398. 2,3,4,6-*Tetra*-O-*benzyl*-D-*glucosyl Chloride and its Use in the Synthesis of the* α- *and* β-*Anomers of* 2-O-D-*Glucosylglycerol and* 4-O-D-*Glucosyl*-D-*ribitol.*

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2,3,4,6-Tetra-*O*-benzyl-D-glucosyl chloride has been synthesised by two methods, the two products being mixtures of the α- and the β-anomer in different proportions. The reactions of this glycosyl chloride have been studied and conditions are described for its use in the synthesis of α- and β-D-glucosides; the α- and β-anomers of 2-*O*-D-glucosylglycerol and 4-*O*-D-glucosyl-D-ribitol were synthesised by treating it with 1,3-di-*O*-benzylglycerol and 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-ribitol, respectively.

Several glycosides of glycerol and ribitol have been obtained in these laboratories by hydrolysis of teichoic acids [1] with alkali followed by enzymic dephosphorylation (for a review of teichoic acids see ref. 2). The structures assigned to four of these glycosides, the α- and β-anomers of 4-*O*-D-glucosyl-D-ribitol [3,4] and of 4-*O*-D-glucosaminyl-D-ribitol,[5] have been confirmed by synthesis. All the glycerol compounds obtained from teichoic acids [6-9] contain glycosidic linkages involving position 2 in glycerol. The glycoside obtained by degradation of the intracellular teichoic acid from *Lactobacillus arabinosus* 17-5 has been identified as 2-*O*-α-D-glucopyranosylglycerol.[6] Although this compound had already been prepared by degradation of maltose [10] it was thought that a synthesis from glycerol and D-glucose would be of interest.

The chemical synthesis of α-D-glucopyranosides is generally far more difficult than that of the β-D-anomers. The simplest glucosides are exceptional, thus the α-anomeric forms are easily prepared by the Fischer glycoside synthesis [11] in which a solution of D-glucose in the appropriate alcohol is boiled in the presence of an acid catalyst. Several approaches have been made to the problem of providing a general synthetical route to α-D-glucosides: (1) modification of the Koenigs–Knorr reaction for the synthesis of β-D-glucosides by replacing the silver salt catalyst with either quinoline [12] or a mercuric salt; [13] (2) preparation of stable β-D-glucosyl chlorides in which the 2-substituent does not participate in the displacement of halogen by a hydroxylic compound; [14,15] (3) anomerisation of the acetate of a β-D-glucoside; [16] (4) reaction of 1-*O*-(2′,4′,6′-trimethylbenzoyl)-β-D-glucose with alcohols in the presence of an acid catalyst; [17,18] (5) reaction of Brigl's anhydride (3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucose) [19] at elevated temperatures with some alcohols.[20,21] The last method was used recently in these laboratories in the synthesis of 4-*O*-α-D-glucosyl-D-ribitol.[3]

1 Armstrong, Baddiley, Buchanan, Carss, and Greenberg, *J.*, 1958, 4344.
2 Baddiley, *J. Roy. Inst. Chem.*, 1962, **86**, 366.
3 Sargent, Buchanan, and Baddiley, *J.*, 1962, 2184.
4 Baddiley, Buchanan, and Hardy, *J.*, 1961, 2180.
5 Hardy, Buchanan, and Baddiley, *J.*, 1963, 3360.
6 Critchley, Archibald, and Baddiley, *Biochem. J.*, 1962, **85**, 420.
7 Ellwood, Kelemen, and Baddiley, *Biochem. J.*, 1963, **86**, 213.
8 Wicken and Baddiley, *Biochem. J.*, 1963, **87**, 54.
9 RajBhandary and Baddiley, *Biochem. J.*, 1963, **87**, 429.
10 Charlson, Gorin, and Perlin, *Canad. J. Chem.*, 1956, **34**, 1811; 1957, **35**, 365.
11 Fischer, *Ber.*, 1893, **26**, 2400.
12 Fischer and Bergman, *Ber.*, 1917, **50**, 711.
13 Zemplen and Gerecs, *Ber.*, 1930, **63**, 2720.
14 Brigl, *Z. physiol. Chem.*, 1921, **116**, 1.
15 Wolfrom, Pittet, and Gillam, *Proc. Nat. Acad. Sci. U.S.A.*, 1961, **47**, 700.
16 Lindberg, *Acta Chem. Scand.*, 1949, **3**, 1355.
17 Helferich and Kashelikar, *Chem. Ber.*, 1957, **90**, 2094.
18 Helferich and Johannis, *Annalen*, 1960, **632**, 121.
19 Brigl, *Z. physiol. Chem.*, 1922, **122**, 245.
20 Hickinbottom, *J.*, 1928, 3140.
21 Lemieux and Huber, *J. Amer. Chem. Soc.*, 1956, **78**, 4117.

In the present work an attempt was made to synthesise 2-*O*-α-D-glucosylglycerol (VII) by a modification of the Fischer glycoside synthesis. Since a 1,3-protected glycerol with considerable stability to acid was required for such a reaction, 1,3-di-*O*-benzylglycerol (I) was prepared [22] and characterised. This derivative and 2,3,4,6-tetra-*O*-benzyl-α-D-glucose (II) [23] were heated in toluene with toluene-*p*-sulphonic acid as catalyst, and benzyl groups were removed from the product by hydrogenolysis. Unfortunately, a mixture of *O*-glucosylglycerols had been produced containing about 70% of 1-*O*-glucosylglycerol indicating that the acid conditions of the condensation had caused partial hydrolysis of 1,3-di-*O*-benzylglycerol.

Attention was then given to the preparation of a glucosyl chloride (IV) from 2,3,4,6-tetra-*O*-benzyl-α-D-glucose (II). Two methods were found for bringing about this transformation: (A) by the use of thionyl chloride and (B) by preparing the non-crystalline 1-*O*-acetyl derivative (III) (probably all α-anomer) and treating it with hydrogen chloride in dioxan. Barker and Fletcher [24] have recently obtained 2,3,5-tri-*O*-benzyl-D-ribosyl bromide from 2,3,5-tri-*O*-benzyl-D-ribose by preparing the 1-*O*-*p*-nitrobenzoyl derivative and treating this with hydrogen bromide in methylene chloride. Similarly, the glucosyl chloride (IV) could be synthesised by preparing the 1-*O*-*p*-nitrobenzoyl derivative of 2,3,4,6-tetra-*O*-benzyl-α-D-glucose (both anomers crystallised) and treating it with hydrogen chloride in methylene chloride, but methods (A) and (B) were preferred. The products of reactions (A) and (B) had different properties: the specific rotation of that prepared by method (A) suggested that it was mainly the α-anomer, and indeed on treatment with sodium methoxide it gave crystalline methyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucoside (V); the glucosyl chloride prepared by method (B) was presumably a 1 : 1 mixture of the α- and the β-anomer since treatment with sodium methoxide followed by catalytic hydrogenolysis of the product gave a mixture of approximately equal parts of methyl α- and β-D-glucoside. Both samples of the glucosyl chloride gave a good yield of 1,5-anhydro-D-glucitol (VI) [25] on reduction with lithium aluminium hydride followed by removal of benzyl groups.

The reaction between this chloride and 1,3-di-*O*-benzylglycerol under Koenigs–Knorr conditions was investigated. Glucosyl chlorides are rarely used in Koenigs–Knorr syntheses as they are usually unreactive.[26] However, it has recently been found that the removal of halogen from these compounds by silver ion is much faster if, instead of depending on a heterogeneous reaction with silver carbonate, a mixture of this salt with a little of the soluble silver perchlorate is used.[15] Under these conditions 2,3,4,6-tetra-*O*-benzyl-D-glucosyl chloride (IV) prepared by method (A) reacted smoothly with 1 mol. of 1,3-di-*O*-benzylglycerol (I) in benzene in the presence of anhydrous calcium sulphate. Paper chromatography of the product after hydrogenolysis revealed the presence of glycerol, glucose, and 2-*O*-glucosylglycerol. This mixture was resolved by chromatography on a column of Dowex 1 resin in the hydroxide form; [27] glycerol, 2-*O*-α-D-glucosylglycerol (VII) and the corresponding β-anomer (VIII) were eluted with water in that order while glucose was retained by the resin. These two glucosides were obtained in 42% yield from this reaction, the ratio of α- to β-anomer in the mixture being 2 : 1. When the glucosyl chloride (IV), prepared by method (B) was treated with a threefold excess of 1,3-di-*O*-benzylglycerol, in the same way, a 39% yield of the glucosides was obtained, the ratio of α- to β-anomer being 3·3 : 1. The high yield of α-glucoside in these two reactions indicates that, in the presence of silver perchlorate, the glucosyl chloride (IV) largely dissociates into an ion-pair [15,28] before reaction with the alcohol (I), thus the

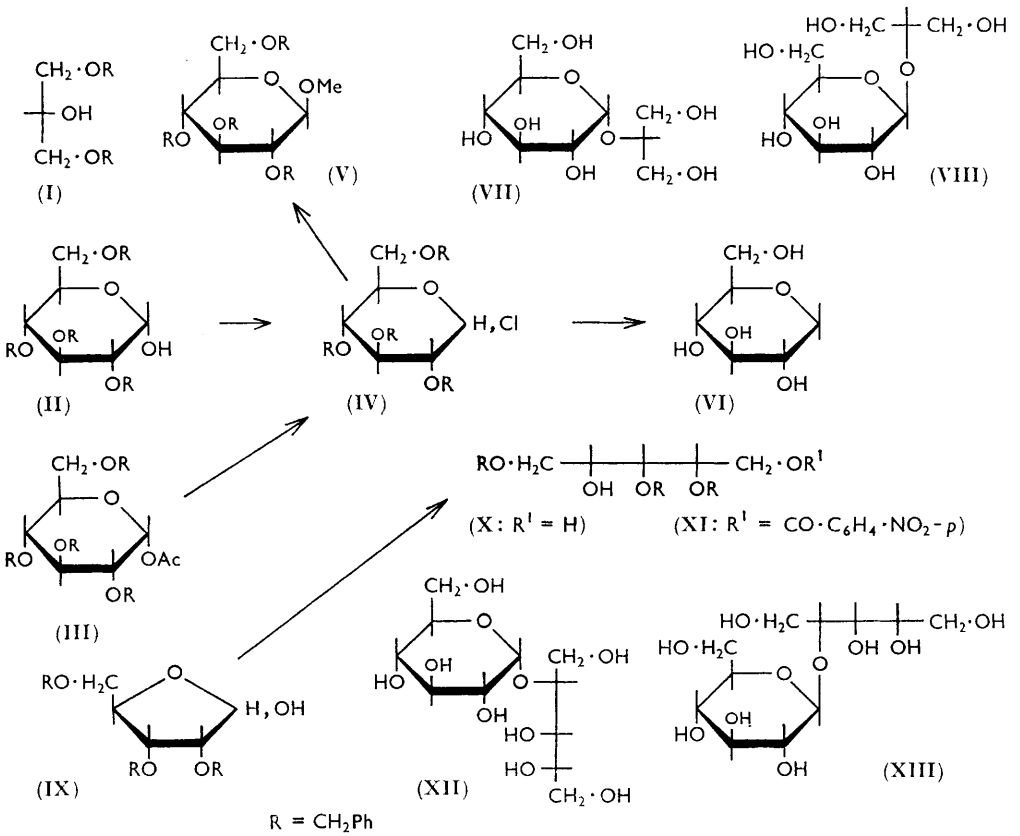
[22] Fairbourne, Gibson, and Stephens, *J.*, 1931, 445.
[23] Schmidt, Auer, and Schmadel, *Chem. Ber.*, 1960, **93**, 556.
[24] Barker and Fletcher, *J. Org. Chem.*, 1961, **26**, 4605.
[25] Ness, Fletcher, and Hudson, *J. Amer. Chem. Soc.*, 1950, **72**, 4547.
[26] Haynes and Newth, *Adv. Carbohydrate Chem.*, 1955, **10**, 207.
[27] Austin, Hardy, Buchanan, and Baddiley, *J.*, 1963, 5350.
[28] Swain and Pegues, *J. Amer. Chem. Soc.*, 1958, **80**, 812.

configuration of the glucosidic linkage in the product does not greatly depend on the configuration at position 1 in the chloride (IV).

Probably the best method described hitherto for the preparation of α-glucosides involves reaction between 3,4,6-tri-*O*-acetyl-2-*O*-nitro-β-D-glucosyl chloride and alcohols in the presence of silver carbonate and silver perchlorate.[15,29] In order to compare the two methods this chloride was treated with 1,3-di-*O*-benzylglycerol in ether under these conditions: after removal of protecting groups 2-*O*-α-D-glucosylglycerol (VII) was obtained in 39% yield, while the β-anomer was not detected. However, although this chloride gives a higher yield of the α-product, it is more difficult to prepare than the glucosyl chloride (IV).

The 2-*O*-α-D-glucosylglycerol prepared in these experiments was characterised as its hexa-*O*-*p*-nitrobenzoyl derivative. This was shown by comparison of melting points and infrared spectra to be identical with *p*-nitrobenzoates prepared from (*a*) the " natural " glucoside isolated from the intracellular teichoic acid of *L. arabinosus*,[6] and (*b*) the *O*-glucosylglycerol obtained by degradation of maltose with sodium periodate and sodium borohydride.[10]

A synthesis of the anomeric 4-*O*-D-glucosyl-D-ribitols (XII and XIII) from the glucosyl chloride (IV) was also carried out. All the suitably protected ribitol derivatives available [3,4] contained an *O*-isopropylidene ring; this might be unstable under Koenigs–Knorr reaction conditions described above, since triphenylmethyl ethers are known to be



unstable under similar conditions,[30] and so a derivative was prepared which did not contain these groups. Tri-*O*-benzyl-D-ribofuranose (IX) has recently been prepared as a syrup [24]

[29] Wolfrom, Thompson, and Lineback, *J. Org. Chem.*, 1963, **28**, 860.
[30] Bredereck, Wagner, Faber, Ott, and Rauther, *Chem. Ber.*, 1959, **92**, 1135.

which has been crystallised in these laboratories (P. R. H. Speakman, unpublished). A sample of this was reduced with sodium borohydride to the corresponding ribitol derivative (X) the structure of which was proved by methylation followed by hydrogenolysis to 1,4-di-*O*-methyl-D-ribitol; this consumed 1 mol. of sodium periodate without formation of formaldehyde. The ribitol derivative (X) was converted into its crystalline 1-*O*-*p*-nitrobenzoyl derivative (XI) which was treated with 2 mol. of the glucosyl chloride (IV) prepared by method (A). The product, after deacylation with methanolic ammonia was chromatographed on alumina. Three of the fractions from this column were subjected to catalytic hydrogenolysis over palladium and the products were identified as trehalose, 4-*O*-β-D-glucosyl-D-ribitol (XIII) and the corresponding α-anomer (XII), almost free from each other. The trehalose fraction was further separated into αα- and αβ-trehalose by chromatography on Dowex 1 resin in the hydroxide form.[27] 4-*O*-β-D-Glucosyl-D-ribitol [12·8% yield from the *p*-nitrobenzoate (XI)] was crystallised in the anhydrous form and as its hemihydrate and gave a crystalline octa-acetate. All these materials were identical to previously synthesised compounds [4] and to the glucoside (and its octa-acetate) obtained by degradation of the wall teichoic acid from *Bacillus subtilis*.[31] The 4-*O*-α-D-glucosyl-D-ribitol (22·2% yield) was crystallised in the anhydrous form which slowly absorbed moisture giving the stable hydrate; this was identified with previously synthesised material [3] and with one of the glucosides obtained by degradation of the wall teichoic acid from *L. arabinosus*.[32] The formation of trehalose in this experiment was expected since an excess of the glucosyl chloride had been used.

These Koenigs–Knorr reactions demonstrate that 2,3,4,6-tetra-*O*-benzyl-D-glucosyl-chloride, in the presence of silver carbonate and silver perchlorate, will react with alcohols (even secondary alcohols where steric-hindrance effects might be expected) to give mixtures of α- and β-D-glucosides in good yield. Since these αβ-mixtures are very easily separated by chromatography on Dowex 1 resin in the hydroxide form,[27] this chloride should provide a useful addition to the existing methods of synthesising α-glucosides.

Experimental

Infrared spectra were determined on potassium bromide discs. Chromatographic separations were carried out on either silica gel (British Drug Houses), neutral silica (silica gel neutralised with dilute ammonia and dried at 140°), alumina (Savory and Moore), or Dowex 1 resin (2% cross-linkages, 200—400 mesh, OH^- form).

Paper Chromatography.—Whatman No. 1 or 4 paper was used with the solvent system butan-1-ol–ethanol–water–ammonia (*d* 0·88) (40 : 10 : 49 : 1) [33] descending. The periodate–Schiff,[34,35] silver nitrate–sodium hydroxide,[36] and aniline phthalate reagents [37] were used as sprays where appropriate.

1,3-*Di*-O-*benzylglycerol*.—This was prepared from technical dichloropropanol (British Drug Houses) by the method of Fairbourne *et al.*[22] Fractional distillation gave a syrup, b. p. 180—182°/1·5—2 mm., in 75% yield. This material, on treatment with *p*-phenylazobenzoyl chloride in pyridine gave a crystalline p-*phenylazobenzoate*, m. p. 50°, from ethanol (Found: C, 75·1; H, 6·2. $C_{30}H_{28}N_2O_4$ requires C, 75·0; H, 5·9%), while reaction with α-naphthyl isocyanate in light petroleum (b. p. 100—120°) gave an α-*naphthylcarbamate*, m. p. 102°, from ethanol (Found: C, 76·0; H, 6·2. $C_{28}H_{27}NO_4$ requires C, 76·2; H, 6·2%). A sample of di-*O*-benzylglycerol (0·93 g.) was methylated with methyl sulphate in acetone in the presence of sodium hydroxide by the method of Myers *et al.*[38] The product, a pale yellow syrup (0·91 g., 94%), was hydrogenated during 20 hr., palladium (from 1·0 g. of oxide) in ethanol (40 ml.) being used. Filtration

[31] Armstrong, Baddiley, and Buchanan, *Biochem. J.*, 1960, **76**, 610.
[32] Archibald, Baddiley, and Buchanan, *Biochem. J.*, 1961, **81**, 124.
[33] Hirst, Hough, and Jones, *J.*, 1949, 928.
[34] Baddiley, Buchanan, Handschumacher, and Prescott, *J.*, 1956, 2818.
[35] Hardy and Buchanan, *J.*, 1963, 5881.
[36] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[37] Partridge, *Nature*, 1949, **164**, 443.
[38] Glen, Myers, and Grant, *J.*, 1951, 2568.

and evaporation to dryness gave 2-*O*-methylglycerol as a syrup (0·32 g., 95%) which was characterised as its di-*O*-*p*-nitrobenzoyl derivative, m. p. 154°, from ethyl acetate (Fairbourne *et al.*[22] give m. p. 154—155°). It was shown, by estimating [39] formaldehyde produced on oxidation with periodate, that the syrupy monomethylglycerol contained less than 3% of 1-*O*-methylglycerol.

Methyl 2,3,4,6-*Tetra*-O-*benzyl*-β-D-*glucoside*.—A mixture of methyl β-D-glucoside (0·54 g.), benzyl chloride (30 ml.), dioxan (30 ml.), and powdered potassium hydroxide (12 g.) was stirred at 100° for 2 hr. Benzyl chloride (30 ml.), dioxan (20 ml.), and powdered potassium hydroxide (12 g.) were added and stirring at 100° was continued for a further 6 hr. On cooling, the mixture was filtered through " Hyflo " silica, and the filtrate was concentrated to an oil which was dissolved in chloroform (150 ml.). The chloroform solution was washed with 2N-hydrochloric acid (100 ml.) and water (2 × 100 ml.), dried (Na_2SO_4), and evaporated to dryness at 160°/1·5 mm. The syrup crystallised from light petroleum (b. p. 60—80°) giving the *glucoside* as needles (0·58 g., 37%), m. p. 65—66°. After recrystallisation from ethanol it had m. p. 68—69°, $[\alpha]_D$ +11° (*c* 5·3 in dioxan) (Found: C, 75·5; H, 6·6; OMe, 6·0. $C_{35}H_{38}O_6$ requires C, 75·8; H, 6·8; OMe, 5·6%).

2,3,4,6-*Tetra*-O-*benzyl*-α-D-*glucose*.—This was prepared from methyl α-D-glucoside by a slight modification of the method of Schmidt *et al.*;[23] the benzylation proceeded more smoothly in dioxan, giving a higher yield of the required product.

1-O-*Acetyl*-2,3,4,6-*tetra*-O-*benzyl*-D-*glucose*.—The above tetra-*O*-benzylglucose (4·04 g.) was added to a mixture of acetic anhydride (10 ml.) and pyridine (15 ml.). After 2 days at room temperature the *acetate* was isolated as a syrup (4·15 g., 95%) by use of chloroform. It had $[\alpha]_D$ +51° (*c* 4·2 in C_6H_6) (Found: C, 74·3; H, 6·5. $C_{36}H_{38}O_7$ requires C, 74·3; H, 6·6%).

2,3,4,6-*Tetra*-O-*benzyl*-1-O-p-*nitrobenzoyl*-D-*glucose*.—2,3,4,6-Tetra-*O*-benzyl-α-D-glucose (4·46 g.) and *p*-nitrobenzoyl chloride (1·93 g.) were added to pyridine (30 ml.), and the mixture was shaken first at room temperature for 14 hr. and then at 37° for 28 hr. The p-*nitrobenzoate* was isolated with chloroform. Crystallisation from methylene chloride–ethanol gave the α-anomer as needles (4·1 g.), m. p. 126—127°, $[\alpha]_D$ +69° (*c*, 6·7 in dioxan) (Found: C, 71·0; H, 5·9; N, 1·8. $C_{41}H_{39}NO_9$ requires C, 71·4; H, 5·7; N, 2·0%); Tate and Bishop [40] give m. p. 126—127°, $[\alpha]_D$ +72°, for this compound. Concentration of the mother-liquors yielded a crystalline mass which, after recrystallisation from ethanol–ether and then from light petroleum (b. p. 60—80°), gave needles (0·9 g.) presumably of the β-anomer, m. p. 96—98°, $[\alpha]_D$ −26° (*c*, 6·0 in dioxan) (Found: C, 71·9; H, 5·8; N, 2·0%).

2,3,4,6-*Tetra*-O-*benzyl*-D-*glucosyl Chloride*.—(a) *From* 2,3,4,6-*tetra*-O-*benzyl*-α-D-*glucose*. This benzyl ether (2·91 g.) was added to pure thionyl chloride (10 ml.), and the solution was kept at 70° for 3 hr. After concentrating *in vacuo*, the residue was freed from traces of thionyl chloride by adding toluene and then evaporating to dryness. This procedure was carried out three times giving the *glucosyl chloride* as a dark yellow syrup (2·94 g.) (Found: Cl, 6·4; S, 0. $C_{34}H_{35}ClO_5$ requires Cl, 6·35%). This material could be purified by use of charcoal or neutral silica giving a syrup, $[\alpha]_D$ +95° (*c* 4·0 in C_6H_6), but this step is not usually necessary and in the reactions described below the coloured product was used.

(b) *From* 1-O-*acetyl*-2,3,4,6-*tetra*-O-*benzyl*-D-*glucose*. This acetate (1·57 g.) was dissolved in a freshly prepared 4% solution of hydrogen chloride in dioxan (60 ml.), and the resulting solution was kept at 37° for 30 hr. and then evaporated to dryness *in vacuo*. The residue was freed from traces of hydrogen chloride and acetic acid by evaporation with toluene (30 ml.); evaporation was carried out 3 times, leaving the glucosyl chloride as a yellow syrup (1·26 g., 78%), $[\alpha]_D$ +66° (*c*, 6·3 in C_6H_6) (Found: Cl, 6·15. $C_{34}H_{35}ClO_5$ requires Cl, 6·35%).

Reactions of 2,3,4,6-*Tetra*-O-*benzyl*-D-*glucosyl Chloride*.—(a) *With sodium methoxide*. A sample of the chloride (2·02 g.), prepared from 2,3,4,6-tetra-*O*-benzyl-α-D-glucose, was dissolved in anhydrous methanol (100 ml.) containing sodium methoxide (from 0·2 g. of sodium). After 2 days at room temperature the solution was neutralised (solid carbon dioxide) and evaporated to dryness. The residue was extracted with benzene (50 ml.) and the extract was washed with water, dried (Na_2SO_4), and evaporated to a syrup (1·8 g.). This was crystallised from ethanol giving, after recrystallisation, needles (0·9 g., 44%), $[\alpha]_D$ +11° (*c*, 5·0 in dioxan), m. p. 68—69°, undepressed in admixture with authentic methyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucoside.

[39] Hanahan and Olley, *J. Biol. Chem.*, 1958, **231**, 813.
[40] Tate and Bishop, *Canad. J. Chem.*, 1963, **41**, 1801.

In a similar experiment the syrupy product (1·11 g. from 1·2 g. of glucosyl chloride) was chromatographed on alumina (50 g.). Ether–benzene (1 : 9) eluted the glucosides (0·88 g.) which were then hydrogenated during 20 hr., palladium (from 0·6 g. of oxide) in ethanol–dioxan (150 ml., 1 : 1) being used. After filtration the solution was evaporated to dryness and the residue was dissolved in water (20 ml.); the aqueous solution was washed with chloroform and evaporated to a syrup (0·3 g., 72%), $[\alpha]_D$ −9° (*c*, 5·0 in H_2O). Examination of this material on paper showed that it contained only methyl β- and α-D-glucopyranosides ($R_{Glucose}$ 2·1 and 2·3, respectively). The specific rotation of the mixture indicated that the ratio of β- to α-anomer was approximately 7 : 1.

In another experiment a sample of 2,3,4,6-tetra-*O*-benzyl-D-glucosyl chloride (1·25 g.), prepared from 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-glucose, was treated with sodium methoxide; the glucosides were isolated by chromatography on alumina, and then hydrogenated as before. The resulting mixture of anomeric methyl glucosides (0·32 g., 75%) had $[\alpha]_D$ +62° (*c*, 5·1 in H_2O), indicating that it contained approximately equal amounts of the two anomers. The mixture was resolved by applying its aqueous solution to a column (10 cm. × 1·7 cm.) of Dowex 1 (OH^-) resin and then eluting with water.[27] The eluate was collected in fractions (8 ml.); fractions 4 and 5 contained methyl α-D-glucoside, m. p. 164—165°, $[\alpha]_D$ +152° (*c*, 3·5 in H_2O), while fractions 6—8 contained a similar amount of methyl β-D-glucoside, m. p. 106—108°, $[\alpha]_D$ −28° (*c*, 4·0 in H_2O).

(b) *With lithium aluminium hydride.* 2,3,4,6-Tetra-*O*-benzyl-D-glucosyl chloride (2·13 g.; prepared from the corresponding 1-*O*-acetyl derivative) and lithium aluminium hydride (0·42 g.) were added to anhydrous ether (50 ml.), and the mixture was stirred at room temperature for 3 hr. Lithium aluminium hydride (0·4 g.) was added and stirring was continued for a further 1·5 hr. Ethyl acetate was added cautiously to decompose excess of hydride and, after a little 2N-hydrochloric acid and ether (50 ml.) had been added, the solution was washed with 2N-hydrochloric acid (2 × 100 ml.) and water (150 ml.), dried (Na_2SO_4), and evaporated to a syrup (1·69 g.). This was chromatographed on alumina (60 g.); benzene eluted material (1·15 g.) with $[\alpha]_D$ +28° (*c*, 5·5 in dioxan) which was hydrogenated during 20 hr., palladium (from 0·4 g. of oxide) in ethanol–dioxan (50 ml., 3 : 2) being used. Filtration and evaporation to dryness gave 1,5-anhydro-D-glucitol (0·35 g., 54%), which on recrystallisation from ethanol had m. p. 136—138°, $[\alpha]_D$ +41° (*c*, 3·5 in H_2O) (Found: C, 43·6; H, 7·4. Calc. for $C_6H_{12}O_5$: C, 43·9; H, 7·4%). It was compared with an authentic sample of 1,5-anhydro-D-glucitol prepared from 2,3,4,6-tetra-O-acetyl-α-D-glucosyl bromide by the method of Ness *et al.*;[25] the two samples had the same mobility on paper ($R_{Glycerol}$ 0·65), possessed identical infrared spectra, and had the same m. p., undepressed in admixture.

When this experiment was carried out on a sample of the glucosyl chloride prepared from 2,3,4,6-tetra-*O*-benzyl-α-D-glucose, the same anhydroglucitol, m. p. 137—139°, was produced in about the same yield.

Synthesis of 2-O-D-*Glucopyranosylglycerol.*—(a) *By using* 2,3,4,6-*tetra*-O-*benzyl*-D-*glucosyl chloride derived from the corresponding* 1-O-*acetyl derivative.* 1,3-Di-*O*-benzylglycerol (2·0 g.), silver carbonate (2·5 g.), and Drierite (7·5 g., regular grade) were added to pure, dry benzene (25 ml.) and the mixture was stirred overnight in the dark. Silver perchlorate (0·08 g.) was added and half of a solution of the glucosyl chloride (1·2 g., 0·29 mol.) in benzene (25 ml.) was introduced during 2 hr. with stirring in the dark. Stirring was continued for a further 24 hr. and then the remainder of this solution was added during 3 hr. After the mixture had been stirred in the dark for a further 24 hr. it was filtered through " Hyflo " silica and the filtrate was thoroughly washed with water (2 × 100 ml.), dried (Na_2SO_4), and evaporated to a syrup (3·33 g.). This was hydrogenated during 48 hr., palladium (from 1 g. of oxide) in ethanol–dioxan (150 ml., 1 : 1) being used. After filtration, the solution was evaporated to dryness and the residue was examined on paper. This showed the presence of glycerol, glucose, 2-*O*-D-glucopyranosylglycerol ($R_{Glucose}$ 1·2), and another component ($R_{Glucose}$ 0·45). An aqueous solution of this mixture was applied to a column (18 cm. × 1·7 cm.) of Dowex 1 (OH^-) resin which was then kept overnight. Compounds were eluted with water, the eluate being collected in fractions (25—30 ml.);[27] fractions 1 and 2 contained glycerol (0·41 g.), fraction 3 contained pure 2-*O*-α-D-glucopyranosylglycerol, and fractions 4 and 5 contained 2-*O*-β-D-glucopyranosylglycerol. The α-anomer (0·17 g., 30%), $[\alpha]_D$ +122° (*c*, 3·0 in H_2O) (Charlson *et al.*[10] give $[\alpha]_D$ +120°), was converted by means of benzoyl chloride in pyridine into its hexa-*O*-benzoyl derivative, m. p. 134° (from methylene chloride–ethanol), $[\alpha]_D$ +96° (*c*, 4·0 in $CHCl_3$) (Found: C, 69·9;

H, 4·9. Calc. for $C_{51}H_{42}O_{14}$: C, 69·7; H, 4·8%). The m. p. of this derivative was not depressed on mixing it with authentic material, m. p. 137—138°, $[\alpha]_D$ +96°, provided by Dr. A. S. Perlin, and the infrared spectra of the two samples were identical. Recrystallisation from methylene chloride–ethanol could give this compound in a different crystalline form, m. p. 97°, $[\alpha]_D$ +92° (*c*, 3·8 in $CHCl_3$). The slightly impure β-anomer (0·073 g.) was purified as its hexa-*O*-acetyl derivative (0·098 g.), m. p. 127—129°, $[\alpha]_D$ −15° (*c*, 7·2 in $CHCl_3$) (Found: C, 49·9; H, 6·0. Calc. for $C_{21}H_{30}O_{14}$: C, 49·8; H, 6·0%); Carter [41] gives m. p. 128°, $[\alpha]_D$ −15°, for this compound. The amount of pure hexa-*O*-acetyl derivative corresponds to a 9% yield of 2-*O*-β-D-glucosylglycerol from 2,3,4,6-tetra-*O*-benzyl-D-glucosyl chloride.

(b) *By using* 2,3,4,6-*tetra*-O-*benzyl*-D-*glucosyl chloride derived from* 2,3,4,6-*tetra*-O-*benzyl*-α-D-*glucose*. 1,3-Di-*O*-benzylglycerol (0·7 g.), Drierite (18 g.), and silver carbonate (4 g.) were added to pure, dry benzene (80 ml.), and the mixture was stirred overnight in the dark. Silver perchlorate (0·1 g.) was added and a solution of the glucosyl chloride (1·4 g., 1 mol.) in benzene (60 ml.) was introduced during 4 hr. with constant stirring in the dark. The mixture was stirred for a further 48 hr. and then filtered through " Hyflo " silica. The filtrate was washed with water and evaporated to dryness. The residue (1·79 g.) was chromatographed on alumina (100 g.); elution with benzene (900 ml.) and benzene–ether (1800 ml., 49 : 1) gave material (1·29 g.) which was hydrogenated during 48 hr., by using palladium (from 0·83 g. of oxide) in ethanol–dioxan (150 ml., 1 : 1). Filtration and evaporation gave a syrup (0·36 g.) which was shown by paper chromatography to be largely 2-*O*-D-glucosylglycerol. It was dissolved in a little water and chromatographed on a column (25 cm. × 1·7 cm.) of Dowex 1 (OH^-) resin; material was eluted with water, and the eluate was collected in fractions (25 ml.). Fractions 4 and 5 contained pure 2-*O*-α-D-glucosylglycerol (0·175 g., 28%) which was converted by means of *p*-nitrobenzoyl chloride in pyridine [10] into its hexa-*O*-*p*-nitrobenzoyl derivative, m. p. 188—189° (from methylene chloride–ethanol) (Found: C, 53·3; H, 3·2; N, 7·3. Calc. for $C_{51}H_{36}N_6O_{26}$: C, 53·55; H, 3·2; N, 7·4%). Fractions 6 and 7 contained slightly impure 2-*O*-β-D-glucosylglycerol which was purified by conversion into its hexa-*O*-acetyl derivative, m. p. 127—129°. The amount of this compound (0·177 g.) corresponds to a 14% yield of the β-glucoside from 2,3,4,6-tetra-*O*-benzyl-D-glucosyl chloride.

The hexa-*O*-*p*-nitrobenzoyl derivative of 2-*O*-α-D-glucosylglycerol had the same m. p. as a sample provided by Dr. A. S. Perlin and a sample prepared by Dr. P. Critchley [6] from an *O*-glucosylglycerol obtained from the intracellular teichoic acid of *L. arabinosus*. All samples possessed identical infrared spectra and no depression of m. p. was observed on mixing them. Recrystallisation from acetone–ethanol could give this compound in a different crystalline form, m. p. 165—168°.

(c) *By using* 3,4,6-*tri*-O-*acetyl*-2-O-*nitro*-β-D-*glucosyl chloride*. 1,3-Di-*O*-benzylglycerol (2·04 g.), silver carbonate (2 g.), Drierite (3 g.), and anhydrous ether (100 ml.) were mixed. To this mixture was added silver perchlorate (0·1 g.) and the β-glucosyl chloride [15,29] (1·0 g., 0·36 mol.) with constant stirring in the dark; stirring was continued for 9 hr. After filtration through " Hyflo " silica, the solution was evaporated to a syrup (2·73 g.) which was hydrogenated in ethanol (200 ml.) during 48 hr., 10% palladium on charcoal (8·0 g.) being used. Filtration and evaporation gave a syrup (1·04 g.) which was dissolved in methanol (20 ml.), sodium methoxide (from 0·03 g. of sodium) in methanol (15 ml.) was added, and the solution was kept at room temperature for 2 days. After neutralisation (solid carbon dioxide) the solution was evaporated to dryness. Chromatography showed the presence of glucose, glycerol 2-*O*-D-glucosylglycerol, and another component ($R_{Glucose}$ 0·45). The mixture in a little water was applied to a column (20 cm. × 1·7 cm.) of Dowex 1(OH^-) resin, and material was eluted with water, the eluate being collected in fractions (50 ml.). Fraction 2 contained glycerol, while fractions 3 and 4 contained syrupy 2-*O*-α-D-glucosylglycerol (0·27 g., 39%), $[\alpha]_D$ +110° (*c*, 6·0 in H_2O), characterised as its hexa-*O*-*p*-nitrobenzoyl derivative, m. p. 188—189°.

2,3,5-*Tri*-O-*benzyl*-D-*ribofuranose*.—D-Ribose (10 g.) was dissolved in methanol (200 ml.) containing sulphuric acid (1 ml.). After 1 hr. at room temperature the solution was neutralised ($BaCO_3$) and filtered through " Hyflo " silica. The filtrate was evaporated to a syrup which was shown by paper chromatography to contain the anomeric methyl ribofuranosides and some ribose. It was dissolved in water (150 ml.) and Dowex 1 (OH^-) resin (50 ml.) was added. After being shaken overnight at room temperature the mixture was applied to a short column

[41] Carter, *Ber.*, 1930, **63**, 1684.

of Dowex 1 (OH^-) resin. When the aqueous solution had passed through the resin, the column was washed with water (450 ml.), and the eluate and washings were evaporated to a yellow syrup (10 g.) which was free from ribose.

Syrupy methyl 2,3,5-tri-*O*-benzyl-β-D-riboside, prepared from this material by the method of Barker and Fletcher,[24] was dissolved in a mixture of dioxan (500 ml.) and 0·12N-hydrochloric acid (125 ml.), and the solution was boiled gently for 7 hr., distillate (135 ml.) being collected during that time. The residual solution was concentrated *in vacuo* to a yellow syrup which solidified after the addition of a crystal of 2,3,5-tri-*O*-benzyl-D-ribofuranose. Recrystallisation from light petroleum (b. p. 60—80°) gave crystals (13·7 g.), m. p. 48—50°, $[\alpha]_D$ +39° (*c*, 2·0 in dioxan); Speakman and Hughes (unpublished) give m. p. 50—51°, $[\alpha]_D$ +42°, for this compound. The mother-liquors were evaporated to a syrup which was treated with more dioxan and dilute hydrochloric acid as described above. After evaporation to dryness, the remaining syrup solidified on addition of a crystal of the benzyl ether. Recrystallisation gave material (7·3 g.), m. p. 47—49°. The yield from D-ribose was 71%.

2,3,5-*Tri*-O-*benzyl*-D-*ribitol*.—The above ribose benzyl ether (6·79 g.) and sodium borohydride (1·05 g.) were dissolved in ethanol (80 ml.), and the solution was kept at room temperature for 24 hr. After acetic acid had been added to pH 5, the solution was evaporated to dryness. The residue was shaken with a mixture of chloroform (150 ml.) and N-hydrochloric acid (150 ml.), and the chloroform layer was washed with a further quantity (100 ml.) of this acid and water (150 ml.) and then dried (Na_2SO_4). Evaporation to dryness gave 2,3,5-*tri*-O-*benzyl*-D-*ribitol* (6·7 g.) as a syrup, $[\alpha]_D$ +14° (*c*, 4·8 in dioxan).

The ribitol derivative was characterised as 4-O-*acetyl*-2,3,5-*tri*-O-*benzyl*-1-O-*triphenylmethyl*-D-*ribitol* by treatment of a pyridine solution with triphenylmethyl chloride (1 mol.) and then with acetic anhydride (cf. ref. 4). Isolated by use of chloroform and crystallised from ethanol, this derivative had m. p. 80°, $[\alpha]_D$ +15° (*c*, 3·9 in dioxan) (Found: C, 80·2; H, 6·4. $C_{47}H_{46}O_6$ requires C, 79·9; H, 6·6%).

1,4-*Di*-O-*methyl*-D-*ribitol*.—2,3,5-Tri-*O*-benzyl-D-ribitol (0·6 g.) and powdered potassium hydroxide (1·7 g.) were added to dioxan (20 ml.), and the mixture was stirred at 45°. Methyl sulphate (1·7 ml.) was added during 45 min. and stirring was continued for 3 hr. More potassium hydroxide (1·7 g.) was added, followed by methyl sulphate (1·5 ml.) and dioxan (12 ml.) during 1·5 hr.; the temperature was raised to 60° and stirring was continued for a further 4 hr. The solution was filtered through " Hyflo " silica and evaporated to a syrup which was shaken with a mixture of chloroform (100 ml.) and water (100 ml.). The chloroform layer was dried (Na_2SO_4) and evaporated to dryness; the residue was dissolved in benzene and chromatographed on alumina (30 g.). Elution with benzene (150 ml.) gave a syrup (0·49 g.) which was hydrogenated during 24 hr., palladium (from 0·44 g. of oxide) being used in ethanol (20 ml.). Filtration and evaporation to dryness gave 1,4-*di*-O-*methyl*-D-*ribitol* as a syrup (0·19 g., 74%). It consumed 1 molar equivalent of sodium metaperiodate, determined by the method of Dixon and Lipkin,[42] without formation of formaldehyde and it gave a crystalline *tri*-O-p-*nitrobenzoyl derivative*, m. p. 168—170° (Found: C, 53·5; H, 3·8; N, 6·8. $C_{28}H_{25}N_3O_{14}$ requires C, 53·6; H, 4·0; N, 6·7%).

2,3,5-*Tri*-O-*benzyl*-1-O-p-*nitrobenzoyl*-D-*ribitol*.—2,3,5-Tri-*O*-benzyl-D-ribitol (6·8 g.) and *p*-nitrobenzoyl chloride (3·0 g., 1 mol.) were dissolved in methylene chloride (30 ml.). Pyridine (10 ml.) was slowly added and the solution was kept for 4 days at room temperature. Water (1 ml.) was added and after 2 hr. the solution was diluted with chloroform (60 ml.), washed with 2N-sulphuric acid (2 × 100 ml.) and water, dried (Na_2SO_4), and evaporated to a syrup. The syrup was dissolved in a little benzene and chromatographed on silica (200 g.); benzene–ether (19 : 1) eluted a syrup which gave crystals (6·3 g., 73%) of the p-*nitrobenzoate* [from ether–light petroleum (b. p. 40—60°)], m. p. 75—76°, $[\alpha]_D$ −18° (*c*, 5·8 in dioxan), $[\alpha]_D$ −21° (*c*, 1·8 in C_6H_6), (Found: C, 69·65; H, 5·6; N, 2·5. $C_{33}H_{33}NO_8$ requires C, 69·5; H, 5·8; N, 2·5%); ether eluted 2,3,5-tri-*O*-benzyl-D-ribitol (0·65 g.).

Reaction between 2,3,5-*Tri*-O-*benzyl*-1-O-p-*nitrobenzoyl*-D-*ribitol and* 2,3,4,6-*Tetra*-O-*benzyl*-D-*glucosyl Chloride*.—The above p-*nitrobenzoate* (2·38 g.), silver carbonate (4·87 g.), and Drierite (20 g.) were added to benzene (80 ml.), and the mixture was stirred at room temperature in the dark for 12 hr. Silver perchlorate (0·2 g.) was added and a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucosyl chloride (2·17 g., 0·93 mol.; prepared by the action of thionyl chloride on 2,3,4,6-tetra-*O*-benzyl-α-D-glucose) in benzene (50 ml.) was added during 8 hr. with stirring in the dark.

[42] Dixon and Lipkin, *Analyt. Chem.*, 1954, **26**, 1092.

Stirring was continued for a further 12 hr. and then more of the glucosyl chloride (2·17 g.) in benzene (50 ml.) was added during 4 hr. After being stirred in the dark for a further 48 hr. the mixture was filtered through " Hyflo " silica; the filtrate was washed with water (3 × 200 ml.), dried (Na_2SO_4, $NaHCO_3$), and evaporated to a syrup (6·18 g.). This was dissolved in methanol (200 ml.), previously saturated at 0° with dry ammonia, and the solution was kept at room temperature overnight. Ammonia solution (10 ml.; *d* 0·88) was added with stirring and the solution was kept for a further 24 hr. Evaporation to dryness gave a residue which was extracted with warm benzene (2 × 200 ml.); this extract was clarified by filtration and concentrated to a syrup (5·51 g.). This, in a little benzene, was chromatographed on alumina (240 g.) and compounds were eluted as follows: benzene–ether (19 : 1) eluted a syrup (0·97 g.)—product 1; benzene–ether (9 : 1) eluted a second syrup (0·7 g.)—product 2; benzene–ether (1 : 1) eluted a third syrup (1·63 g.)—product 3. These products were hydrogenated separately and the glucosides were isolated and identified as described below.

(a) *Product* 1. This was hydrogenated during 20 hr. over palladium (from 0·8 g. of oxide) in ethanol–dioxan (150 ml., 1 : 1). Filtration and evaporation to dryness gave material (0·32 g.) which had the same mobility on paper as trehalose ($R_{Glucose}$ 0·47). It was dissolved in water (1 ml.) and applied to a column (22 cm. × 1·7 cm.) of Dowex 1 (OH^-) resin; material was eluted with water, the eluate being collected in fractions (20 ml.). Fractions 8—11 contained αα-trehalose (0·06 g.) which on recrystallisation from aqueous ethanol had $[\alpha]_D$ +177° (*c* 1·0 in H_2O) and a softening point between 100—135°. It was converted into its crystalline octa-acetate $[\alpha]_D$ +160° (*c* 2·1 in $CHCl_3$), m. p. and mixed m. p. 99—101°; Lemieux and Bauer [43] give $[\alpha]_D$ +163°, m. p. 101°, for this compound. Fractions 13—19 contained αβ-trehalose (0·13 g.) which on recrystallisation from aqueous ethanol had $[\alpha]_D$ +97° (*c* 1·0 in H_2O) and softened between 137—148°. Its crystalline octa-acetate had m. p. 141—142°, $[\alpha]_D$ +84° (*c*, 2·3 in $CHCl_3$); Haworth and Hickinbottom [44] give softening point 145—150°, $[\alpha]_D$ +95° for the disaccharide and m. p. 140—141°, $[\alpha]_D$ +85°, for its acetate.

(b) *Product* 2. This was hydrogenated during 20 hr., palladium (from 0·5 g. of oxide) being used in ethanol–dioxan (150 ml.). Filtration and evaporation to dryness gave material (0·23 g.) with $[\alpha]_D$ −10° (*c*, 4·5 in H_2O). Chromatography on paper showed the presence of a major component with the mobility of a 4-*O*-D-glucopyranosyl-D-ribitol ($R_{Ribitol}$ 0·55). Crystallisation from methanol–ether gave microcrystals (0·124 g.), m. p. 133—135° $[\alpha]_D$ −17° (*c*, 5·0 in H_2O). The infrared spectra of this material and authentic 4-*O*-β-D-glucopyranosyl-D-ribitol,[4] m. p. 134—137°, $[\alpha]_D$ −22°, were indistinguishable. Recrystallisation from aqueous ethanol, on the other hand, gave needles, $[\alpha]_D$ −19° (*c*, 5·0 in H_2O) (Found: C, 40·5; H, 7·2. Calc. for $C_{11}H_{33}O_{10},0{\cdot}5H_2O$: C, 40·9; H, 7·2%), m. p. 132—134°, undepressed in admixture with authentic 4-*O*-β-D-glucosyl-D-ribitol hemihydrate; [4,31] the infrared spectra of the two were identical. The mother-liquors from these crystallisations were evaporated to dryness and the residue, in a little water, was chromatographed on a column (12 cm. × 1·7 cm.) of Dowex 1 (OH^-) resin; elution was with water, the eluate being collected in fractions (15 ml.). Fractions 19—24 contained pure 4-*O*-β-D-glucosyl-D-ribitol (0·043 g.), m. p. 134—135° (from aqueous ethanol), $[\alpha]_D$ −19° (*c*, 2·4 in H_2O), while fractions 15—17 contained a little (0·006 g.) of the corresponding α-anomer. Thus, crystalline 4-*O*-β-D-glucosyl-D-ribitol (0·167 g.) was obtained in 12·8% yield from 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-ribitol.

A sample of the anhydrous glucoside was converted into its octa-*O*-acetyl derivative, m. p. 98° (from ethanol) undepressed in admixture with an authentic specimen [4] (Found: C, 50·1; H, 5·9. Calc. for $C_{27}H_{38}O_{18}$: C, 49·8; H, 5·9%).

(c) *Product* 3. This material was hydrogenated in ethanol–dioxan (150 ml.) during 20 hr., palladium (from 0·9 g. of oxide) being used. Filtration and evaporation gave a syrup (0·49 g.), $[\alpha]_D$ +91° (*c*, 4·9 in H_2O). Examination on paper showed only a compound with the mobility of a 4-*O*-D-glucosyl-D-ribitol. Crystallisation from aqueous ethanol gave, after recrystallisation from this solvent, needles (0·15 g.), $[\alpha]_D$ +104° (*c*, 2·0 in H_2O), which gave an infrared spectrum identical to that of anhydrous 4-*O*-α-D-glucosyl-D-ribitol.[3] When exposed to the atmosphere this material slowly absorbed moisture forming the more stable monohydrate (Found: C, 39·9; H, 7·2. Calc. for $C_{11}H_{22}O_{10},H_2O$: C, 40·0; H, 7·2%), m. p. 119—121°, undepressed in admixture with an authentic sample of this hydrate [32] which had m. p. 122—124°; the infrared spectra of these samples were identical. The mother-liquors were evaporated to dryness and

[43] Lemieux and Bauer, *Canad. J. Chem.*, 1954, **32**, 240.
[44] Haworth and Hickinbottom, *J.*, 1931, 2847.

the residue, in a little water, was applied to a column (23 cm. × 1·7 cm.) of Dowex 1 (OH^-) resin [28] and elution was carried out with water. The first 50 ml. of eluate was discarded and further fractions (20 ml.) were collected. Fractions 3—6 contained pure 4-*O*-α-D-glucopyranosyl-D-ribitol which crystallised from aqueous ethanol as rosettes of fine needles (0·14 g.). This material, after absorption of moisture from the atmosphere, had m. p. 122—124°, $[\alpha]_D$ +107° (*c*, 1·4 in H_2O); Archibald *et al.*[32] give m. p. 125—126°, $[\alpha]_D$ +106°, for this compound. Thus, crystalline 4-*O*-α-D-glucosyl-D-ribitol (0·29 g.) was produced in 22·2% yield from 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-ribitol. A sample of the anhydrous glucoside was converted into its crystalline *octabenzoate*, m. p. 83—86° (from ethanol–ether), $[\alpha]_D$ +63° (*c*, 3·8 in dioxan) (Found: C, 69·8; H, 5·0. $C_{67}H_{54}O_{18}$ requires C, 70·2; H, 4·75%).

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