Synthesis of 3-*O*-(6-Deoxy-6-sulpho-α-D-glucopyranosyl)-1,2-di-*O*-hexadecanoyl-L-glycerol, 'Sulphoquinovosyl Diglyceride' [1]

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3-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-tosyl-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-L-glycerol was treated with potassium thioacetate and the product was hydrolysed with base to give the corresponding 6-deoxy-6-thio-derivative which on oxidation with iodine gave the crystalline disulphide. The isopropylidene groups were hydrolysed and the product was acylated with hexadecanoyl chloride in pyridine to give crystalline bis-[3-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyrancsyl)-1,2-di-*O*-hexadecanoyl-L-glycerol]-6,6-disulphide. Oxidation of this compound with 3-chloroperbenzoic acid and subsequent hydrogenolysis of the benzyl groups gave the title compound with properties similar to those of a sample prepared by hydrogenation of the natural 'sulphoquinovosyl diglyceride.'

A NEW sulphur-containing glycolipid ('the plant sulpholipid', 'sulphoquinovosyl diglyceride') was isolated from plants by Benson and his co-workers in 1959 [2] and it was later shown to be a component of the photosynthetic tissues of plants and micro-organisms.[3-5] 'Sulphoquinovosyl diglyceride' accounts for approximately half of the sulphur in the leaf [6] and is thus a major component of the sulphur cycle in Nature.[5]

Structural studies [7-10] showed that the compound was a 1,2-di-*O*-acyl-3-*O*-(6-deoxy-6-sulpho-α-D-glucopyranosyl)-L-glycerol and this was confirmed by an *X*-ray crystallographic study [11] of the rubidium salt of the deacylated glycolipid. The fatty acid distribution in the natural lipid from various sources has been examined [12,13] and as in most phospholipids and glycolipids [14] a spectrum of fatty acids was found. Hexadecanoic acid was found to be present predominantly on the C-2 hydroxy-group of the glycerol moiety and mono-, di-, and tri-enic derivatives of octadecanoic acid occurred preferentially on the C-1 hydroxy-group of glycerol.

Miyano and Benson [10,15] reported the synthesis of 3-*O*-(6-deoxy-6-sulpho-α-D-glucopyranosyl)-L-glycerol (13) from allyl 6-deoxy-6-sulpho-α-D-glucopyranoside by hydroxylation of the double bond with permanganate and preferential crystallisation of the cyclohexylamine salt of the required isomer but 'sulphoquinovosyl diglyceride' has not previously been synthesised.

We have recently [16] described the synthesis of the crystalline 3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-L-glycerol (3) from 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (1) by way of the bis(but-2-enyl)glycerol glucoside (2). Compound (3) was prepared as an intermediate for the synthesis [16] of a monoglucosyl diglyceride which is the parent compound of a series of glycolipids present in *Streptococci*.[17] Compound (3) was also suitably substituted for a synthesis of 'sulphoquinovosyl diglyceride' and we describe here the synthesis of 1,2-di-*O*-hexadecanoyl-3-*O*-(6-deoxy-6-sulpho-α-D-glucopyranosyl)-L-glycerol (12)—a saturated 'sulphoquinovosyl diglyceride'.

RESULTS AND DISCUSSION

3-*O*-(2,3,4-Tri-*O*-benzyl-α-D-glucopyranosyl)-L-glycerol (3) [16] was prepared by a modified procedure which avoided column chromatography and which allowed the recovery of the excess of 1,2-di-*O*-(but-2-enyl)-L-glycerol used in the synthesis. Compound (3) was converted into the isopropylidene derivative (4) [16] which gave the toluene-*p*-sulphonate (5). The sulphonate group was displaced by the action of potassium thioacetate in methanol to give the crystalline thioacetate (6). Alkaline hydrolysis of compound (6) gave the thiol (7) which was oxidised with iodine to give the crystalline disulphide (8). In the replacement reaction with potassium thioacetate, partial conversion of the thioacetate (6) into the thiol (7) and the disulphide (8) was sometimes observed and these compounds were all resolved by t.l.c. Since purification by crystallisation was most readily carried out with the thioacetate (6) the contents of the mother liquors were treated with lithium aluminium hydride in ether. This converted compounds (6) and (8) into the thiol (7) which was acetylated with acetic anhydride in pyridine to give a further crop of the thioacetate (6).

Acidic hydrolysis of the isopropylidene derivative (8) gave the crystalline tetrol (9) which on acylation with hexadecanoyl chloride in pyridine gave the crystalline palmitate (10). The disulphide (10) was oxidised with 3-chloroperbenzoic acid to give the crude sulphonate (11) which was hydrogenolysed over palladium-charcoal in glacial acetic acid to give the crude saturated 'sulphoquinovosyl diglyceride' (12) which was purified by chromatography on silica gel. The product which analysed as a trihydrate had a similar optical rotation to that reported [12] for a hydrogenated natural 'sulphoquinovosyl diglyceride' (containing mainly hexadecanoyl and octadecanoyl groups) and co-chromatographed with a sample of this hydrogenated natural lipid (kindly supplied by Dr E. Heinz) on thin layer chromatograms.

The dihexadecanoyl derivative is not a major species* of natural 'sulphoquinovosyl diglycerides' but it is present in some species of plants [12] and micro-organisms.[13] A route for the synthesis of unsaturated 'sulphoquinovosyl diglycerides' using the same intermediates has been proposed.[1]

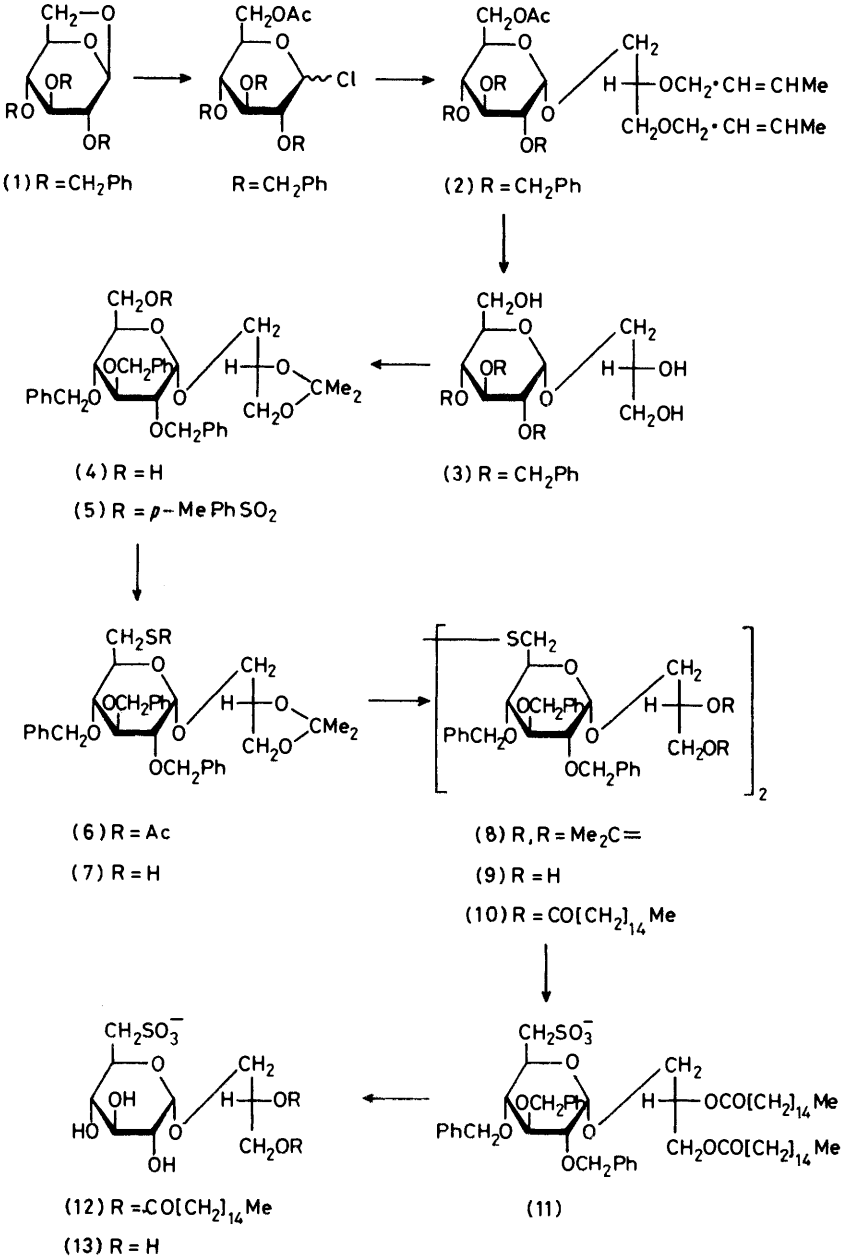
* A recent publication [18] indicated that it is a major component (29%) of the 'sulphoquinovosyl diglycerides' of the thylakoid membrane of rice plants (*Oryza sativa*).

EXPERIMENTAL

Solvents were evaporated off under reduced pressure. The light petroleum used had b.p. 40—60 °C unless otherwise stated. Optical rotations were measured at 21—24 °C with a Bendix automatic polarimeter. T.l.c. was carried out on microscope slides coated with silica gel G unless otherwise stated.

3-O-(2,3,4-*Tri*-O-*benzyl*-α-D-*glucopyranosyl*)-L-*glycerol* (3).[16]—The crude 3-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-1,2-di-*O*-(but-2-enyl)-L-glycerol (2) containing the excess of 1,2-di-*O*-(but-2-enyl)-L-glycerol was prepared as described previously [16] and the excess of the glycerol derivative was distilled from the product by heating at 150 °C and 2 mmHg and re-used in subsequent preparations. The crude product (2) remaining was then treated with potassium t-butoxide in dimethyl sulphoxide, to remove the acetyl and but-2-enyl groups, and worked up as described previously.[16] An ether solution of the crude product (3) was kept at 4 °C for 2 days and the crystalline compound (3) [*ca.* 30% yield from (1)] was recovered by filtration.

3-O-(6-S-*Acetyl*-2,3,4-*tri*-O-*benzyl*-6-*deoxy*-6-*thio*-α-D-*glucopyranosyl*)-1,2-O-*isopropylidene*-L-*glycerol* (6).—A solution of 3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-1,2-*O*-isopropylidene-L-glycerol (4) [16] (3.2 g) and toluene-*p*-sulphonyl chloride (3 g) in dry pyridine (50 ml) was kept at 40 °C for 24 h. T.l.c. [toluene–acetone (10 : 1)] then showed complete conversion of the alcohol (4) (R_F 0.2) into the tosylate (5) (R_F 0.5). Ice and ether were added to the mixture and the ether extract was washed with 2N-hydrochloric acid at 0 °C (to remove the pyridine) and then with



saturated potassium chloride solution and sodium hydrogen carbonate solution. The ether extract was dried ($MgSO_4$) and evaporated to give the tosylate (5) (3.9 g) as a syrup. This was taken up in dry methanol (50 ml) and potassium thioacetate (6 g, Fluka) was added. The solution was heated under reflux for 1.5 h, after which time t.l.c. [ether–light petroleum (1 : 1), on commerical silica gel plates (Merck No. 5721)] showed complete conversion of the tosylate (5) (R_F 0.45) into the thioacetate (6) (R_F 0.6) together with traces of the corresponding thiol (7) (R_F 0.75) and the disulphide (8) (R_F 0.5). The solution was cooled and diluted with ether, washed with saturated potassium chloride solution, and dried ($MgSO_4$). Evaporation of the solvents gave a crystalline product which was recrystallised from ethyl acetate–light petroleum (b.p. 60—80 °C) to give the *thioacetate* (6) (2.6 g), m.p. 79—81 °C, $[\alpha]_D$ +52.5° (*c* 1 in $CHCl_3$) (Found: C, 67.3; H, 6.7; S, 5.3. $C_{35}H_{42}O_8S$ requires C, 67.5; H, 6.8; S, 5.15%).

Bis-[3-O-(2,3,4-*tri*-O-*benzyl*-6-*deoxy*-α-D-*glucopyranosyl*)-1,2-O-*isopropylidene*-L-*glycerol*]-6,6-*disulphide* (8).—A mixture of the thioacetate (6) (1.8 g) and sodium hydroxide (1 g) in methanol (25 ml) was heated under reflux for 30 min after which time t.l.c. [ether–light petroleum (1 : 1), on commercial silica gel plates (Merck No. 5721)] showed complete conversion of the thioacetate (6) (R_F 0.6) into the thiol (7) (R_F 0.75) together with a small quantity of the disulphide (8) (R_F 0.5). Water (25 ml) was added and most of the methanol was evaporated off. Solid carbon dioxide was added to convert the sodium hydroxide into carbonate and the products were extracted into ether. Iodine was added in small portions until the colour persisted in the ether layer and t.l.c. (as above) then showed complete conversion of the thiol (7) into the disulphide (8). The ether layer was separated and washed with saturated potassium chloride solution and with sodium thiosulphate solution and dried (K_2CO_3). The solvent was evaporated off and the crystalline product (1.6 g) was recrystallised from ether–light petroleum at 4 °C to give the *disulphide* (8) (1.3 g), m.p. 85—86 °C, $[\alpha]_D$ +110° (*c* 1 in $CHCl_3$) (Found: C, 68.2; H, 6.7; S, 5.5. $C_{66}H_{78}O_{14}S_2$ requires C, 68.4; H, 6.8; S, 5.5%).

Bis-[3-O-(2,3,4-*tri*-O-*benzyl*-6-*deoxy*-α-D-*glucopyranosyl*)-L-*glycerol*]-6,6-*disulphide* (9).—A solution of the isopropylidene derivative (8) (1.3 g) in methanol (45 ml), N-hydrochloric acid (5 ml), and benzene (12 ml) was heated under reflux for 15 min after which time t.l.c. [toluene–acetone (1 : 1)] showed complete conversion of compound (8) into the alcohol (9) (R_F 0.45). An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. The product was extracted from the residue with chloroform and the solution dried ($MgSO_4$) and evaporated to give a solid residue. Recrystallisation from ethyl acetate–light petroleum (b.p. 60—80 °C) (1 : 1) gave the *tetrol* (9) (1 g), m.p. 155—157 °C, $[\alpha]_D$ +122° (*c* 1 in $CHCl_3$) (Found: C, 66.6; H, 6.55; S, 6.0. $C_{60}H_{70}O_{14}S_2$ requires C, 66.8; H, 6.5; S, 5.9%).

Bis-[3-O-(2,3,4-*tri*-O-*benzyl*-6-*deoxy*-α-D-*glucopyranosyl*)-1,2-*di*-O-*hexadecanoyl*-L-*glycerol*]-6,6-*disulphide* (10).—Hexadecanoyl chloride (1.2 g, Fluka) was added to a solution of the tetrol (9) (660 mg) in dry pyridine (20 ml) and the solution was kept at 20 °C for 12 h. T.l.c. [ether–light petroleum (1 : 3)] then showed a major product (R_F 0.5). Water (1 ml) was added to decompose the excess of hexadecanoyl chloride and the solution was kept at 20 °C for 2 h. The solution was diluted with ether and washed with 1N-hydrochloric acid and saturated potassium chloride solution and dried ($MgSO_4$). The product was chromatographed on neutral alumina (50 g, Merck, pre-washed with methyl formate in ether) and elution with ether gave the crystalline product (990 mg, free from the hexadecanoic acid) which was recrystallised from ether (20 ml) at 4 °C to give the *palmitate* (10) (730 mg), m.p. 58—60 °C, $[\alpha]_D$ +61.2° (*c* 1 in $CHCl_3$) (Found: C, 72.8; H, 9.5; S, 3.3. $C_{124}H_{190}O_{18}S_2$ requires C, 73.3; H, 9.4; S, 3.15%).

3-O-(6-*Deoxy*-6-*sulpho*-α-D-*glucopyranosyl*)-1,2-*di*-O-*hexadecanoyl*-L-*glycerol* (12).—3-Chloroperbenzoic acid (750 mg) was added to a stirred mixture of the disulphide (10) (490 mg), dichloromethane (25 ml), anhydrous sodium acetate (50 mg), and water (0.2 ml). After 3 h at 20 °C, t.l.c. [chloroform–methanol (8 : 1) on commerical silica gel plates (Merck No. 5721)] showed complete conversion of compound (10) (R_F 1.0) into a major product (R_F 0.7) together with small amounts of more polar by-products. The mixture was dried by the addition of sodium sulphate, filtered, and chromatographed on silica gel. Elution with chloroform–methanol (50 : 1) removed the 3-chlorobenzoic acid and 3-chloroperbenzoic acid and a little of the product and elution with chloroform–methanol (1 : 1) gave the major product together with the more polar by-products (350 mg). This was taken up in glacial acetic acid and treated with hydrogen over 10% palladium on charcoal for 12 h at atmospheric pressure. The mixture was filtered through Celite and the catalyst was washed with chloroform–methanol–water (65 : 25 : 4) and the filtrate evaporated to give the crude product (230 mg). T.l.c. [chloroform–methanol–water (65 : 25 : 4) on commerical silica gel plates (Merck No. 5721)] showed complete conversion of the starting material (R_F 0.9, together with more-polar impurities R_F 0.75—0.85) into a major product (R_F 0.7, with small amounts of less-polar impurities R_F 0.75—0.85). The product was chromatographed on silica gel and elution with chloroform–methanol (19 : 1) removed most of the less-polar impurities and elution with chloroform–methanol–water (65 : 25 : 4) gave the pure *product* (12) [100 mg, 25% from compound (10)] as a white solid which co-chromatographed on t.l.c. [in both chloroform–methanol–water (65 : 25 : 4) and acetone–benzene–water (70 : 23 : 8) on commercial silica gel plates (Merck No. 5721)] with a sample of a hydrogenated natural ' sulphoquinovosyl diglyceride ' (kindly supplied by Dr E. Heinz), $[\alpha]_D$ +34.7° (*c* 0.5 in pyridine after warming to dissolve and cooling to ambient temperature) {lit.,[12] $[\alpha]_D$ +33.2° (*c* 0.34 in pyridine) for a hydrogenated sample of natural ' sulphoquinovosyl diglyceride ' containing mainly hexadecanoic and octadecanoic acids} (Found: C, 56.3; H, 9.2; S, 4.1. $C_{41}H_{77}NaO_{12}S,3H_2O$ requires C, 56.5; H, 9.6; S, 3.7%).

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