418. *Synthesis of* 4-O-α-D-*Glucopyranosyl*-D-*ribitol, a Degradation Product of the Ribitol Teichoic Acid from the Walls of* Lactobacillus arabinosus.

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4-*O*-α-D-Glucopyranosyl-D-ribitol has been synthesised by an unambiguous route and is identical with the major mono-D-glucosylribitol isolated by degradation of the ribitol teichoic acid from *Lactobacillus arabinosus*. The successful procedure involved reaction between 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucose (Brigl's anhydride) and 1-*O*-benzoyl-5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol (V), followed by removal of protecting groups.

IN chemical studies of teichoic acids from bacteria the degradation which has proved most useful has been alkali hydrolysis followed by enzymic dephosphorylation of the monoesters produced.[1-5] The hydrolysis with alkali follows the expected course for a phosphodiester with a hydroxyl group suitably placed for participation. The products after dephosphorylation have usually crystallised or given crystalline derivatives; their structures can be determined by standard procedures of oligosaccharide chemistry. Under these conditions the ribitol teichoic acid from *Lactobacillus arabinosus* 17-5 walls gives two monoglucosides and a diglucoside of ribitol.[4] Periodate oxidation has shown that these are the 3- and 4-mono-α-D-glucosides and the 3,4-di-α-D-glucoside of D-ribitol. We have now synthesised one of the monoglucosides, 4-*O*-α-D-glucopyranosyl-D-ribitol (VIII), by a procedure developed from an earlier synthesis of the β-compound [6] obtained from *Bacillus subtilis* ribitol teichoic acid.

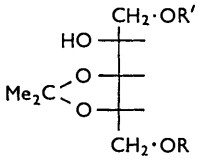
Four methods have been used successfully for the synthesis of disaccharides containing α-glucosyl linkages: (1) anomerisation of the acetate of a β-glucoside; [7] (2) reactions of Brigl's anhydride (3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucose); [8-11] (3) the Koenigs–Knorr reaction in the presence of mercuric cyanide; [12] (4) the use of a glucosyl halide in which participation of the substituent at $C_{(2)}$ is prevented.[13,14] [It is possible that the synthesis of nigerose by Haq and Whelan [13] is of type (2).]

In the present work route (2) has been examined most fully. It is known that Brigl's anhydride reacts with a relatively hindered hydroxy-compound at a high temperature to form α-glucosides.[15] The preparation of suitable derivatives of ribitol having only one free hydroxyl group was described earlier.[6] When Brigl's anhydride [16] and the triphenylmethyl ether (II) were heated in benzene at 100° for 113 hr., and when protecting groups were removed from the products, very little ribitol glucoside was detected by paper chromatography. The dibenzoate (III) was prepared from the alcohol (I) by treatment with 2 mol. of benzoyl chloride in pyridine, followed by chromatographic purification.

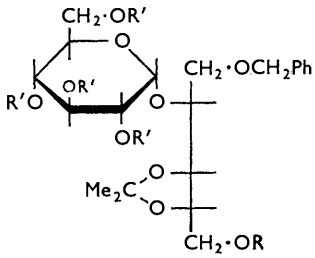
[1] Baddiley, *Proc. Chem. Soc.*, 1959, 177.
[2] Armstrong, Baddiley, and Buchanan, *Biochem. J.*, 1960, **76**, 610.
[3] Kelemen and Baddiley, *Biochem. J.*, 1961, **80**, 246.
[4] Archibald, Baddiley, and Buchanan, *Biochem. J.*, 1961, **81**, 124.
[5] Baddiley, Buchanan, Hardy, Martin, RajBhandary, and Sanderson, *Biochim. Biophys. Acta*, 1961, **52**, 406.
[6] Baddiley, Buchanan, and Hardy, *J.*, 1961, 2180.
[7] Lindberg, *Acta Chem. Scand.*, 1949, **3**, 1355.
[8] Haworth and Hickinbottom, *J.*, 1931, 2847; Lemieux and Bauer, *Canad. J. Chem.*, 1954, **32**, 340.
[9] Lemieux, *Canad. J. Chem.*, 1953, **31**, 949.
[10] Lemieux and Huber, *J. Amer. Chem. Soc.*, 1953, **75**, 4118; 1956, **78**, 4117.
[11] Haq and Whelan, *Nature*, 1956, **178**, 1221.
[12] Matsuda, *Chem. and Ind.*, 1958, 1627.
[13] Haq and Whelan, *J.*, 1958, 1342.
[14] Wolfrom, Pittet, and Gillam, *Proc. Nat. Acad. Sci. U.S.A.*, 1961, **47**, 700.
[15] Lemieux, *Adv. Carbohydrate Chem.*, 1954, **9**, 1.
[16] Brigl, *Z. physiol. Chem.*, 1921, **116**, 1.

It was heated with Brigl's anhydride at 90—95° for 116 hr.; after removal of acyl and isopropylidene groups, a ribitol glucoside with the correct properties was observed which was not hydrolysed by a β-glucosidase.

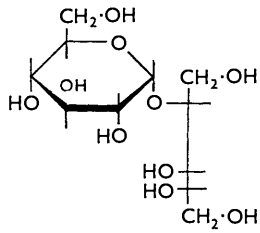
The method finally chosen was the condensation of Brigl's anhydride with the monobenzoate (V) of the benzyl ether (IV).[6] The mixture of products containing the glycoside



(I; R = R′ = H)
(II; R = R′ = CPh_3)
(III; R = R′ = COPh)
(IV; R = H, R′ = CH_2Ph)
(V; R = COPh, R′ = CH_2Ph)



(VI; R = COPh, R′ = COMe)
(VII; R = R′ = H)



(VIII)

(VI) was de-*O*-acylated catalytically. Some de-*O*-benzoylated starting material (IV) was removed from an aqueous solution by extraction with chloroform, and the remainder, which contained the glucoside (VII), was subjected to catalytic hydrogenolysis over palladium and to careful acid hydrolysis. Paper chromatography showed the presence of a compound with the properties of the glucoside (VIII), together with glucose, ribitol, and other products with lower R_F values. Some purification was achieved by chromatography on charcoal–Celite; the fraction eluted with 8% ethanol was treated with β-glucosidase to hydrolyse any β-glucosides, and the mixture of compounds was purified by chromatography on either thick paper or a charcoal–Celite column. The 4-*O*-α-D-glucopyranosyl-D-ribitol isolated by either of these procedures was identical with the material from *L. arabinosus* teichoic acid.[4] The compound, when first crystallised from ethanol, is very hygroscopic, and has an indefinite melting point and an infrared spectrum (KBr disc) which is not well resolved. On storage (more rapidly on nucleation) the glucoside is converted into a stable hydrate,[4] m. p. 125—126°, which has a different, and better resolved, infrared spectrum. This behaviour was observed with both the synthetic compound and that prepared from the teichoic acid. The stable hydrate could not be obtained directly by nucleation of supersaturated solutions in aqueous ethanol; the β-compound also exists in two forms.[6]

No other monoglucoside of ribitol was detected amongst the products after treatment with β-glucosidase. A compound with an R_F value slightly lower than the glucoside was probably kojibiose; it reacted with aniline phthalate and with alkaline silver nitrate, gave a yellow colour with the periodate–Schiff reagents, and had the same R_F value as trehalose. The presence of kojibiose is not surprising in view of its reported synthesis from Brigl's anhydride.[11]

In earlier model experiments an attempt was made to anomerise nona-*O*-acetylcellobiitol by using titanium tetrachloride. Although the acetate of cellobiose is resistant to anomerisation at the glycosidic linkage,[7] it was thought that the opening of the second ring, as in cellobi-itol, might assist the reaction. The experiment was unsuccessful, and the reaction was not attempted with the acetate of 4-*O*-β-D-glucosyl-D-ribitol. Lindberg has suggested that the lack of reactivity of cellobiose octa-acetate is due to electronic effects,[17] and our results support his view. The acetate of cellobi-itol is reported here crystalline for the first time.

[17] Lindberg, *Acta Chem. Scand.*, 1949, **3**, 1350.

Experimental

Infrared spectra were determined on potassium bromide discs. Silicic acid (Mallinckrodt) was used for chromatography. Evaporations were carried out under reduced pressure at bath temperatures not exceeding 45°.

Paper Chromatography.—Whatman No. 1 or No. 4 paper was used with the following solvent systems: A, Butan-1-ol–ethanol–water–ammonia (*d* 0·88) (40 : 10 : 49 : 1) [18] descending. B, Propan-1-ol–water (7 : 3) ascending. The periodate–Schiff reagents,[19] aniline phthalate,[20] and silver nitrate [21] were used as sprays where appropriate.

1,5-*Di*-O-*benzoyl*-2,3-O-*isopropylidene*-D-*ribitol.*—2,3-*O*-Isopropylidene-D-ribitol [6] (1·1 g.) in pyridine (8 c.c.) was cooled to 0° and benzoyl chloride (1·35 c.c., 2 ml.) added dropwise during 10 min. Cooling was continued for 10 min. and the mixture was kept at room temperature overnight. The product was isolated by using chloroform, giving a pale yellow syrup (2·1 g.). The syrup (1·8 g.) was dissolved in benzene and chromatographed on silica (180 g.). Benzene–ether (9 : 1) eluted the *tribenzoate* (0·23 g.) which crystallised from light petroleum as prisms, m. p. 80·5—82°. The compound was also obtained as needles, m. p. 95°, having a slightly different infrared spectrum, $[\alpha]_D^{23}$ −30·6° (*c* 2·99 in chloroform) (Found: C, 69·2; H, 5·6. $C_{29}H_{28}O_8$ requires C, 69·0; H, 5·6%). Benzene–ether (3 : 1) eluted the *dibenzoate* as a viscous syrup (1·15 g., 50%), $[\alpha]_D$ −18·0° (*c* 2·0 in chloroform) (Found: C, 65·6; H, 6·3. $C_{22}H_{24}O_7$ requires C, 66·0; H, 6·0%).

1-O-*Benzoyl*-5-O-*benzyl*-2,3-O-*isopropylidene*-D-*ribitol.*—5-*O*-Benzyl-2,3-*O*-isopropylidene-D-ribitol [6] (0·89 g.) in pyridine (7 c.c.) was cooled to 0°, and benzoyl chloride (0·375 c.c., 1 mol.) was added dropwise during 10 min. After a further 10 min. at 0° the mixture was set aside overnight at room temperature. Isolation, with chloroform, yielded a nearly colourless syrup (1·17 g.) which was dissolved in benzene and chromatographed on silica (100 g.). Elution was with benzene–ether (19 : 1 and 3 : 1). The latter solvent eluted two compounds, the second being the required *benzoate* which was a viscous syrup (0·8 g., 65%), $[\alpha]_D$ −23·6° (*c* 2·5 in ethanol) (Found: C, 68·7; H, 6·6. $C_{22}H_{26}O_6$ requires C, 68·4; H, 6·8%). The infrared spectrum showed bands at 3484 (OH) and 1719 $cm.^{-1}$ (ester carbonyl).

4-O-α-D-*Glucopyranosyl*-D-*ribitol.*—The above monobenzoate (0·932 g.) and Brigl's anhydride [22] (0·7 g., 1·01 mol.) were heated in a sealed tube at 90—97° for 115 hr. The clear, dark brown syrup was dissolved in methanol (15 c.c.), 0·1M-sodium methoxide in methanol (15 c.c.) was added, and the mixture was kept at room temperature overnight. The solution was neutralised with carbon dioxide, evaporated to dryness, and shaken with water (40 c.c.) and chloroform (40 c.c.). The emulsion was broken by centrifugation and the aqueous layer extracted five more times with chloroform. The aqueous layer was hydrogenolysed at atmospheric pressure with palladium black (0·8 g. of PdO). The filtered solution was evaporated to a syrup which was dissolved in ethanol (95 c.c.) and N-sulphuric acid (5 c.c.), and then boiled under reflux for 45 min. The solution was neutralised ($BaCO_3$), filtered through " Hyflo Supercel " silica, and evaporated. The resulting syrup was dissolved in water (5 c.c.) and applied to a column of Norit A (30 g., washed with acid) and Celite-545 (15 g.). The column was washed with water (900 c.c.), 0·5% ethanol (1800 c.c.), and 8% ethanol (3000 c.c.). The 8% ethanol fraction was evaporated to a syrup (0·21 g.) which was examined by paper chromatography in solvent A. It contained a little glucose together with larger amounts of ribitol glucosides. The syrup was dissolved in water (3·5 c.c.) and treated with a solution of β-glucosidase (L. Light and Co., 35 mg.) in water (3 c.c.) at 37° for 48 hr., the mixture being covered by a layer of toluene. Paper chromatography in solvent A showed that ribitol and glucose had been liberated and that a relatively large amount of a ribitol monoglucoside was present.

One-third of the enzyme hydrolysate was chromatographed on Whatman 3MM paper in solvent A. Elution of the appropriate zone with water gave a syrup (18 mg.) which slowly crystallised when seeded with a sample of the glucoside hydrate from *L. arabinosus* teichoic acid; the infrared spectra were identical. It was recrystallised from ethanol containing a little water, and it formed large rosettes of needles which were hygroscopic and gave an infrared

[18] Hirst, Hough, and Jones, *J.*, 1949, 928.
[19] Baddiley, Buchanan, Handschumacher, and Prescott, *J.*, 1956, 2818.
[20] Partridge, *Nature*, 1949, **164**, 443.
[21] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[22] Lemieux and Huber, *Canad. J. Chem.*, 1953, **31**, 1040; Hickinbottom, *J.*, 1928, 3140

spectrum identical with that of freshly crystallised material from teichoic acid; on storage of the material the infrared spectrum altered to that of the stable hydrate.

Two-thirds of the enzyme hydrolysate was chromatographed on Norit A (13 g.)–Celite-545 (9 g.). Ribitol and glucose were eluted with 1% ethanol (2·8 l.), the disaccharide fraction with 5% ethanol (2·5 l.), and the trisaccharide fraction with 10% ethanol (2·2 l.). The disaccharide fraction (84 mg.) gave two spots when chromatographed on paper in solvents A and B. The faster spot had properties identical with that of the required glucoside. The slower spot gave a yellow colour with the periodate–Schiff reagents and a weak aniline phthalate reaction; it was probably kojibiose.[11] The syrup crystallised from ethanol as needles (35 mg., 6·5%) which were chromatographically homogeneous and behaved in the same way as the glucoside from *L. arabinosus* teichoic acid. The infrared spectrum was that of the newly crystallised form, and after several days the spectrum had changed to that of the stable hydrate. When recrystallised from ethanol, and permitted to absorb moisture, it had m. p. 125—126°, undepressed on admixture with the *glucoside* from *L. arabinosus* teichoic acid, m. p. 125—126° (Found: C, 40·6; H, 7·3. $C_{11}H_{22}O_{10},\frac{1}{2}H_2O$ requires C, 40·9; H, 7·2%).

Nona-O-*acetylcellobi-itol*.—The *compound* was prepared by acetylation of cellobi-itol (2 g.) with acetic anhydride (13·5 c.c.) and fused sodium acetate (0·67 g.) at 100° for 2 hr. Seed crystals were first obtained from an unsuccessful anomerisation with titanium tetrachloride in chloroform. It had m. p. 105—107° (from ethanol), $[\alpha]_D$ +18·8° (*c* 2·5 in $CHCl_3$) (Found: C, 50·2; H, 6·0. $C_{30}H_{42}O_{20}$ requires C, 49·9; H, 5·9%).

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