Synthesis of 3-*O*-{6-*O*-[6-*O*-(α-D-Galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-1,2-di-*O*-stearoyl-L-glycerol, a 'Trigalactosyl Diglyceride'

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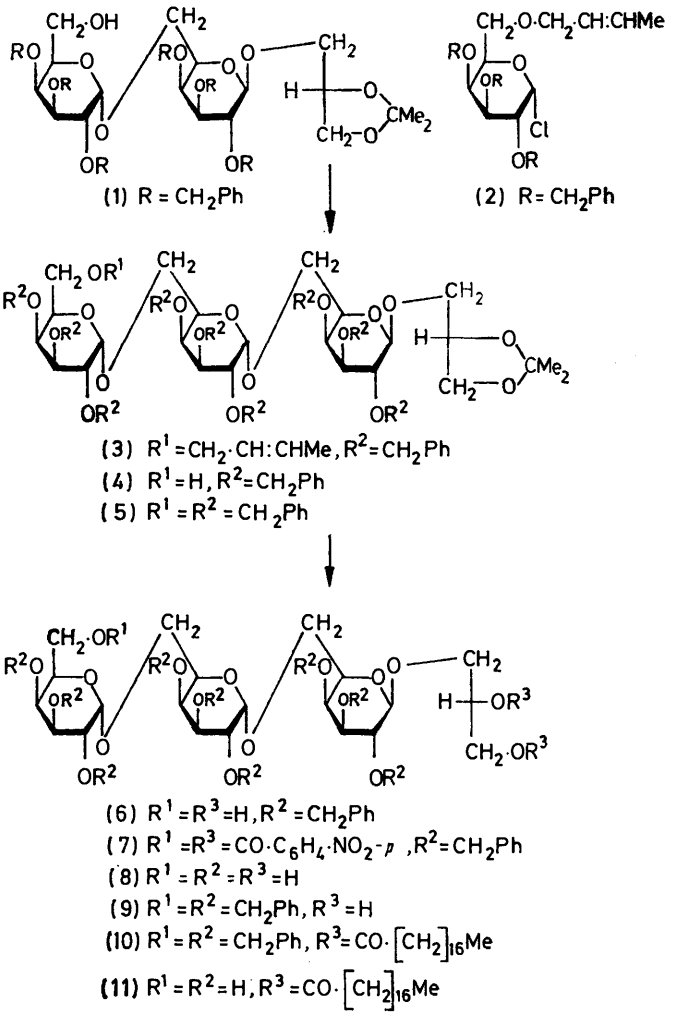
2,3,4-Tri-*O*-benzyl-6-*O*-but-2-enyl-α-D-galactopyranosyl chloride was condensed with 3-*O*-[6-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl]-1,2-*O*-isopropylidene-L-glycerol under conditions shown previously to give predominantly 1,2-*cis*-glycosides. The product was converted into the crystalline tris-*p*-nitrobenzoate of 3-*O*-{6-*O*-[6-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl]-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl}-L-glycerol which was further elaborated to afford the title compound.

'TRIGALACTOSYL DIGLYCERIDES' have been isolated from potato tubers [1] and from spinach chloroplasts [2] and tentatively identified [1] as 1,2-di-*O*-acyl-3-*O*-{6-*O*-[6-*O*-(α-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-L-glycerols. A similar material has been shown [3] to be present in *Mycoplasma pneumoniae* and this shows [3] serological activity similar to that of the trigalactosyl diglycerides of spinach.

We have recently [4a] described the synthesis of a 'digalactosyl diglyceride' {3-*O*-[6-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-1,2-di-*O*-stearoyl-L-glycerol} by way of the intermediate 3-*O*-[6-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl]-1,2-*O*-isopropylidene-L-glycerol (1). Compound (1) is suitably substituted for the addition of a further molecule of galactose and, applying the method which we have previously described [4] for the preparation of 1,2-*cis*-glycosides, we have condensed the protected galactosyl chloride (2) [5] with compound (1) to give a product containing the protected trigalactosyl glycerol (3). This method for 1,2-*cis*-glycoside synthesis usually gives [4] *ca.* 10% of the 1,2-*trans*-glycoside in the product and in our previous work [4] with this reaction the separation of the *trans*-glycoside was readily achieved because of the ease of crystallisation of the perbenzylated disaccharides. However compound (3) did not crystallise from the mixture and therefore the but-2-enyl group was cleaved from the crude product (3), by the action [6] of potassium t-butoxide in dimethyl sulphoxide, to give the crude alcohol (4). Compound (4) and various derivatives were also non-crystalline and the crude alcohol (4) was therefore converted into the crude triol (6) from which the crystalline tris-*p*-nitrobenzoate (7) was obtained. Alkaline hydrolysis of compound (7) and subsequent hydrogenolysis gave the trigalactosyl glycerol (8), which had an optical rotation similar to that reported [7] for a trigalactosyl glycerol isolated from potatoes and tentatively characterised as compound (8).

For the preparation of the trigalactosyl diglyceride, compound (7) was hydrolysed with base to give the triol (6), which was converted into the isopropylidene derivative (4). Compound (4) was converted into the perbenzyl ether (5) and the isopropylidene group was subsequently removed by acidic hydrolysis to give the diol (9), which was acylated with stearoyl chloride in



pyridine to give the ester (10). Hydrogenolysis of the ester (10) gave the trigalactosyl diglyceride (11) which

[1] T. Galliard, *Biochem. J.*, 1969, **115**, 335.
[2] D. E. Webster and S. B. Chang, *Plant Physiol.*, 1969, **44**, 1523.
[3] G. E. Kenny and R. M. Newton, *Ann. New York Acad. Sci.*, 1973, **225**, 54.
[4] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, (*a*) 1975, **1521**; (*b*) 1974, **1446**; (*c*) 1974, **1835**; (*d*) 1975, **361**.
[5] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1975, 364.
[6] P. A. Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1972, **1535**.
[7] B. Urbas, *Canad. J. Chem.*, 1968, **46**, 49.

ran concurrently on t.l.c. with material present in commercial dehydrated potato or in ' leaf protein concentrate '.[8] The ' leaf protein concentrate ' contains quite high concentrations of the mono-, di-, and tri-galactosyl diglycerides.

The serological activity of the synthetic trigalactosyl diglyceride will be compared with that of the presumed trigalactosyl diglyceride [3] of *Mycoplasma pneumoniae*.

EXPERIMENTAL

General experimental details were as described previously.[5]

3-O-{6-O-[6-O-(2,3,4-*Tri*-O-*benzyl*-6-O-p-*nitrobenzoyl*-α-D-*galactopyranosyl*)-2,3,4-*tri*-O-*benzyl*-α-D-*galactopyranosyl*]-2,3,4-*tri*-O-*benzyl*-β-D-*galactopyranosyl*}-1,2-*di*-O-p-*nitrobenzoyl*-L-*glycerol* (7).—A mixture of 2,3,4-tri-*O*-benzyl-6-*O*-but-2-enyl-α-D-galactopyranosyl chloride (2) [5] (2.1 g, 4 mmol), 3-*O*-[6-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl]-1,2-*O*-isopropylidene-L-glycerol (1) [4a] (2.1 g, 2.1 mmol), dry tetraethylammonium chloride (0.63 g, 3.8 mmol), and dry triethylamine (1.3 ml, 9 mmol) in dry dichloroethane (20 ml) was heated under reflux for 24 h. T.l.c. (ether–light petroleum, 2 : 1) then showed the presence of a major product (R_F 0.5), a small amount of a presumed unsaturated product (R_F 0.7) [from the dehydrochlorination of compound (2)], a trace of the galactosyl chloride (2) (R_F 0.8), a trace of alcohol (R_F 0.4) [from the hydrolysis of the galactosyl chloride (2)] and some of the aglycone (1) (R_F 0.2). Dry triethylamine (0.5 ml, 3.5 mmol) and the chloride (2) (0.8 g, 1.5 mmol) in dry dichloroethane (6 ml) were added and the mixture was heated under reflux for a further 17 h, after which only a trace of the aglycone (1) was present (as observed by t.l.c.). Water (0.5 ml) was added and the mixture was heated under reflux for 1 h to decompose the excess of galactosyl chloride (2). The mixture was diluted with chloroform, washed with water, and dried ($MgSO_4$), and the crude product was chromatographed on alumina. Elution with ether–methanol (199 : 1) gave at first a mixture of the presumed unsaturated product (R_F 0.7) and the glycoside fraction (R_F 0.5) (2 g), and then the pure glycoside fraction (1.75 g).

A solution of the mixed products (R_F 0.5 and 0.7) (2 g) in dioxan–0.1N-hydrochloric acid (9 : 1; 10 ml) was heated at 100 °C for 20 min. T.l.c. (ether–light petroleum, 2 : 1) then showed that the glycoside fraction (R_F 0.5) had been completely converted into a more polar product (R_F 0.05), whereas some of the presumed unsaturated product (R_F 0.7) was still present. An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. The product (1.95 g) was extracted from the residue with chloroform and chromatographed on alumina; elution with ether–methanol (99 : 1) gave the presumed unsaturated product (0.33 g), which was recrystallised from light petroleum (b.p. 60—80°) to give 2,3,4-*tri*-O-*benzyl*-6-O-*but*-2-*enyl*-1-*deoxy*-D-lyxo-*hex*-1-*enopyranose*, m.p. 64—66°, $[\alpha]_D^{25}$ −48.8° (*c* 0.9 in $CHCl_3$) (Found: C, 76.8; H, 6.9. $C_{31}H_{34}O_5$ requires C, 76.5; H, 7.0%). A portion of this compound was treated with potassium t-butoxide in dry dimethyl sulphoxide at 20 °C to remove [6] the but-2-enyl group. T.l.c. (ether–light petroleum, 1 : 1) showed conversion of the starting material (R_F 0.8) into a product (R_F 0.1) which still gave a positive test for unsaturation with the potassium permanganate spray reagent.

The pure glycoside fraction (1.75 g) from the initial condensation reaction was treated with potassium t-butoxide in dry dimethyl sulphoxide at room temperature for 1 h. T.l.c. (ether–light petroleum, 2 : 1) showed complete conversion of the starting material (R_F 0.5) into a major product (R_F 0.4) [crude alcohol (4)] together with minor contaminants (R_F 0, 0.2, and 0.7). The product was isolated in the usual way [6] and chromatographed on alumina; elution with ether removed the trace contaminant (R_F 0.7) and elution with ether–methanol (199 : 1) gave the crude alcohol (4) (0.8 g). This was dissolved in N-hydrochloric acid–dioxan (1 : 9; 10 ml) and heated under reflux for 10 min; t.l.c. (ether–light petroleum, 4 : 1) then showed complete conversion of the starting material (R_F 0.7) into a single product (R_F 0.2). An excess of sodium hydrogen carbonate was added, the solvents were evaporated off, and the product was extracted with chloroform. The product was then treated with *p*-nitrobenzoyl chloride (1.6 g) in dry pyridine (10 ml) at 20 °C for 1 h; t.l.c. (as above) then showed complete conversion of the starting material (R_F 0.2) into a product (R_F 0.8). This was isolated in the usual way and recrystallised from ethanol–ethyl acetate to give the *tris*-p-*nitrobenzoate* (7) (0.73 g), m.p. 125.5—127.5°, $[\alpha]_D$ +63.8° (*c* 0.8 in $CHCl_3$) (Found: C, 68.6; H, 5.6; N, 2.4. $C_{105}H_{101}N_3O_{27}$ requires C, 68.65; H, 5.5; N, 2.3%).

3-O-{6-O-[6-O-(α-D-*Galactopyranosyl*)-α-D-*galactopyranosyl*]-β-D-*galactopyranosyl*}-1,2-*di*-O-*octadecanoyl*-L-*glycerol* (11).—A solution of the tris-*p*-nitrobenzoate (7) (0.71 g) in dioxan (6 ml) and N-sodium hydroxide in methanol (6 ml) was heated at 50 °C until t.l.c. (ether–light petroleum, 4 : 1) showed complete conversion of compound (7) (R_F 0.8) into the triol (6) (R_F 0.2). Solid carbon dioxide was added, the solvents were evaporated off, and the product (0.63 g) was extracted with chloroform.

A portion of the triol (6) in glacial acetic acid–methanol (1 : 1) was treated with hydrogen in the presence of palladium–charcoal for 15 h. T.l.c. (butan-1-ol–acetic acid–water, 1 : 2 : 1) then showed a single product (R_F 0.4). The product (8) was isolated in the usual way and obtained as a solid glass, $[\alpha]_D$ +117° (*c* 1.2 in H_2O) {lit.,[7] $[\alpha]_D^{25}$ +114° (*c* 2.0 in H_2O) for a compound tentatively identified as (8)}.

A solution of the triol (6) (0.5 g) in dry acetone (50 ml) containing toluene-*p*-sulphonic acid (25 mg) was kept at 20 °C for 1 h. T.l.c. (ether–light petroleum, 4 : 1) then showed complete conversion of the triol (6) (R_F 0.2) into the isopropylidene derivative (4) (R_F 0.7). An excess of sodium hydrogen carbonate was added, the solvent was evaporated off, and the product was extracted with chloroform. Compound (4) was treated with benzyl chloride and sodium hydride in *NN*-dimethylformamide at 50 °C until t.l.c. (toluene–acetone, 9 : 1) showed complete conversion of the alcohol (4) (R_F 0.4) into the perbenzyl ether (5) (R_F 0.6), which was isolated in the usual way. A solution of compound (5) in N-hydrochloric acid–dioxan (1 : 9; 10 ml) was heated under reflux for 10 min; t.l.c. (toluene–acetone, 4 : 1) then showed complete conversion of compound (5) (R_F 0.8) into the diol (9) (R_F 0.6). An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. The product was extracted with chloroform and treated with stearoyl chloride (0.5 ml) in dry pyridine (10 ml) at 20 °C until t.l.c. (toluene–acetone, 9 : 1) showed conversion of the diol (9) (R_F 0.3) into the bis-

[8] N. W. Pirie, *Nature*, 1975, **253**, 239.

stearoyl ester (10) (R_F 0.9). The product was isolated in the usual way and chromatographed on neutral alumina. Elution with ether gave the ester (10) (0.2 g), which was dissolved in chloroform–methanol–acetic acid (5 : 5 : 1; 11 ml) and treated with hydrogen in the presence of palladium–charcoal for 12 h. T.l.c. (chloroform–methanol–water, 4 : 2 : 0·3) then showed a major product (R_F 0.7) together with trace contaminants (R_F 0.1 and 0.5) (possibly due to deacylation) and the product was isolated in the usual way. Recrystallisation from methanol gave *compound* (11) (47 mg), which softened over a wide temperature range and gave a meniscus at 220—230°; $[\alpha]_D$ +61.6° (*c* 0.9 in pyridine) (Found: C, 60.1; H, 9.8 $C_{57}H_{106}O_{20}$,-2MeOH requires C, 60.3; H. 9.8%). This material ran concurrently on t.l.c. (in several solvent systems) with samples of ' trigalactosyl diglyceride ' present in commercial dehydrated potato and ' leaf protein concentrate '.

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