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Note

Synthesis of kojitriose

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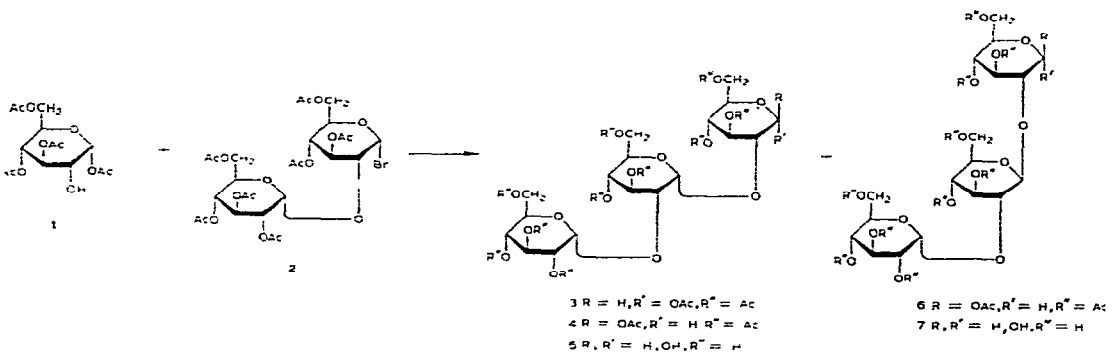
O-α-D-Glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→2)-D-glucopyranose (kojitriose, **5**) is the carbohydrate component of the membrane teichoic acid isolated[1] from *Streptococcus faecalis* strain 8191. Because of the limited availability of **5**, its structure was determined solely on the basis of a chromatographic study of the components of the partial acid hydrolyzate and of those obtained by reduction with sodium borohydride, and the physical constants were not given[1]. The chemical synthesis of **5** is now described.

Initial attempts to synthesize α-kojitriose hendecaacetate (**3**) by the reaction of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose[2] (**1**), under halide ion-catalyzed conditions[3], with hepta-*O*-acetyl-α-kojibiosyl bromide[4] (**2**) (having a nonparticipating α-D-glucopyranosyl group at O-2) in dichloromethane were not successful; no reaction occurred between **1** and **2** at room temperature, even after 10 days. After this work had been undertaken, a similar observation was reported[5] on the halide ion-catalyzed condensation of benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside with **2** in dichloromethane.



Condensation of **1** with **2** in acetonitrile in the presence of mercuric cyanide and mercuric bromide[2], a method that usually gives[6] a mixture of 1,2-*cis*- and *trans*-glycosides, proceeded readily, to afford a mixture that was difficult to separate by chromatography and fractional recrystallization. The mixture was sequentially treated with acetic anhydride–pyridine, hydrogen bromide–acetic acid, and mercuric

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acetate–acetic acid, and the resulting mixture of products was fractionated by chromatography on a column of silica gel, to give, in 42% yield, a mixture of two isomeric, trisaccharide derivatives, namely, β-kojitriose hendecaacetate (**4**) and *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→2)-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (**6**). Fractional recrystallization effected a separation of this mixture, to afford **4** and **6** in 23 and 12% yield, respectively. The n.m.r. spectra of **4** and **6** in chloroform-*d* each showed the H-1 resonance, respectively at δ 5.78 as a doublet (*J* 7.5 Hz) and at δ 5.82 as a doublet (*J* 7.0 Hz). The magnitudes of the coupling constants were indicative of the β configuration of C-1 in **4** and **6**. A comparison of the molecular rotation (+119,000°) of **4** with the sum of the molecular rotations of the constituents (1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[7], +9,000°, and α-kojibiose octaacetate[2], +103,500°) (sum = +112,500°) suggested the α-D configuration of the (middle) D-glucopyranosyl residue in **4**. In a similar way, the β-D configuration of the (middle) D-glucosyl residue of **6** was indicated by the results of comparison of its molecular rotation (+75,900°) with the sum of the molecular rotations of the constituents (1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose[7], +9,000°; β-kojibiose octaacetate[8], +76,700°) (sum = +85,700°). *O*-Deacetylation of **4** and **6**, respectively, furnished **5** and *O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-D-glucopyranose (**7**), both compounds being obtained as the crystalline monohydrate.

The n.m.r. spectrum of **5** in deuterium oxide at 60° showed six doublets, at δ 5.58 (*J* 3.5 Hz), 5.45 (*J* 3.5 Hz), 5.28 (*J* 3.5 Hz), 5.18 (*J* 3.5 Hz), 5.10 (*J* 3.5 Hz), and 4.79 (*J* 7.0 Hz), in the region[9] for anomeric- and inter-sugar, anomeric-proton resonances. This observation indicated that the inter-sugar, anomeric protons (H-1′ and H-1″) of **5** display the effect of mutarotation[9], and each of the protons shows two separate signals for the α and β anomers, but these resonances could not be specifically differentiated, except that the peak at δ 4.79 was assigned to H-1-β. The n.m.r. spectrum of **7** in deuterium oxide at 60° was rather simple, and exhibited four doublets, at δ 5.43 (*J* 3.5 Hz), 5.41 (*J* 3.5 Hz), 4.74 (*J* 7.5 Hz), and 4.72 (*J* 7.5 Hz), which could be assigned to H-1-α, H-1″, H-1′, and H-1-β, respectively, by taking advantage of their relative peak-intensities. Methylation[10] of both compound **5** and **7**, followed by hydrolysis, reduction with sodium borohydride, and acetylation, gave, from each, a 2:1 mixture of the peracetates of 3,4,6-tri- and 2,3,4,6-tetra-*O*-methyl-D-glucitol (g.l.c.), which proved that **5** and **7** is each a trisaccharide containing only linear (1→2)-interglucosidic linkages. Partial hydrolysis of **5** with acid produced D-glucose and kojibiose, which were identified by p.c. D-Glucose, kojibiose, and sophorose were detected by p.c. in the partial, acid hydrolyzate of **7**. These results confirmed the assigned, interglucosidic linkages in **5** and **7**. However, the anomeric configurations of crystalline **5** and **7** were not clearly determined. In water, **5** showed a slight upward mutarotation, whereas **7** exhibited a slight downward mutarotation. This suggested the β and α configuration of C-1 for **5** and **7**, respectively, but did not exclude the possibility of an anomeric mixture richer in the β and α anomers for **5** and **7**, respectively, than in the equilibrium mixture of the anomers in water.

EXPERIMENTAL

General methods. — Unless otherwise stated, the general experimental conditions were the same as those described previously[11]. Gas-liquid chromatography was performed with a Hitachi gas chromatograph 063, using a column of 3% of ECNSS-M on Gas-Chrom A (100–120 mesh) at an operating temperature of 180°. Retention times are given relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity. Descending, paper chromatography was performed on Whatman No. 1 paper in 6:4:3 (v/v) 1-butanol–pyridine–water, with detection with *p*-anisidine phthalate[12].

O-(*2,3,4,6-Tetra*-O-*acetyl*-α-D-*glucopyranosyl*)-(*1*→*2*)-O-(*3,4,6-tri*-O-*acetyl*-α-D-*glucopyranosyl*)-(*1*→*2*)-*1,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranose* (**4**) *and* O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*glucopyranosyl*)-(*1*→*2*)-O-(*3,4,6-tri*-O-*acetyl*-β-D-*glucopyranosyl*)-(*1*→*2*)-*1,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranose* (**6**). — Compound **1** (2.49 g, 7.1 mmol) was dissolved in dry acetonitrile (40 mL) containing mercuric cyanide (0.90 g, 3.6 mmol) and mercuric bromide (1.29 g, 3.6 mmol), and **2** (5.0 g, 7.1 mmol) was added. The mixture was stirred for 6 h at room temperature, and then evaporated to a syrup which was dissolved in chloroform. The solution was washed successively with water, aqueous potassium bromide, and water, dried (Na_2SO_4), and evaporated. The residual syrup was acetylated with 1:1 (v/v) acetic anhydride–pyridine (30 mL) overnight at room temperature, and the solvents were removed by repeated codistillation with toluene. The resulting syrup was dissolved in acetic acid (18 mL), and treated with a saturated (at 0°) solution of hydrogen bromide in acetic acid (18 mL) for 1 h at room temperature. The solution was diluted with dichloromethane, washed successively with ice–water, aqueous sodium hydrogencarbonate, and water, dried ($MgSO_4$), and evaporated. The residue was treated with a solution of mercuric acetate (7.5 g) in acetic acid (70 mL) for 5 h at room temperature. The solution was diluted with chloroform, washed with water, dried (Na_2SO_4), and evaporated to a syrup which was fractionated on a column of silica gel (400 g). Elution with 2:1 (v/v) benzene–ethyl acetate removed the impurities (mono- and di-saccharide derivatives). Subsequent elution with 1:1 (v/v) benzene–ethyl acetate gave a mixture of **4** and **6** (2.89 g, 42%). Crystallization of the mixture from ethanol–chloroform afforded **4** (1.58 g, 23%), m.p. 187–188°, $[\alpha]_D^{25}$ +123.1° (*c* 2.2, chloroform); t.l.c.: R_F 0.28 (1:1, v/v, benzene–ethyl acetate).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.80; H, 5.54.

The mother liquor obtained after the removal of **4** was evaporated, and the residue was crystallized from ethanol to give **6** (0.82 g, 12%), m.p. 179–180°, $[\alpha]_D^{25}$ +78.5° (*c* 1.7, chloroform); t.l.c.: R_F 0.23 (1:1, v/v, benzene–ethyl acetate).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.69; H, 5.63. Found: C, 49.85; H, 5.56.

O-α-D-*Glucopyranosyl*-(*1*→*2*)-O-α-D-*glucopyranosyl*-(*1*→*2*)-D-*glucopyranose* (**5**). — A solution of **4** (1.3 g) in anhydrous methanol (30 mL) was treated with 0.5M methanolic sodium methoxide (1 mL). The solution was kept for 2 h at room temperature, made neutral with Amberlite IR-120 (H^+) ion-exchange resin, filtered, and

evaporated. Crystallization from aqueous methanol gave **5** as a monohydrate (631 mg, 90%), m.p. 228–230° (dec.), $[\alpha]_D^{25}$ +150.2 (5 min)→+156.1° (48 h, constant; *c* 1.7, water).

Anal. Calc. for $C_{18}H_{32}O_{16} \cdot H_2O$: C, 41.38; H, 6.56. Found: C, 41.29; H, 6.50.

Methylation[10] of a portion of **5**, followed by hydrolysis, reduction with sodium borohydride, and acetylation, gave compounds that had the retention times of the peracetates of 2,3,4,6-tetra-*O*-methyl-D-glucitol (*T* 1.00, 33%) and 3,4,6-tri-*O*-methyl-D-glucitol (*T* 1.97, 66%).

A solution of **5** (20 mg) in 50mM sulfuric acid (2 mL) was heated for 90 min at 100°, the acid neutralized with barium carbonate, the suspension filtered, and the filtrate evaporated to a syrup, in which D-glucose, kojibiose (R_{Glc} 0.67), and unhydrolyzed **5** (R_{Glc} 0.45) were identified by p.c.

O-α-D-Glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→2)-D-glucopyranose (**7**). — Compound **6** (0.7 g) was *O*-deacetylated, as just described, and crystallization of the residue from aqueous methanol gave **7** as the monohydrate (347 mg, 92%), m.p. 182–183° (dec.), $[\alpha]_D^{25}$ +78.5→+72.8° (48 h, constant; *c* 1.4, water).

Anal. Calc. for $C_{18}H_{32}O_{16} \cdot H_2O$: C, 41.38; H, 6.56. Found: C, 41.27; H, 6.61.

Successive methylation of a portion of **7**, hydrolysis, reduction with sodium borohydride, acetylation, and g.l.c. of the resulting products gave peaks corresponding to the peracetates of 2,3,4,6-tetra-*O*-methyl-D-glucitol (*T* 1.00, 33%) and 3,4,6-tri-*O*-methyl-D-glucitol (*T* 1.97, 66%).

Partial, acid hydrolysis of **7**, as described for **5**, gave a syrup, in which D-glucose, sophorose (R_{Glc} 0.75), kojibiose (R_{Glc} 0.68), and unhydrolyzed **7** (R_{Glc} 0.52) were detected by p.c.

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