed by distillation with ethanol. The remaining syrup was crystallized from ethanol (10 ml), yield 0.95 g. Recrystallization from ethanol gave pure **10**, m.p. 149.5–150.5°; $[\alpha]_D$ −5.5° (*c* 4.3, chloroform); R_f (t.l.c., solvent A) 0.39; p.m.r. ($CDCl_3$): τ 4.00 (1H doublet, $J_{1,2}$ = 1.5 Hz, H-1), 5.00 (1H doublet, $J_{1',2'}$ = 1.8 Hz, H-1'), 7.82–8.00 (21H, OAc's), 8.60 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Anal. Calcd. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.37; H, 5.98.

4-O-α-D-Mannopyranosyl-α-L-rhamnopyranose (11)

The peracetate of the disaccharide (**10**) (1.2 g) was deacetylated and the product (0.6 g) crystallized neat after long standing. Pure **11** was obtained by recrystallization from a 1:1 mixture of methanol and 2-propanol (20 ml/g), m.p. 143–145°; $[\alpha]_D$ +53.0° mutarotating to +60.3° in 2 h (*c* 1.0, water); $R_{glucose}$ (solvent C) 1.0; p.m.r. (D_2O,

external TMS): τ 4.90 (0.6H doublet, $J_{1,2}$ = 1.5 Hz, H-1 of α-L form), 5.03 (1H doublet, $J_{1',2'}$ = 1.8 Hz, H-1'), 5.16 (0.4 H doublet, $J_{1,2}$ = 1.0 Hz, H-1 of β-L form), 8.71 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Anal. Calcd. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 43.91; H, 6.86.

Gas–liquid chromatography of the per(trimethylsilyl) disaccharide (column a at 240°) gave one peak (80%) at 8.5 min and a second peak at 11.7 min (per(trimethylsilyl) sucrose 14.5 min) (14).

The free disaccharide (**11**) (0.1 g) was dissolved in sodium acetate buffer (0.3 *M*, p*H* 4.5, 2 ml) and incubated at 37° with 2 ml of crude α-mannosidase (6). The disaccharide (**11**) was 80% cleaved in 10 h and completely cleaved in 22 h as shown by paper chromatography (solvent C). Under these conditions methyl β-D-mannopyranoside (3) was not cleaved but methyl α-D-mannopyranoside (commercial preparation) was hydrolyzed. The products from the disaccharide were reduced, acetylated, and identified by g.l.c.–m.s. (2, 9) as an equimolecular mixture of L-rhamnitol pentaacetate and D-mannitol hexaacetate (m.p. 120–122°; lit. (15) m.p. 125°)

4-O-α-D-Mannopyranosyl-L-rhamnitol (12)

The free disaccharide (**11**) (0.5 g) was reduced with sodium borohydride (0.2 g) to give **12**; yield 0.5 g; $[\alpha]_D$ +57° (*c* 3.5, water); $R_{glucose}$ (solvent C) 0.5; p.m.r. (D_2O, external TMS): τ 4.89 (1H doublet, $J_{1',2'}$ = 1.9 Hz, H-1'), 8.73 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Gas–liquid chromatography of the per(trimethylsilyl) alditol (column a at 240°) gave one peak at 11.5 min (per(trimethylsilyl)sucrose, 12.9 min) (14).

The alditol **12** (0.3 g) was acetylated with pyridine (10 ml) and acetic anhydride (10 ml) to give 4-*O*-α-D-mannopyranosyl-L-rhamnitol octaacetate (**13**) (0.55 g, crystallized from ethanol (5 ml)). Recrystallization from ethanol gave pure **13**, m.p. 84–85°; $[\alpha]_D$ −4.4° (*c* 1.9, chloroform); R_f (t.l.c., solvent A) 0.33; p.m.r. ($CDCl_3$): τ 4.98 (1H doublet, $J_{1',2'}$ = 1.6 Hz, H-1'), 7.84–8.02 (24H, OAc's), 8.63 (3H doublet, $J_{5,6}$ = 6 Hz, CH_3).

Anal. Calcd. for $C_{28}H_{40}O_{18}$: C, 50.60; H, 6.07. Found: C, 50.39; H, 6.13.

Gas–liquid chromatography of the peracetylated alditol (**13**) (column a at 270°) gave one peak at 7.0 min (sucrose octaacetate, 10.0 min).

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