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SYNTHESIS OF GLUCOSYLGLYCEROLS AND DIGLUCOSYLGLYCEROLS, AND THEIR IDENTIFICATION IN SMALL AMOUNTS

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ABSTRACT

The four *O*-β-D-glucopyranosyl(1→*x*)-*O*-β-D-glucopyranosyl(1→1)-D-glycerols (*x* = 2, 3, 4, or 6) have been synthesized and shown to be distinguishable by paper and gas–liquid chromatographic techniques. Synthesis of the four *O*-D-glucopyranosyl-(1→1)-glycerols and *O*-α-kojibiosyl-(1→1)-L-glycerol showed that anomers, but not all diastereomers, are distinguishable by these methods. The application of these results to general problems in structural carbohydrate chemistry is outlined, and the procedure has been used in the identification of small amounts of glycosides derived from bacterial glycolipids.

INTRODUCTION

Glycosylglycerols and diglycosylglycerols have been isolated as deacylation products of glycolipids from Gram-positive bacteria[1] and plants[2], and as degradation products of various carbohydrates[3–5] and teichoic acids[6]. For three of the glycolipids, confirmation of structures derived from degradative studies has been provided by synthesis[7–9]. However, as the amount of glycoside isolated from bacteria[10] is usually small, a microscale method of identification, based on a comparison with synthetic compounds, seemed desirable.

DISCUSSION

Gas–liquid chromatography of glycosyl- and diglycosyl-glycerols (obtained from bacterial glycolipids[10]), as their trimethylsilyl ethers[11] on SE52 at 185° and 255°, respectively, indicated that this method might be useful for the identification of related glycosides, especially when taken in conjunction with paper chromatography. The procedure has been examined for a particularly accessible series, the *O*-β-D-glucopyranosyl-*O*-β-D-glucopyranosyl-(1→1)-D-glycerols, and for diastereomeric glucosyl- and diglucosyl-glycerols. The results (Table I) indicated that the four positional isomers of diglucosylglycerol are distinguishable by g.l.c. and paper chromatography, and provide proof that the glycoside obtained by deacylation of the glycolipids of *Bacillus subtilis*, *Staphylococcus aureus* H, *Staph. aureus* B4, *Staph. saprophyticus* I2,

Staph. lactis (*albus*) N.C.T.C. 7944, and *Staph. lactis* I3 is, in all cases, *O*-β-gentiobiosyl-(1→1)-glycerol (**1**). The glycoside from *Lactobacillus casei* A.T.C.C. 7469 is identical to that[7] from *Pneumococcus* 1-192R, A.T.C.C. 12213, and was shown

TABLE I

PAPER AND GAS–LIQUID CHROMATOGRAPHIC PROPERTIES OF SYNTHETIC AND NATURAL DIGLYCOSYLGLYCEROLS

Glycoside	R_{Glc}[a]	T[b]
From *Pneumococcus glycolipid*[26] (**2**)	0.43	1.00
From *Lactobacillus casei* glycolipid[10]	0.43	1.01
Synthetic Gal-Glc-Gly[7] (**2**)	0.43	1.00
O-β-Sophorosyl-(1→1)-D-glycerol (**10**)	0.65	0.93
O-α-Sophorosyl-(1→1)-D-glycerol (**9**)	0.57	1.06
O-Laminaribiosyl-(1→1)-D-glycerol (**8**)	0.79	0.91
O-β-Cellobiosyl-(1→1)-D-glycerol (**6**)	0.43	1.10, 0.87[c]
O-β-Gentiobiosyl-(1→1)-D-glycerol (**7**)	0.49	1.33
From *B. subtilis* glycolipid[10]	0.49	1.33
From *Staph. aureus* H glycolipid[10]	0.49	1.33
From *Staph. aureus* B4 glycolipid	0.49	1.33
From *Staph. saprophyticus* I2 glycolipid[10]	0.49	1.33
From *Staph. lactis* (albus) glycolipid[10]	0.49	1.33
From *Staph. lactis* I3 glycolipid[8]	0.49	1.33
From *Micrococcus lysodeikticus* glycolipid[10]	0.51	0.76
From *Strep. faecalis* glycolipid[10]	0.53	1.06
O-α-Kojibiosyl-(1→1)-L-glycerol (**4**)	0.52	1.07

[a]Paper-chromatographic mobility relative to that of glucose (Ref. 24). [b]Elution time of *O*-trimethylsilyl derivative relative to that of glycoside **2**, 13.25 min. [c]Impurity, presumably of α-cellobioside, present as 4% of preparation.

previously[7] by synthesis to be *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→1)-D-glycerol (**2**). The chemical degradation of the glycoside from *L. casei* confirms this structure[12]. The glycoside from the glycolipid of *Streptococcus faecalis* 9, characterized[13] for a different strain as *O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→1)-glycerol (**3**) (presumably the D-glycerol form, by biosynthetic reasoning), chromatographed identically with the synthetic diastereomer of L-glycerol (**4**), suspected as a degradation product of a novel teichoic acid under investigation in this laboratory[14]. Thus, it appears that the method will not distinguish diastereomeric glycerol glycosides. The same conclusion was reached with the four synthetic *O*-D-glucopyranosyl-(1→1)-glycerols (Table II).

Isomers of *O*-β-D-glucopyranosyl-*O*-β-D-glucopyranosyl-(1→1)-D-glycerol were synthesized by condensation of the appropriate hepta-*O*-acetyldisaccharide bromides and methylenebis-2-*O*-(3-*O*-benzoyl-D-glycerol)[9] (**5**) in methylene chloride in the presence of mercuric oxide and mercuric bromide[15]. Hepta-*O*-acetyl-α-sophorosyl bromide gave a high proportion of the α-sophoroside, in accordance with the previous conclusion that the reason for the usual production of *trans*-glycosides in the Koenigs–Knorr synthesis is the participation of a neighbouring acyl group on C-2 of the

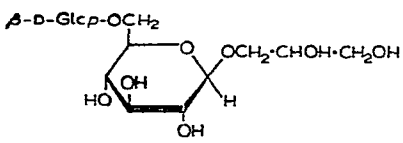
acylglycosyl halide[7,16–18]. It is interesting that the β-sophoroside trimethylsilyl ether emerged from the g.l.c. column before the α-sophoroside derivative, and that the β-sophoroside had the lower melting point and the faster mobility on paper chromatography. Construction of the molecular models indicated that the β-glycoside is the more hindered of the two anomers.

TABLE II

PAPER AND GAS–LIQUID CHROMATOGRAPHIC PROPERTIES OF SYNTHETIC AND NATURAL GLYCOSYLGLYCEROLS

Glycoside	R_{Glc}[a]	T[b]
O-α-Glucosyl-(1→1)-D-glycerol	1.00	1.00
O-β-Glucosyl-(1→1)-D-glycerol	1.02	1.36
O-α-Glucosyl-(1→1)-L-glycerol	1.00	1.00
O-β-Glucosyl-(1→1)-L-glycerol	1.00	1.36
O-α-Galactosyl-(1→1)-D-glycerol	0.86	0.97
O-β-Galactosyl-(1→1)-D-glycerol	0.85	1.08
From *Pneumococcus* minor glycolipid[26]	1.00	1.00
From *Staph. aureus* B4 minor glycolipid	1.01	1.36
From partial acidic hydrolysis of 8	—	1.00, 1.36

[a]Paper-chromatographic mobility relative to that of glucose (Ref. 24). [b]Elution time of *O*-trimethylsilyl derivative relative to that of *O*-α-D-glucopyranosyl-(1→1)-D-glycerol, 17.05 min.



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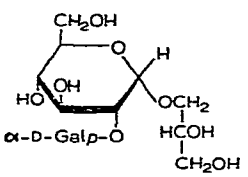

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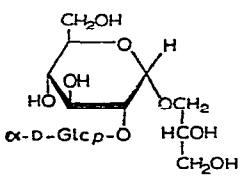

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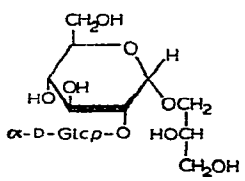

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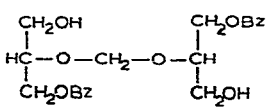

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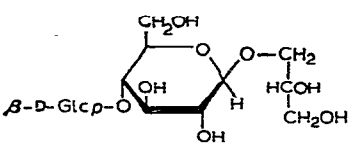

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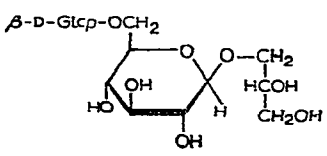

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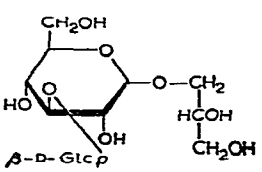

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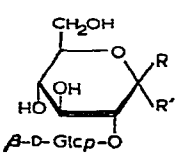

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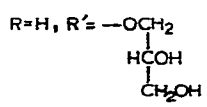

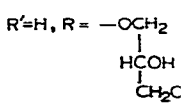

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The β-cellobioside **6** and β-gentiobioside **7** were easily prepared in a pure condition[8]. On the other hand, the laminaribioside **8** was obtained as a mixture of the α- and β-anomers, and these were not resolved by g.l.c. or paper chromatography. This may be due to the steric effect between the bifunctional aglycon **5** and the hepta-*O*-acetyl-α-laminaribiosyl bromide in which the tetra-*O*-acetyl-D-glucopyranosyl substituent is still near the C-1 reaction centre. Support for the view that an aglycon can be partly responsible for influencing the anomeric configuration in the synthesis of a glycoside comes from a study of the reaction between compound **5** and tetra-*O*-acetyl-α-D-glucopyranosyl bromide, when 29% of β-D-glucoside and 6% of α-D-glucoside were obtained. Wickberg's synthesis[9] of the corresponding anomeric galactosides was repeated, and his similar observation was confirmed. By contrast, condensation of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with 2,3-di-*O*-benzyl-L-glycerol yielded only a trace of the α-D-glucoside, a result consistent with Wickberg's findings[9] in the synthesis of the corresponding anomeric L-glycerol galactosides. We synthesized *O*-α-D-glucopyranosyl-(1→1)-L-glycerol by condensation of 2,3-di-*O*-benzyl-L-glycerol with 1 molar proportion of 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride[19]. Subsequent addition of a second molar proportion of the chloride to the reaction mixture yielded *O*-α-D-glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→1)-L-glycerol (**4**). Sequential addition of hexose residues in this way has found use recently in the synthesis of substituted cerebrosides[20,21], but this is the first report of the synthesis of *cis*-glycosidic linkages by this procedure.

The g.l.c. and paper-chromatographic procedure described here has been used successfully on small amounts (less than 2 mg) of a number of glycosides of glycerol obtained from earlier work[10] on the structure of bacterial glycolipids. When appropriate samples of synthetic glycosides are available, it could be used for the identification of glycosylglycerols obtained from teichoic acids and from the degradation of other carbohydrates, especially in the application of the Smith degradation[23] to polysaccharides. The method would also be valuable in the identification of glycosylglycerols obtained by partial hydrolysis of diglycosylglycerols with acid and in the determination of purity of anomeric glycerol glycosides. Its sensitivity is shown in an examination of the glycoside preparation from the total glycolipid of *Staph. aureus* B4; g.l.c. of the trimethylsilyl ethers revealed the presence of *O*-β-D-glucopyranosyl-(1→1)-glycerol and *O*-β-D-gentiobiosyl-(1→1)-glycerol (**1**) in the molar ratio 1:100.

O-β-D-Glucopyranosyl-(1→1)-D-glycerol, the L-glycerol diastereomer, and *O*-β-gentiobiosyl-D-glycerol (**7**) were examined by Prof. E. F. Gale as possible[22] bacterial 'amino acid incorporation factors'. The first glycoside was highly active, whereas the other two were totally inactive.

EXPERIMENTAL

General Methods. — Gas–liquid chromatography of glycosylglycerols as their trimethylsilyl ethers, prepared by addition of Pierce 'TRI-SIL' reagent (1 ml/1 mg

of glycoside), was carried out with a Pye Series 104 Chromatograph on a column (150 cm) of 3% SE-52 on Celite (80–100 mesh) with N_2 as carrier[11] at a pressure of 1.06 Kg/cm^2 and a temperature of 185°. Diglycosylglycerols were examined at 255°. Mixtures arising from partial hydrolysis of diglycosylglycerols with acid were examined by a programme using an initial period of 30 min at 185°, followed by maximal rate of column heating to 255° and a final period of 30 min.

Paper chromatography of glycosides was performed on Whatman No. 1 paper in the solvent system[24] butyl alcohol–pyridine–water (6:4:3 by vol.). Compounds were detected by the periodate–Schiff reagents for α-glycols[25] and the modified[26] silver nitrate reagent for sugars and polyols[27].

Thin-layer chromatography of glycoside acetates was performed on Silica gel G (Merck) with the solvent system benzene–ethanol (19:1). Compounds were detected by spraying the plates with 4% sulphuric acid followed by heating in an oven for 20 min at 150°.

*Methylenebis-2-*O*-(3-*O*-benzoyl-*D*-glycerol)* (**5**). — This compound, prepared by the literature method[7], had m.p. 107°, $[\alpha]_D$ −61 ±1° (*c* 0.8, chloroform).

*2,3-Di-*O*-benzyl-*L*-glycerol.* — This compound, prepared as required by acid hydrolysis[9] of its 1-trityl ether, had m.p. 85°, $[\alpha]_D$ −9° (*c* 0.8, chloroform).

*3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranosyl bromide.* — Methyl 4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-glucopyranoside[28] (1.43 g) was dissolved in acetic anhydride (4 ml), a mixture of acetic anhydride and sulphuric acid (14:1 by vol., 4 ml) was added, and the acetolysis product was recovered[29]. The syrup was dissolved in 47% hydrogen bromide in acetic acid (10 ml) and kept for 3 h at room temperature. The mixture was poured into ice–water (20 ml), and the product was extracted with chloroform (3 × 20 ml). The combined extracts were washed with ice-cold, saturated, aqueous sodium hydrogen carbonate (3 × 20 ml), dried (Na_2SO_4), and evaporated to dryness. The residue was recrystallized twice from ethyl acetate to give the bromide (1.13 g, 69%), m.p. 192°, $[\alpha]_D$ +94 ±2° (*c* 0.5, chloroform).

*2,4,6-Tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranosyl bromide.* — Laminaribiose was synthesized[30], and the resulting reaction mixture was chromatographed on a column (35 × 6 cm) of charcoal–Celite (1:1, w/w)[31]. D-Glucose was removed by elution with water (10 l), and then portions (3 l) of increasing concentrations of ethanol in water (respectively 2, 4, 6, 8, and 10%, v/v) were applied to the column. Laminaribiose (1.5 g), containing a trace of D-glucose, was obtained from the 8% ethanol fraction. The material could not be crystallized, presumably due to the D-glucose impurity, and it was rechromatographed. The pure disaccharide was converted, in the usual manner, into the hepta-*O*-acetyl bromide *via* its crystalline octa-acetate. It had m.p. 175°, $[\alpha]_D$ +80 ±2° (*c* 0.8, chloroform).

*2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-α-*D*-glucopyranosyl bromide.* — This was prepared by bromination of cellobiose octa-acetate. It had m.p. 184°, $[\alpha]_D$ +93 ±2° (*c* 1.0, chloroform).

3,4,6-Tri-O-acetyl-β-D-glucopyranosyl chloride. — This was prepared[19] from the 2-trichloroacetate[32]. It had m.p. 134°, $[\alpha]_D$ +41 ±1° (*c* 0.4, chloroform).

O-β-D-Glucopyranosyl-O-β-D-glucopyranosyl-(1→1)-D-glycerols. — These were prepared by a general procedure. A mixture of compound **5** (500 mg), mercuric oxide (400 mg), mercuric bromide (40 mg), and Drierite (1.5 g) was stirred in dry methylene chloride (5 ml) for 2 h. A solution of the appropriate acetylated disaccharide bromide (1.85 g) and iodine (300 mg) in dry methylene chloride (7 ml) was added during 5 min, and the mixture was stirred for 72 h at room temperature. The glycosides were recovered[9] and separated[33] on a column (30 × 2.5 cm) of Dowex-1 (OH^- form) resin.

The sophorosyl bromide yielded *O-β-D*-glucopyranosyl-(1→2)-*O-α-D*-glucopyranosyl-(1→1)-D-glycerol (**9**) (160 mg, 16%), which, after two recrystallizations from moist ethanol, had m.p. 190°, $[\alpha]_D$ +66.5 ±1.0° (*c* 0.1, water) [*Anal.* Calc. for $C_{15}H_{28}O_{13}$: C, 43.25; H, 6.8. Found: C, 43.25; H, 6.85]; and *O-β-D*-glucopyranosyl-(1→2)-*O-β-D*-glucopyranosyl-(1→1)-D-glycerol (**10**) (283 mg, 27.5%), which, after two recrystallisations from moist ethanol, had m.p. 186°, $[\alpha]_D$ −20.0 ±1.0° (*c* 0.1, water) [*Anal.* Calc. for $C_{15}H_{28}O_{13}$: C, 43.25; H, 6.8. Found: C, 43.3; H, 6.9].

"The laminaribiosyl bromide (1.59 g) yielded 45 mg (4.8%) of gum **8**, $[\alpha]_D$ +10.0 ±0.2° (*c* 1.5, water)." The nona-acetate was also non-crystalline and, when examined by t.l.c., gave two compounds, R_F 0.22 and 0.235. A degradation described later shows that the preparation is a mixture of the two anomeric laminaribiosides.

The cellobiosyl bromide (1.93 g) gave a mixture of non-crystalline cellobiosides (139 mg, 12%), $[\alpha]_D$ −8.7° (*c* 1.2, water), estimated by g.l.c. of their trimethylsilyl ethers to be present in the ratio $\beta:\alpha$ = 93:7. The isomers were not separable by chromatography on a column of Dowex-1 (OH^- form) resin, but the preparation ran on a paper chromatogram as an intense spot having a faint tail. The mixture afforded nona-*O*-acetyl-*O-β-D*-glucopyranosyl-(1→4)-*O-β-D*-glucopyranosyl-(1→1)-D-glycerol which, after four recrystallizations from ethanol, had m.p. 148°, $[\alpha]_D$ −5.8 ±1.0° (*c* 0.5, chloroform).

Anal. Calc. for $C_{33}H_{46}O_{22}$: C, 49.9; H, 5.7. Found: C, 49.9; H, 5.8.

Saponification of the acetate and g.l.c. investigation of the liberated glycoside as its trimethylsilyl ether showed the preparation to contain 4% of the α-anomer as impurity (Table I).

The preparation of nona-*O*-acetyl-*O-β-D*-glucopyranosyl-(1→6)-*O-β-D*-glucopyranosyl-(1→1)-D-glycerol has already been published[8].

The anomeric O-D-*galactopyranosyl-(1→1)-D-glycerols.* — These were prepared as described in the literature[9] and separated by chromatography on a column of Dowex-1 (OH^- form) resin. The α-anomer had m.p. 152°, $[\alpha]_D$ +158 ±3° (*c* 0.3, water); the β-anomer had m.p. 138–40°, $[\alpha]_D$ 0 ±1° (*c* 1.0, water).

The anomeric O-D-*glucopyranosyl-(1→1)-D-glycerols.* — These were prepared from tetra-*O*-acetyl-α-D-glucopyranosyl bromide (2.25 g) as described above for the corresponding galactosides.

The α-anomer (82 mg, 6%) had $[\alpha]_D$ +93 ±2° (*c* 0.2, water) and was character-

ized as hexa-*O*-acetyl-*O*-α-D-glucopyranosyl-(1→1)-D-glycerol, crystallized twice from aqueous ethanol, m.p. 90°, $[\alpha]_D$ +87 ±2° (*c* 0.9, methanol).

Anal. Calc. for $C_{21}H_{30}O_{14}$: C, 49.9; H, 5.9. Found: C, 49.95; H, 5.7.

The β-anomer (397 mg, 29%) had $[\alpha]_D$ −32 ±1° (*c* 0.5, water) and was characterized as hexa-*O*-acetyl-*O*-β-D-glucopyranosyl-(1→1)-D-glycerol; crystallized twice from ethanol, m.p. 144°, $[\alpha]_D$ −4.4 ±1.0° (*c* 0.4, chloroform).

Anal. Calc. for $C_{21}H_{30}O_{14}$: C, 49.9; H, 5.9. Found: C, 49.8; H, 5.8.

O-β-D-*Glucopyranosyl-(1→1)-*L*-glycerol.* — This was prepared from tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.5 g) and 2,3-di-*O*-benzyl-L-glycerol (2.5 g) as described[9] for the corresponding galactoside. Chromatography of the crude glycoside mixture on a column (30 × 2.5 cm) of Dowex-1 (OH^- form) resin gave 12.4 mg of impure α-D-glucoside and 1.19 g (51%) of pure β-D-glucoside, $[\alpha]_D$ −24.5 ±0.5° (*c* 0.4, water), which was characterized as hexa-*O*-acetyl-*O*-β-D-glucopyranosyl-(1→1)-L-glycerol, crystallized twice from ethanol, m.p. 107°, $[\alpha]_D$ −54.6 ±1.0° (*c* 0.5, chloroform).

Anal. Calc. for $C_{21}H_{30}O_{14}$: C, 49.9; H, 5.9. Found: C, 49.9; H, 6.1.

O-α-D-*Glucopyranosyl-(1→1)-*L*-glycerol.* — A solution of 2,3-di-*O*-benzyl-L-glycerol (0.5 g) in dry ether (20 ml) was stirred in the dark for 2 h with silver carbonate (1.5 g), silver perchlorate (60 mg), and Drierite (2 g). A solution of the 3,4,6-tri-*O*-acetyl-β-D-glucosyl chloride (0.62 g, 1 mol. prop.) in ether (10 ml) was added in one portion, and the mixture was stirred for 4 h at room temperature. The reaction mixture was filtered through a pad of Celite, and the filtrate was evaporated to a syrup. This was redissolved in methanol (30 ml) and hydrogenated in the presence of 10% palladium-on-charcoal (0.5 g) until reaction was complete. After filtration, a little sodium methoxide in methanol was added to the filtrate, and the mixture was kept overnight. Water (60 ml) was added, and the solution was passed through a short column (10 ml) of Dowex-50 (H^+ form) resin, and the eluate was evaporated. Chromatography of the crude glycoside mixture on a column (20 × 3 cm) of Dowex-1 (OH^- form) resin yielded 141 mg (30%) of pure α-anomer, $[\alpha]_D$ +142 ±3° (*c* 0.3, water); and 64 mg (14%) of pure β-anomer. The α-anomer was characterized as hexa-*O*-acetyl-*O*-α-D-glucopyranosyl-(1→1)-L-glycerol; crystallised twice from aqueous ethanol, m.p. 56°, $[\alpha]_D$ +89 ±2° (*c* 1.5, chloroform).

Anal. Calc. for $C_{21}H_{30}O_{14}$: C, 49.9; H, 5.9. Found: C, 49.9; H, 6.2.

O-α-D-*Glucopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→1)-*L*-glycerol* (**4**). — The first step of the preparation described above was repeated on a scale four times greater. After the addition of a molar proportion of the chloride, the reaction mixture was stirred in the dark for 24 h, and then a further amount of silver carbonate (6.0 g) was added, followed by a solution of the chloride (2.48 g, 1 mol. prop.) in dry ether (40 ml). The reaction mixture was stirred for a further 72 h, and the glycosides were recovered as described above.

Examination of the glycoside mixture by paper chromatography revealed the presence of glycerol and glucosylglycerol in small proportions, and larger proportions of two diglucosylglycerols. The crude mixture (2.5 g) was chromatographed on

a column (30 × 3 cm) of Dowex-1 (OH^- form) resin with automatic collection of fractions (20 ml each). Fractions 21–25 yielded 619 mg of material having a large, positive optical rotation which, when examined by paper chromatography, was seen to consist of a mixture of glucosyl- and diglucosyl-glycerol (R_{Glc} 0.52). Examination of a portion (1 mg) of the mixture, after trimethylsilylation, by g.l.c. showed that the glucosylglycerol was entirely β-, whereas the diglucosylglycerol consisted of one major and two minor components.

The mixture was dissolved in water (500 ml), digested with β-D-glucosidase (0.1 g, Koch–Light) for two weeks at 37°, and then passed through a short column (10 ml) of Dowex-1 (OH^- form) resin. The recovered mixture was rechromatographed as described before on Dowex-1 (OH^- form) resin, and fractions 21–25 were again combined and evaporated to yield 503 mg (17%) of material, $[\alpha]_D$ +87 ±2° (*c* 1.1, water). Examination of this material by g.l.c. showed the presence of two diglucosylglycerols in the ratio 15:85. Attempts to purify the major component by crystallization of the glycoside or its acetate were unsuccessful. Degradation, described below, yielded α- and β-D-glucosylglycerols in the ratio, α:β = 77.23.

Degradation of diglycosylglycerols to glycosylglycerols as a test of their purity. — A sample of the appropriate diglycosylglycerol (5 mg) was heated in 0.1N HCl for 40 min at 100° and evaporated *in vacuo* over potassium hydroxide pellets[34]. Trimethylsilylating reagent (1 ml) was added, and portions (2 μl) of the reaction mixture were examined in the g.l.c. programme. The ratio of α-D-glucosylglycerol to β-D-glucosylglycerol was obtained directly from the peaks on the chart. All compounds described as pure in this section showed only one peak in this procedure. The laminaribiosylglycerol **8** gave the ratio α:β-D-glucosylglycerol as 42:58, the 'α-gentiobiosylglycerol' reported earlier[8] gave the ratio α:β-D-glucosylglycerol as 29:71, and the kojibiosylglycerol **4** gave the ratio α:β-D-glucosyiglycerol as 77:23. Samples of pure glycosylglycerols were not anomerized by the partial hydrolysis conditions.

Examination of the lipid from Staph. aureus *B4.* — A sample of lipid was provided by Prof. E. F. Gale. It was dissolved in chloroform–methanol (4 ml, 1:1 v/v), and 0.1N sodium methoxide in methanol (1 ml) was added[35]. After 10 min, water (5 ml) was added, and the mixture was passed through a column (4 ml) of Dowex-50 (H^+ form) resin and then through a column (4 ml) of Dowex-1 (OH^- form) resin. The separated aqueous layer was evaporated to yield 35 mg of a gum. Examination of the material by paper chromatography showed a single compound (R_{Glc} 0.49) which co-chromatographed with the diglucosylglycerol **7**. Examination of a portion (1 mg) of the material by the programmed g.l.c. method showed the presence of *O*-β-D-glucosylglycerol and the *O*-β-gentiobiosylglycerol **1** in the molar ratio 1:100.

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