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

By **Patricia A. Gent and Roy Gigg,*** Laboratory of Lipid and General Chemistry, National Institute for Medical Research, Mill Hill, London NW7 1AA

2,3,4,-Tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride was condensed with 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol, under conditions shown previously to lead to 1,2-*cis*-glycosides, to give crystalline 1,2-*O*-isopropylidene-3-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-but-2-enyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-D-glycerol. Removal of the but-2-enyl group, by the action of potassium t-butoxide in dimethyl sulphoxide, gave the corresponding crystalline alcohol. The alcohol was benzylated and the isopropylidene group was removed by hydrolysis; subsequent acylation with stearoyl chloride and hydrogenolytic removal of the benzyl groups gave the title compound.

' DIGALACTOSYL DIGLYCERIDES ' {1,2-di-*O*-acyl-3-*O*-[6-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-glycerols} were first isolated from the lipids of wheat flour,[1] and were subsequently recognised as major components of the neutral lipid fraction of green plants, where they occur in the chloroplast membranes.[2] Digalactosyl diglycerides are also present in human brain [3] and in micro-organisms.[4]

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[3] D. A. Wenger, K. S. Rao, and R. A. Pieringer, *J. Biol. Chem.*, 1970, **245**, 2513.

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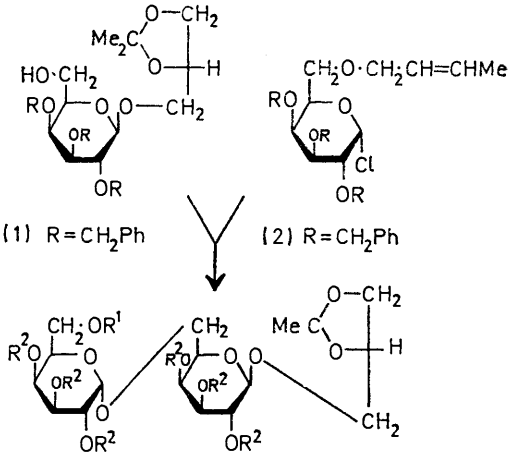
We have recently [5] described a synthesis of a ' galactosyl diglyceride ' [3-*O*-(β-D-galactopyranosyl)-1,2-di-*O*-palmitoyl-L-glycerol] in which 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (1) was used as an intermediate. Applying our previously described [6] 1,2-*cis*-glycoside synthesis to this intermediate (1) we have now achieved a total synthesis of a digalactosyl diglyceride. A partial synthesis of a digalactosyl diglyceride by the deacylation and reacylation of natural digalactosyl diglycerides has been described.[7]

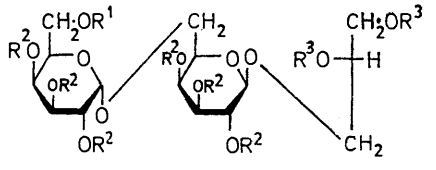


(1) R = CH_2Ph (2) R = CH_2Ph

(3) $R^1 = CH_2{:}CH{:}CHMe$, $R^2 = CH_2Ph$

(4) $R^1 = H$, $R^2 = CH_2Ph$

(5) $R^1 = R^2 = CH_2Ph$



(6) $R^1 = R^3 = H$, $R^2 = CH_2Ph$

(7) $R^1 = R^2 = R^3 = H$

(8) $R^1 = R^2 = CH_2Ph$, $R^3 = H$

(9) $R^1 = R^2 = CH_2Ph$, $R^3 = CO{\cdot}[CH_2]_{16}CH_3$

(10) $R^1 = R^2 = H$, $R^3 = CO{\cdot}[CH_2]_{16}CH_3$

1,2-*O*-Isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol [5] (1) was condensed with 2,3,4-tri-*O*-benzyl-6-*O*-but-2-enyl-α-D-galactopyranosyl chloride [5] (2), under the conditions which we have described [6] previously for 1,2-*cis*-glycoside synthesis, to give crystalline 1,2-*O*-isopropylidene-3-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-but-2′-enyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-glycerol (3). The 1,2-*cis*-glycoside synthesis [6] is carried out in the presence of triethylamine and tetraethylammonium chloride in a sealed tube with dichloromethane as a solvent at 80 °C. To simplify this procedure the condensation of the reactants (1) and (2) was also carried out in refluxing 1,2-dichloroethane (b.p. 83°) in an open flask under dry nitrogen (with otherwise identical conditions) and good yields of the product (3) were also obtained. In this case, where the aglycone is the more valuable intermediate in the reaction mixture, we have also modified the conditions and used the chloride (2) in excess to ensure maximum consumption of the aglycone; previously we have used an excess of the aglycone in this 1,2-*cis*-glycoside synthesis. By conducting the condensation in an open flask it was also easier to follow the reaction by t.l.c. We had suspected [6a] that the glycosyl chloride used in the 1,2-*cis*-glycoside synthesis might react initially with the triethylamine present to give a β-triethylammonium glycoside as described [8a] for related reactions of glycosyl bromides. However by following the reaction with t.l.c. it was shown that an excess of glycosyl chloride was present in the reaction mixture for the duration of the reaction and any reaction of compound (2) with triethylamine must be slow. Kronzer and Schuerch [8b] have observed that tetra-*O*-benzyl-galactopyranosyl chloride did not react with triethylamine at 50 °C. This experiment indicates (but does not prove) that the β-glycosyl chloride is the reactive intermediate, thus allowing one to use a mixture of the α- and β-glycosyl chlorides (which may be produced [9] from a mixture of the anomers of the 1-*O*-*p*-nitrobenzoates by the action of hydrogen chloride in ether–dichloromethane) in the glycoside-forming reaction.

We have also considered using tetraethylammonium bromide (in place of tetraethylammonium chloride). This should lead to the formation of the glycosyl bromide (which is more reactive [10a] than the chloride) from the glycosyl chloride in the reaction mixture and thus avoid the problems [10b] associated with the direct preparation of benzylated glycosyl bromides. This route should also be suitable for the preparation of partially allylated, partially benzylated glycosyl bromides since the reactivity of the unsaturated centres with hydrogen bromide (and contaminating bromine) precludes the normal method of preparation of these compounds with hydrogen bromide.

The protected digalactosylglycerol derivative (3) was treated with potassium t-butoxide in dimethyl sulphoxide, which removed [11] the but-2-enyl group and gave

[5] P. A. Gent and R. Gigg, *J.C S. Perkin I*, 1975, 364.
[6] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, (*a*) 1974, 1446; (*b*) 1974, 1835; (*c*) 1975, 361.
[7] E. Heinz, *Biochim. Biophys. Acta*, 1971, **231**, 537.
[8] (*a*) A. C. West and C. Schuerch, *J. Amer. Chem. Soc.*, 1973, **95**, 1333; (*b*) F. J. Kronzer and C. Schuerch, *Carbohydrate Res.*, 1974, **38**, 273.
[9] C. L. Stevens, G. H. Ransford, J. Němec, J. M. Cahoon, and P. M. Pillai, *J. Org. Chem.*, 1974, **39**, 298.
[10] (*a*) J. M. Berry and G. G. S. Dutton, *Carbohydrate Res.*, 1974, **38**, 339; (*b*) R. U. Lemieux and T. Kondo, *ibid.*, 1974, **35**, C4.
[11] P. A. Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1972, 1535.

the crystalline 1,2-*O*-isopropylidene-3-*O*-[2,3,4-tri-*O*-6-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-glycerol (4). Compound (4) should be a suitable intermediate for the synthesis of the acylated digalactosyl diglycerides which are formed [12] by enzymic transacylation during the isolation of digalactosyl diglycerides and for our projected syntheses of the tri- and tetragalactosyl diglycerides which are the serologically active glycolipids of *Mycoplasma pneumoniae*.[13] Oxidation of compound (4) and subsequent reduction with tritiated sodium borohydride should also give an intermediate suitable for the preparation of labelled digalactosyl diglycerides.

For characterisation, the isopropylidene group of compound (4) was removed by hydrolysis to give the crystalline derivative (6), and the benzyl groups were removed by hydrogenolysis to give 3-*O*-[6-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-glycerol (7). This material was induced to crystallise by seeding with material prepared by hydrolysis of natural digalactosyl diglycerides (kindly supplied by Dr. E. Heinz) and showed properties similar to those reported [1a,7,14] for the same material obtained by basic hydrolysis of natural digalactosyl diglycerides or isolated [15] from natural sources.

For the synthesis of the digalactosyl diglyceride, compound (4) was converted into the per-*O*-benzyl derivative (5) and the isopropylidene group was removed by hydrolysis to give the glycerol derivative (8). Acylation of compound (8) with stearoyl chloride in pyridine gave the ester (9), which was hydrogenolysed to give the digalactosyl diglyceride (10); this showed similar t.l.c. behaviour to an authentic sample of natural digalactosyl diglycerides (kindly supplied by Dr. B. W. Nichols). It was recrystallised from methanol [14] and showed a similar melting behaviour to that reported [14] for a hydrogenated natural digalactosyl diglyceride (containing 97.7% stearic acid and 2.3% palmitic acid) and an optical rotation similar to those reported for hydrogenated natural digalactosyl diglycerides [7] and for semisynthetic digalactosyl diglycerides [7] containing different acyl groups.

We have shown [5] that a glycerol unit can be converted readily into an allyl group by the action of zinc and sodium iodide on the ditosyl derivative. Since the allyl group can be readily removed, by methods described previously,[16] compounds such as (3) and (8) could be used for the preparation of protected reducing disaccharides suitable for conversion into glycosy chlorides. The preparation of a trigalactosyl diglyceride from compound (4) by the addition of another molecule of compound (2) in α-linkage and the preparation of a tetragalactosyl diglyceride by the addition to compound (4) of a disaccharide glycosyl chloride, prepared from compound (8), in α-linkage are under investigation.

EXPERIMENTAL

General experimental details are as described previously.[5]

1,2-O-*Isopropylidene*-3-O-[2,3,4-*tri*-O-*benzyl*-6-O-(2,3,4-*tri*-O-*benzyl*-6-O-*but*-2-*enyl*-α-D-*galactopyranosyl*)-β-D-*galactopyranosyl*]-L-*glycerol* (3).—A mixture of 2,3,4-tri-*O*-benzyl-6-*O*-but-2-enyl-α-D-galactopyranosyl chloride [5] (2) (2.1 g), 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol [5] (1) (1.1 g), dry tetraethylammonium chloride (0.72 g), and dry triethylamine (1.2 ml) in dry dichloroethane (20 ml) was refluxed under dry nitrogen for 17.5 h. T.l.c. (ether–light petroleum, 2 : 1) then indicated the presence of a small amount of the alcohol (1) (R_F 0.1) and the chloride (2) (R_F 0.85), a major product (R_F 0.6), and minor products (R_F 0.75 and 0.5), together with some of the free sugar derived from hydrolysis of the chloride (2) (R_F 0.4). Water (0.5 ml) was added and the mixture was refluxed for a further 45 min to decompose the excess of chloride (2). The solution was washed with water and dried ($MgSO_4$). The crude product (3.6 g) was chromatographed on alumina; elution with ether–light petroleum (1 : 1) removed the minor product (R_F 0.75) and elution with ether–light petroleum (2 : 1) gave the major *product* (3) (R_F 0.6) (1.3 g), which was recrystallised from light petroleum (b.p. 60—80°); yield 1.05 g; m.p. 94—95°; $[\alpha]_D$ +33.3° (*c* 0.5 in $CHCl_3$) (Found: C, 73.1; H, 7.1. $C_{64}H_{74}O_{13}$ requires C, 73.1; H, 7.1%).

1,2-O-*Isopropylidene*-3-O-[2,3,4-*tri*-O-*benzyl*-6-O-(2,3,4-*tri*-O-*benzyl*-α-D-*galactopyranosyl*)-β-D-*galactopyranosyl*]-L-*glycerol* (4).—A solution of compound (3) (1 g) and potassium t-butoxide (1 g) in dry dimethyl sulphoxide (20 ml) was kept at 20 °C for 3 h; t.l.c. (ether–light petroleum, 2 : 1) then indicated almost complete conversion of the but-2-enyl ether (3) (R_F 0.6) into a single product (R_F 0.1). The product (0.96 g) was isolated in the usual way [6b] and chromatographed on alumina; elution with ether removed a trace of compound (3) and elution with ether–methanol (99 : 1) gave the pure *product* (4) which was recrystallised from ethyl acetate–light petroleum (b.p. 60—80°); yield 0.54 g; m.p. 132—134°, $[\alpha]_D$ +24.3° (*c* 0.5 in $CHCl_3$) (Found: C, 72.1; H, 6.8. $C_{60}H_{68}O_{13}$ requires C, 72.3; H, 6.9%).

3-O-[2,3,4-*Tri*-O-*benzyl*-6-O-(2,3,4-*tri*-O-benzyl-α-D-*galactopyranosyl*)-β-D-*galactopyranosyl*]-L-*glycerol* (6).—A solution of compound (4) in N-hydrochloric acid–methanol (1 : 9) (10 ml) was heated under reflux for 10 min; t.l.c. (ether) then indicated complete conversion of the isopropylidene derivative (4) (R_F 0.95) into the product (R_F 0.35). An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. The residue was extracted with chloroform and the product was recrystallised from ethyl acetate–light petroleum (b.p. 60—80°) to give the *triol* (6) (0.33 g), m.p. 117—119°, $[\alpha]_D$ +15.2° (*c* 0.49 in $CHCl_3$) (Found: C, 70.2; H, 6.8. $C_{57}H_{64}O_{13}$,EtOH requires C, 70.1; H, 6.9%).

3-O-[6-O-(α-D-*Galactopyranosyl*)-β-D-*galactopyranosyl*]-L-*glycerol* (7).—A solution of compound (6) (282 mg) in glacial acetic acid (10 ml) was treated with hydrogen over 10%

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[16] J. Gigg and R. Gigg, *J. Chem. Soc.* (*C*), 1966, 82; R. Gigg and C. D. Warren, *ibid.*, 1968, 1903; P. A. Gent and R. Gigg, *J.C.S. Chem. Comm.*, 1974, 277.

palladium–charcoal. When uptake had ceased (1 h), t.l.c. (n-butanol–acetic acid–water, 5 : 4 : 1) indicated the presence of a major product (R_F 0.15) which was isolated in the usual way and crystallised (after seeding) from aqueous ethanol to give *compound* (7) (40 mg), m.p. and mixed m.p. [with material prepared by the hydrolysis of a natural digalactosyl diglyceride (obtained from Dr. E. Heinz)] 191—193°, $[\alpha]_D$ +86.2° (*c* 1 in H_2O) {lit.,[1a] m.p 182—183°, $[\alpha]_D^{27}$ +86.4°; lit.,[15a] m.p. 194—196.5°, $[\alpha]_D^{20}$ +90° (*c* 1.8 in H_2O); lit.,[15b] m.p. 194—195°, $[\alpha]_D^{27}$ +87.7° (*c* 1.7 in H_2O); lit.,[7] m.p. 187—188°, $[\alpha]_D^{21}$ +88.8° (*c* 1.2 in H_2O)}.

3-O-[6-O-(α-D-*Galactopyranosyl*)-β-D-*galactopyranosyl*]-1,2-*di*-O-*octadecanoyl*-L-*glycerol* (10).—Compound (4) (0.33 g) was treated with benzyl chloride (0.5 ml) and sodium hydride (0.5 g) in dry benzene (20 ml) at reflux for 2.5 h; t.l.c. (ether–light petroleum, 2 : 1) then indicated complete conversion of compound (4) (R_F 0.2) into the perbenzyl ether (5) (R_F 0.65), which was isolated in the usual way. A solution of the ether (5) (0.4 g) in N-hydrochloric acid–dioxan (1 : 9) (10 ml) was heated under reflux for 10 min; t.l.c. (toluene–acetone, 4 : 1) then indicated complete conversion of compound (5) (R_F 0.8) into the diol (8) (R_F 0.3). An excess of sodium hydrogen carbonate was added, the solvents were evaporated off, and the product was extracted with chloroform. A solution of compound (8) (0.35 g) in dry pyridine (10 ml) and octadecanoyl chloride (0.5 ml) was kept at 20 °C for 4.5 h; t.l.c. (toluene–acetone, 4 : 1) then indicated complete conversion of compound (8) (R_F 0.3) into a single product (R_F 0.9). Water (0.5 ml) was added to the mixture, which was stirred at 20 °C for 1 h. The product (contaminated with stearic acid) was isolated in the usual way and chromatographed on neutral alumina; elution with toluene–ether (1 : 1) gave the ester (9) (0.33 g), which was dissolved in chloroform–methanol (1 : 1) (10 ml) and treated with hydrogen over 10% palladium–charcoal (previously washed with acetic acid) until uptake ceased. T.l.c. (chloroform–methanol, 2 : 1) showed a major product (R_F 0.8) together with traces of contaminants, and the crude product (0.2 g) was chromatographed on silica gel (B.D.H.; 60—120 mesh). Elution with chloroform–methanol (20 : 1) removed some contaminants and elution with chloroform–methanol (10 : 1) gave the major product (110 mg), which was recrystallised from methanol to give the *digalactosyl diglyceride* (10), softening point 77° and forming a meniscus at 225—230°, $[\alpha]_D$ +36.3° (*c* 1 in pyridine) (Found: C, 64.1; H, 10.0. $C_{51}H_{96}O_{15}$ requires C, 64.5; H, 10.2%) {lit.,[14] softening point 135° and forming a meniscus at 188—189° for a digalactosyl diglyceride (containing 97.7% stearic acid and 2.3% palmitic acid) prepared by the hydrogenation of natural material; lit.,[7] $[\alpha]_D^{21}$ +37.8° (*c* 1.35 in pyridine) (for a hydrogenated natural digalactosyl diglyceride), $[\alpha]_D^{21}$ +38.9 and +37.1° (pyridine) (for semisynthetic digalactosyl diglycerides containing oleoyl, and oleoyl + palmitoleoyl groups, respectively)}.

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