The Allyl Ether as a Protecting Group in Carbohydrate Chemistry. Part 10.[1] Synthesis of 3-*O*-(β-D-Galactopyranosyl 3-sulphate)-2-*O*-hexadecanoyl-1-*O*-hexadecyl-L-glycerol, 'Seminolipid'[2]

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

3-*O*-Allyl-L-glycerol was converted, by way of the 1-*O*-trityl derivative, into 3-*O*-allyl-2-*O*-(but-2-enyl)-L-glycerol, which was alkylated with hexadecyl bromide and sodium hydride in *NN*-dimethylformamide. The product was converted, by the action of potassium t-butoxide in dimethyl sulphoxide, into 1-*O*-hexadecyl-3-*O*-(prop-1-enyl)-L-glycerol which was alkylated with 'crotyl bromide' and the product was hydrolysed to give 2-*O*-(but-2-enyl)-1-*O*-hexadecyl-L-glycerol. This was condensed with acetobromogalactose to give the β-galactoside which was converted into crystalline 2-*O*-(but-2-enyl)-1-*O*-hexadecyl-3-*O*-(3,4-*O*-isopropylidene-β-D-galactopyranosyl)-L-glycerol. This, on benzylation followed by acidic hydrolysis, gave 3-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-2-*O*-(but-2-enyl)-1-*O*-hexadecyl-L-glycerol which was converted, by way of the *O*-dibutylstannylidene derivative, into 3-*O*-(3-*O*-allyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-2-*O*-(but-2-enyl)-1-*O*-hexadecyl-L-glycerol. Benzylation of this compound and subsequent treatment with potassium t-butoxide in dimethyl sulphoxide gave 3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(prop-1-enyl)-β-D-galactopyranosyl]-1-*O*-hexadecyl-L-glycerol which was acylated with hexadecanoyl chloride in pyridine. The prop-1-enyl group was cleaved by the action of mercury(II) chloride to give crystalline 3-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2-*O*-hexadecanoyl-1-*O*-hexadecyl-L-glycerol. This compound was sulphated with the pyridine–sulphur trioxide complex and the benzyl groups were removed by catalytic hydrogenolysis in glacial acetic acid to give 'seminolipid' with properties similar to those reported for the natural material. 'Desulphato-seminolipid' [3-*O*-(β-D-galactopyranosyl)-2-*O*-hexadecanoyl-1-*O*-hexadecyl-L-glycerol] was also prepared by catalytic hydrogenolysis of 3-*O*-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2-*O*-hexadecanoyl-1-*O*-hexadecyl-L-glycerol.

A NEW lipid was identified as the major species of glycolipid present in mature mammalian testes and spermatazoa by Canadian[3] and Japanese[4] workers in 1972. It was identified[3,4] as a 1-*O*-alkyl-2-*O*-acyl-3-*O*-(β-D-galactopyranosyl 3-sulphate)glycerol and the complete stereochemistry, as indicated in the title, was later established by the Japanese workers[5] who have named the compound 'seminolipid'. The occurrence of 'seminolipid' only in mature testes indicates some relationship with spermatogenesis.[5,6] A similar compound was also identified as a minor component of brain lipids[7,8a] where it occurs together with the corresponding 1,2-di-*O*-acyl-L-glycerol derivative.[8,9]

Most phospholipid and glycolipid species contain[10] a spectrum of acyl and/or alkyl groups varying in chain length and unsaturation but analyses[3-5] of 'seminolipid' indicated a high degree of uniformity in the acyl and alkyl chains. Thus 'seminolipid' from mature human testes was found[5] to contain 98.3% of hexadecyl groups in the alkyl chain and 96.7% of hexadecanoyl groups in the acyl chain and similar uniformity was found in the hydrocarbon chains of 'seminolipid' from rat testes[3b] although the composition of the hydrocarbon chains in the brain lipid was less uniform.[7]

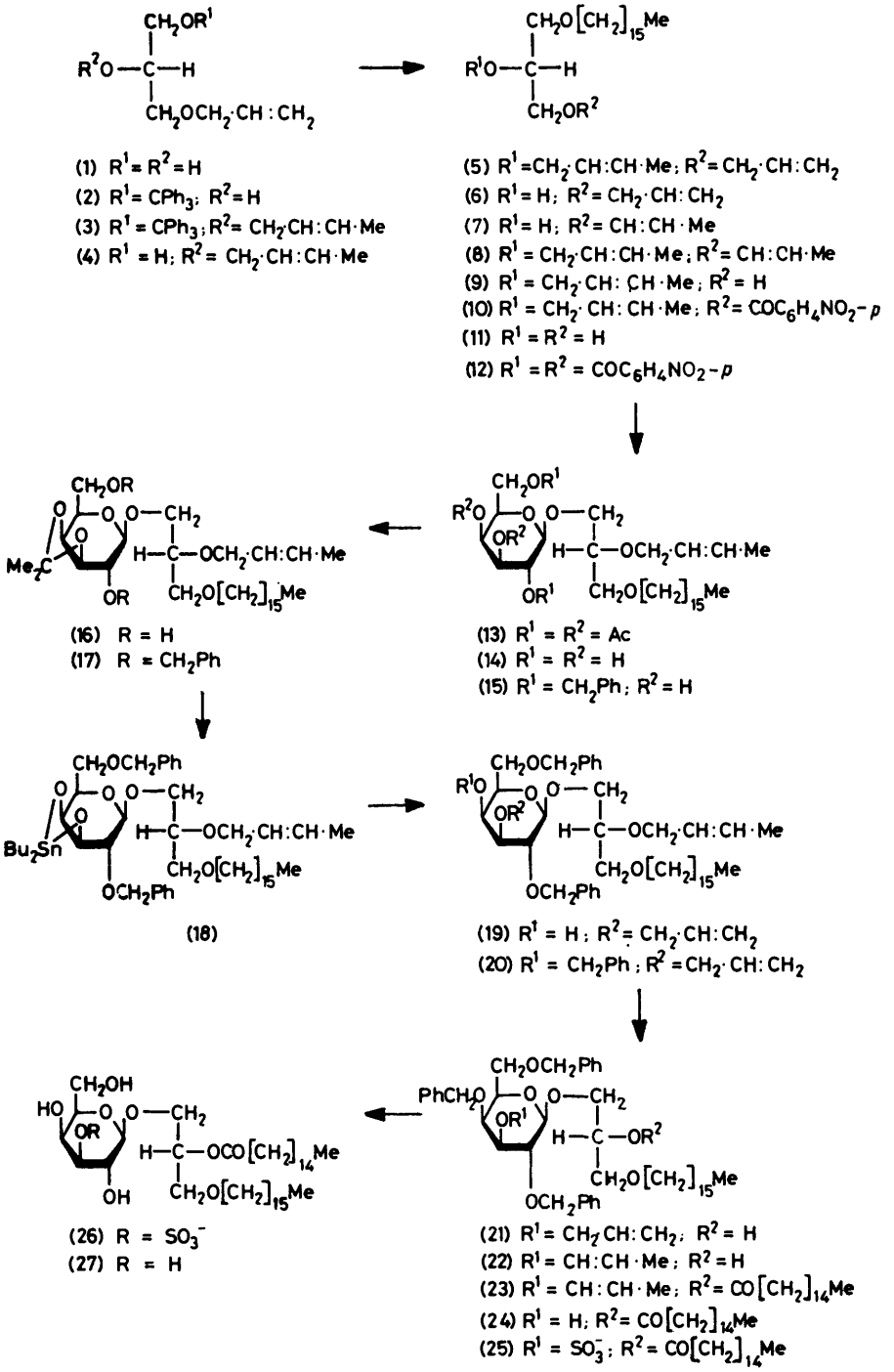
We have recently developed[11] a general procedure for the synthesis of glycolipids and oligosaccharides using allyl ethers for 'temporary' protection and benzyl ethers for 'persistent' protection of hydroxy-groups and we have now applied this method to a synthesis of 'seminolipid'. Allyl, prop-1-enyl, and but-2-enyl ethers were used as 'temporary' protecting groups.

RESULTS AND DISCUSSION

2-*O*-(But-2-enyl)-1-*O*-hexadecyl-L-glycerol (9) appeared to be a suitable intermediate for glycosidation since the but-2-enyl group could be removed at a later stage to allow the introduction of the acyl group. For the synthesis of compound (9), 3-*O*-allyl-L-glycerol[12] (1) was converted into the trityl ether (2) and this was alkylated with 'crotyl bromide' to give the but-2-enyl ether (3), which on acidic hydrolysis gave 3-*O*-allyl-2-*O*-(but-2-enyl)-L-glycerol (4). Compound (4) had similar properties (but opposite optical rotation) to those of the 1,2-di-*O*-allyl-L-glycerol and 1,2-di-*O*-(but-2-enyl)-L-glycerol which we had prepared previously.[13] Alkylation of compound (4) with hexadecyl bromide and sodium hydride in *NN*-dimethylformamide gave the hexadecyl ether (5), and this was treated with potassium t-butoxide in dimethyl sulphoxide, which cleaved[14] the but-2-enyl group and isomerised[15] the allyl group, to give 1-*O*-hexadecyl-3-*O*-(prop-1-enyl)-L-glycerol (7). Compound (7) was alkylated with 'crotyl bromide' to give the but-2-enyl ether (8) which on dilute acidic hydrolysis (to remove the prop-1-enyl group) gave the required alcohol (9) as an oil, which was characterised by conversion into the beautifully crystalline *p*-nitrobenzoate (10). Compound (9) was also converted, by the action of potassium t-butoxide in dimethyl sulphoxide, into 1-*O*-hexadecyl-L-glycerol (chimyl alcohol) (11); the bis-*p*-nitrobenzoate (12) of the chimyl alcohol had the expected properties thus showing that no racemisation had occurred in the manipulation of the glycerol derivatives.

A much shorter route to compound (9) from commercially available 'chimyl alcohol' (11) was also considered. This would involve tritylation of compound (11) followed by conversion into the but-2-enyl ether and subsequent removal of the trityl group. Commercial 'chimyl alcohol' is, however, a mixture of 1-*O*-alkyl-L-glycerols containing only *ca.* 80% of hexadecyl chains and the totally synthetic route, using pure hexadecyl bromide

(prepared from pure hexadecanol by the action of sodium bromide in dimethyl sulphoxide on the corresponding methanesulphonate), was considered more appropriate for establishing the subsequent synthetic pathways. 2-*O*-(But-2-enyl)-1-*O*-hexadecyl-L-glycerol (9) was glycosidated with acetobromogalactose in benzene–nitromethane in the presence of mercury(II) cyanide (Helferich method) to give the crude β-galactoside (13) which was deacetylated with base to give compound (14). Acetonation of the crude galactoside (14) with toluene *p*-sulphonic acid in acetone gave the crude 3,4-*O*-isopropylidene derivative (16). The pure derivative (16) crystallised readily from a solution of the crude product in light petroleum, and this ready crystallisation was highly convenient since the crude product probably contained some 4,6-*O*-isopropylidene derivative, which can be formed [1,16] in the acetonation of galactose derivatives, and also some by-products formed in the gly-



cosidation reaction. The low optical rotation and a doublet in the n.m.r. spectrum at δ 4.18 p.p.m. (J 8.5 Hz) indicated that the crystalline product (16) was the β-galactoside.

Compound (16) was converted into the dibenzyl ether (17) by the action of benzyl bromide and sodium hydride in *NN*-dimethylformamide and the isopropylidene group was readily hydrolysed to give the diol (15). The diol (15) was cleaved by sodium metaperiodate confirming that it was in fact formed by hydrolysis of a 3,4-*O*-isopropylidene- and not a 4,6-*O*-isopropylidene-derivative.

Recently, Augé *et al.*[17] showed that the equatorial-axial *O*-dibutylstannylidene derivative of benzyl 6-*O*-allyl-2-*O*-benzyl-α-D-galactopyranoside was benzylated specifically on the equatorial 3-hydroxy-group when treated with benzyl bromide in *NN*-dimethylformamide. This high regiospecificity in the galactose series was confirmed by Nashed and Anderson[18a,b] when they treated the *O*-dibutylstannylidene derivative of allyl 2,6-di-*O*-benzyl-α-D-galactopyranoside with allyl iodide in *NN*-dimethylformamide to give the equatorial 3-*O*-allyl derivative in high yield. Similar preferential allylation of the equatorial hydroxy-group was observed when the *O*-dibutylstannylidene derivatives of 3,4,5,6-tetra-*O*-benzyl-*myo*-inositol[18a] and methyl 4,6-*O*-benzylidene-α-D-mannopyranoside[18c] were treated with allyl iodide in *NN*-dimethylformamide.

We therefore converted the diol (15) into the *O*-dibutylstannylidene derivative (18) by azeotropic distillation of water from a mixture of the diol (15) and dibutyltin oxide in benzene and, after evaporation of the benzene, the crude product was treated directly with allyl bromide in *NN*-dimethylformamide at 100 °C for 8 h when t.l.c. showed complete conversion of the diol (15) into the 3-*O*-allyl ether (19). A minor product, running slightly ahead of the major product on t.l.c., which might be the corresponding 4-*O*-allyl ether, was separated from the major product by chromatography on alumina. Nashed and Anderson[18] used allyl iodide in this type of reaction because the reaction with allyl bromide was sluggish but we find the latter reagent quite suitable even though the reaction may be slower.

The alcohol (19) was benzylated to give 2-*O*-(but-2-enyl)-3-*O*-(3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-1-*O*-hexadecyl-L-glycerol (20) and this was treated with potassium t-butoxide in dimethyl sulphoxide which cleaved[14] the but-2-enyl group and isomerised[15] the allyl group to give 3-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(prop-1-enyl)-β-D-galactopyranosyl]-1-*O*-hexadecyl-L-glycerol (22). Compound (22) was acylated with hexadecanoyl chloride in pyridine and the prop-1-enyl group was subsequently removed by the action of mercury(II) chloride[15b] before chromatography on neutral alumina (to remove the hexadecanoic acid resulting from the excess of chloride used in the acylation stage), which gave the pure, crystalline alcohol (24). A portion of the crystalline alcohol (24) was hydrolysed with dilute acid to give 2,4,6-tri-*O*-benzyl-D-galactopyranose, identical with the material prepared previously,[14] thus confirming the substitution pattern of the galactose moiety of compound (24). The alcohol (24) was readily sulphated with the pyridine–sulphur trioxide complex[19] in pyridine and the sulphate (25) was isolated as the sodium salt.

It has been reported[20] that the benzyl and benzylidene groups could not be removed from benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside 3-sulphate and from the corresponding methyl glycoside by catalytic hydrogenolysis; difficulty in the hydrogenolysis of benzyl groups in the presence of sulphate esters has also been reported by Turvey.[21] Some initial experiments were therefore conducted on the catalytic debenzylation of the sulphate esters of benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside and benzyl 2,4,6-tri-*O*-benzyl-α-D-galactopyranoside since both these crystalline alcohols were available from previous studies.[22] Hydrogenolysis was conducted in glacial acetic acid which has been shown to be an excellent solvent for this purpose in our previous studies. These compounds were readily debenzylated and the products chromatographed with authentic standards.

The sulphate ester (25) was then debenzylated by the same technique to give the sulphate (26) (' seminolipid ') in excellent yield, the progress of the debenzylation being readily followed by t.l.c. The product (26) co-chromatographed with an authentic sample of ' seminolipid ' provided by Professor Yamakawa and the i.r. spectrum[4a] and optical rotation[5] reported for the natural material were similar to those of the synthetic product.

The non-sulphated analogue of ' seminolipid ', ' desulphato-seminolipid '[4a,5,7] [3-*O*-(β-D-galactopyranosyl)-2-*O*-hexadecanoyl-1-*O*-hexadecyl-L-glycerol] (27), which is a minor glycolipid component of mammalian testes, was also prepared by hydrogenolysis of compound (24).

Recently, Slomiany and his co-workers[23] have isolated a triglucosyl sulphate of 2-*O*-acyl-1-*O*-alkyl-L-glycerol from gastric secretion and saliva. This compound which contains α-linked glucose residues should also be available synthetically from the 2-*O*-(but-2-enyl)-1-*O*-hexadecyl-L-glycerol (9) and 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranosyl chloride[24] by way of the methods which we have developed[11] for 1,2-*cis*-glycoside synthesis and the technique used in this work for the synthesis of glycolipid sulphates.

The 1,2-di-*O*-acyl-L-glycerol analogue of ' seminolipid ' which occurs in the brain[8,9] should also be available from 1,2-di-*O*-(but-2-enyl)-L-glycerol[13] (which we have used previously[24] for the synthesis of a glycoside of 1,2-di-*O*-acyl-L-glycerol) using the techniques described in this work.

EXPERIMENTAL

Solvents were evaporated off under reduced pressure. The light petroleum used had b.p. 40—60 °C. T.l.c. was

carried out on microscope slides coated with silica gel G unless otherwise stated. Optical rotations were measured at 22—24 °C with a Bendix automatic polarimeter.

3-O-*Allyl*-2-O-(*but*-2-*enyl*)-L-*glycerol* (4).—3-*O*-Allyl-L-glycerol (1) [12] (12 g) and triphenylmethyl chloride (34 g) in dry pyridine (100 ml) were set aside at 20 °C for 24 h, after which time t.l.c. (ether) showed complete conversion of compound (1) (R_F 0.5) into the product (2) (R_F 0.95). Methanol (10 ml) and sodium hydrogencarbonate (20 g) were added and the solvents were evaporated off. Toluene was evaporated from the residue and the product (contaminated with triphenylmethyl methyl ether) was extracted from the residue with chloroform. The crude product (2) was taken up in dry *NN*-dimethylformamide (100 ml) containing sodium hydride (6g), and ' crotyl bromide ' (30 g) was added with stirring during 30 min. After 2 h at 20 °C, t.l.c. [ether–light petroleum, (1 : 3)] showed complete conversion of the alcohol (2) (R_F 0.4) into the but-2-enyl ether (3) (R_F 0.8). The crude product was isolated in the usual way and heated under reflux in 1N methanolic hydrogen chloride (400 ml) for 45 min, after which time t.l.c. (as above) showed complete conversion of compound (3) into the alcohol (4) (R_F 0.1). Triethylamine (60 ml) and water (400 ml) were added and the solution was set aside at 20 °C for 3 h to allow the triphenylmethyl methyl ether to crystallise. This was filtered off, washed with 50% aqueous methanol, and the filtrate reduced in volume to *ca.* 100 ml. The product was extracted from the aqueous layer with chloroform, the extract was dried (K_2CO_3), and evaporated to give the crude product (4) (12 g) as an oil. Distillation gave the pure *product* (4), b.p. 99—101 °C at 2 mmHg, $[\alpha]_D$ +28.1° (*c* 2 in $CHCl_3$) (Found: C, 64.3; H, 9.6. $C_{10}H_{18}O_3$ requires C, 64.5; H, 9.7%) {*cf.* $[\alpha]_D$ −27.2° (*c* 1 in $CHCl_3$) for 1,2-di-*O*-allyl-L-glycerol [13] and $[\alpha]_D$ −29.1° (*c* 2.5 in $CHCl_3$) for 1,2-di-*O*-(but-2-enyl)-L-glycerol [13]}.

n-Hexadecyl Bromide.—Methanesulphonyl chloride (30 ml) was added dropwise with stirring, during 30 min, to a solution of n-hexadecanol (B.D.H. Ltd., ' not less than 99% by g.l.c.') (52.6 g) in dry dichloromethane (400 ml) and dry pyridine (100 ml) at 0 °C. After 3 h at 0 °C, water (10 ml) was added slowly with stirring. Hydrochloric acid (3N, 500 ml) was then added and the dichloromethane layer was separated, washed with saturated potassium chloride solution and sodium hydrogencarbonate solution and dried ($MgSO_4$). T.l.c. [ether–light petroleum (1 : 2)] of the extract showed complete conversion of the alcohol (R_F 0.45) into the methanesulphonate (R_F 0.5) which was used without further purification. A stirred mixture of the methanesulphonate (15 g) and sodium bromide (30 g) in dry dimethyl sulphoxide (100 ml) was set aside at 100 °C for 45 min. After cooling, the mixture was diluted with water (400 ml) and the oily product was extracted with light petroleum. The extract was dried (K_2CO_3) and passed through a column of alumina (300 g) to remove traces of the alcohol and methanesulphonate. The light petroleum eluate was evaporated to give n-hexadecyl bromide (15 g), b.p. 176 °C at 6 mmHg, n_D^{20} 1.462 5 (lit.,[25] b.p. 157—160 °C at 3 mmHg, n_D^{20} 1.462 3).

2-O-(*But*-2-*enyl*)-1-O-*hexadecyl*-L-*glycerol* (9).—3-*O*-Allyl-2-*O*-(but-2-enyl)-L-glycerol (4) (6.9 g), n-hexadecyl bromide (21 g), and sodium hydride (3 g) in dry *NN*-dimethylformamide (70 ml) were stirred at 20 °C for 24 h. T.l.c. [ether–light petroleum (1 : 3)] then showed complete conversion of compound (4) (R_F 0.1) into a product (R_F 0.8) together with the excess of hexadecyl bromide (R_F 0.95). Methanol was added to destroy the excess of sodium hydride, the solution was diluted with water (200 ml), and extracted with ether. The ether extract was washed (saturated potassium chloride solution), dried (K_2CO_3) and evaporated to dryness. The crude product (freed from traces of methanol by evaporation of benzene) (22 g) was chromatographed on alumina (550 g). Elution with light petroleum removed the excess of hexadecyl bromide and elution with ether–light petroleum (1 : 1) gave the hexadecyl ether (5) (15 g, 98%). The product was added to a solution of potassium t-butoxide (8 g) in dry dimethyl sulphoxide (150 ml) and the mixture was stirred at 50 °C. The progress of the reaction was followed by t.l.c. [ether–light petroleum (1 : 3)] and, although compound (5) did not appear to be very soluble, this showed a rapid conversion of compound (5) (R_F 0.8) into the alcohol (6) (R_F 0.4) and a slower conversion of compound (6) into the prop-1-enyl ether (7) (R_F 0.5). When the conversion into the prop-1-enyl ether (7) was completed (*ca.* 5 h), the solution was diluted with water (200 ml) and extracted with ether. The ether extract was washed (saturated potassium chloride solution), dried (K_2CO_3), and evaporated in the presence of a small amount of potassium carbonate to avoid the possible cyclisation of the prop-1-enyl ether into a propylidene acetal.[26] The crude product (7) (12.5 g) was added to a mixture of sodium hydride (3 g) in dry *NN*-dimethylformamide (140 ml) and ' crotyl bromide ' (10 g) was then added to the mixture with stirring at 20 °C. After 2 h, t.l.c. (as above) showed complete conversion of compound (7) (R_F 0.5) into the but-2-enyl ether (8) (R_F 0.9). Methanol was added to destroy the excess of sodium hydride, the solution was diluted with water (200 ml), and extracted with ether. The extract was washed (saturated potassium chloride solution), dried (K_2CO_3), and evaporated to give the product (8) (15 g). The crude product (8), acetone (90 ml), and 1N hydrochloric acid (10 ml) were heated under reflux for 20 min, after which time t.l.c. (as above) showed complete conversion of the prop-1-enyl ether (8) (R_F 0.9) into the alcohol (9) (R_F 0.3). An excess of sodium hydrogencarbonate was added and the solvents were evaporated off. The product was extracted from the residue with ether, the extract was dried (K_2CO_3), and evaporated to give compound (9) (13.1 g) which was chromatographed on alumina. Elution with ether–light petroleum (1 : 1) and ether removed a small amount of impurity and elution with ether–methanol (49 : 1) gave the pure product (9) [10.5 g, 76% yield from compound (4)] as a syrup. Compound (9) (750 mg) was converted into the *p*-nitrobenzoate in the usual way, and the product was recrystallised from methanol to give 2-*O*-(*but*-2-*enyl*)-1-O-*hexadecyl*-3-O-p-*nitrobenzoyl*-L-*glycerol* (10) (800 mg) as needles, m.p. 52—54 °C, $[\alpha]_D$ −3.4° (*c* 2 in $CHCl_3$) (Found: C, 69.3; H, 9.45; N, 2.7. $C_{30}H_{49}NO_6$ requires C, 69.3; H, 9.5; N, 2.7%).

A portion of compound (9) (355 mg) was treated with potassium t-butoxide (500 mg) in dry dimethyl sulphoxide (5 ml) at 50 °C until t.l.c. [ether–light petroleum (1 : 3)] showed complete conversion of the but-2-enyl ether (9) (R_F 0.3) into the diol (11) (R_F 0). The product (297 mg) was isolated in the usual way and crystallised from light petroleum to give 1-*O*-hexadecyl-L-glycerol (11) (176 mg), m.p. 66—68 °C (lit.,[27] m.p. 63—64 °C). This was converted into the bis-*p*-nitrobenzoate in the usual way; recrystallisation of the product from methanol gave compound (12), m.p. 60—62 °C, $[\alpha]_D$ −33.3° (*c* 1 in $CHCl_3$)

(Found: C, 64.3; H, 7.3; N, 4.5. Calc. for $C_{33}H_{46}N_2O_9$: C, 64.5; H, 7.5; N, 4.6%); lit.,[27] m.p. 52 °C, $[\alpha]_D$ −29.2° (*c* 8.3 in tetrachloroethane); lit.,[28] $[\alpha]_D$ −31.2° (*c* 1 in $CHCl_3$) for the bis-*p*-nitrobenzoate of 1-*O*-octadecyl-L-glycerol.

2-O-(*But*-2-*enyl*)-1-O-*hexadecyl*-3-O-(3,4-O-*isopropylidene*-β-D-*galactopyranosyl*)-L-*glycerol* (16).—A mixture of 2-*O*-(but-2-enyl)-1-*O*-hexadecyl-L-glycerol (9) (9.3 g), acetobromogalactose (25 g), and mercury(II) cyanide (17 g) in dry benzene (100 ml) and dry nitromethane (100 ml) was stirred at 40 °C for 9 h after which time t.l.c. [ether–light petroleum (1 : 1)] showed complete conversion of the alcohol (9) (R_F 0.6) into a major product (R_F 0.5) together with other minor products. The mixture was diluted with benzene (200 ml) and washed with water and saturated sodium hydrogencarbonate solution. The benzene extract was dried ($MgSO_4$) and evaporated to give the crude product (13) (33.2 g). This was taken up in a solution of sodium hydroxide (20 g) in methanol (250 ml) and water (10 ml) and the solution was heated under reflux for 15 min. Solid carbon dioxide was added to the cooled solution which was then evaporated to dryness. The crude product (14) was extracted from the residue with ethyl acetate and the extract was evaporated to dryness; toluene and ethanol were evaporated from the residue to remove the last traces of water, and t.l.c. [chloroform–methanol (9 : 1)] then showed a major product (R_F 0.4) with other slow running by-products derived from the excess of acetobromogalactose. The crude product (14) (25 g) was added to a solution of toluene-*p*-sulphonic acid (5 g) in dry acetone (1 000 ml) and the mixture was stirred at 20 °C for 6 h, after which time t.l.c. (ether) showed conversion of compound (14) into a major product (R_F 0.5). Triethylamine (10 ml) and sodium hydrogencarbonate (5 g) were added and the solvent was evaporated off. The product (17 g) was extracted from the residue with ether and chromatographed on alumina (380 g). Elution with ether–methanol (49 : 1) gave the major product (9.7 g) (together with small amounts of less polar impurities) which crystallised readily from light petroleum to give the *isopropylidene derivative* (16) (6 g), m.p. 63—65 °C, $[\alpha]_D$ +12.2° (*c* 2 in $CHCl_3$) (Found: C, 67.2; H, 10.5. $C_{32}H_{60}O_8$ requires C, 67.1; H, 10.6%). The 270 MHz ^1H n.m.r. spectrum showed a doublet at **4.18** p.p.m. (*J* 8.5 Hz) which was attributed to the anomeric proton of a 1,2-*trans*-glycosidic linkage.

3-O-(2,4,6-*Tri*-O-*benzyl*-β-D-*galactopyranosyl*)-2-O-*hexadecanoyl*-1-O-*hexadecyl*-L-*glycerol* (24).—A mixture of the diol (16) (4 g), sodium hydride (4 g), and benzyl bromide (3.2 ml) in dry *NN*-dimethylformamide (75 ml) was stirred at 20 °C for 4 h, after which time t.l.c. [ether–light petroleum (1 : 1)] showed complete conversion of the diol (16) (R_F 0) into the dibenzyl ether (17) (R_F 0.85). Methanol (2 ml) was added (to convert the excess of benzyl bromide into methyl benzyl ether) and the solution was stirred at 20 °C for 3 h. An excess of methanol was added (to destroy the sodium hydride) and the solution was diluted with water and extracted with ether. The extract was dried (K_2CO_3) and evaporated to give the crude product (17) (6.75 g) (containing methyl benzyl ether) which was taken up in methanol (720 ml) and the solution was heated under reflux. 1N Hydrochloric acid (80 ml) was added, refluxing was continued for 15 min, and the solution was then cooled to 0 °C. T.l.c. [ether–light petroleum (2 : 1)] showed complete conversion of compound (17) (R_F 1) into the diol (15) (R_F 0.2); an excess of sodium hydrogencarbonate was added, the solvents were evaporated off, and the crude product (15) (6.26 g) was extracted from the residue with ether. The extract was evaporated to dryness and toluene was evaporated from the product to remove traces of water.

Sodium metaperiodate (5 mg) in water (0.5 ml) was added to a solution of compound (15) (12 mg) in methanol (2 ml). After 15 min at 20 °C, t.l.c. [ether–light petroleum (3 : 1)] showed conversion of compound (15) (R_F 0.6) into a product (R_F 0.8), indicating that (15) was a 3,4-diol and not a 4,6-diol (which would have been obtained from a 4,6-*O*-isopropylidene derivative).

A mixture of crude (15) (6.2 g) and di-n-butyltin oxide (1.9 g) in dry benzene (50 ml) was heated under reflux, with azeotropic removal of water in a Dean and Stark apparatus, for 3 h. The benzene was evaporated off and dry *NN*-dimethylformamide (30 ml) and allyl bromide (1 ml) were added to the residual stannylidene derivative (18) and the solution was kept at 100 °C for 8 h. T.l.c. [ether–light petroleum (1 : 1)] then showed complete conversion of compound (15) (R_F 0.2) [which is regenerated from the stannylidene derivative (18) on the t.l.c. plate] into a major product (R_F 0.75). Water (100 ml) was added and the product was extracted with ether. The extract was dried (K_2CO_3) and evaporated to give the crude product (19) (7 g) which was chromatographed on alumina. Elution with ether removed impurities (methyl benzyl ether) and elution with ether–methanol (99 : 1) gave the required product. The first fractions of the ether–methanol eluate contained a small amount of a slightly less polar product (t.l.c.) and were not combined with the later fractions which were free from other products. The pure product (19) (2.5 g, 48%) was benzylated with sodium hydride and benzyl bromide in *NN*-dimethylformamide in the usual way. T.l.c. [ether–light petroleum (1 : 2)] showed complete conversion of compound (19) (R_F 0.4) into the product (20) (R_F 0.75). Methanol was added to ensure complete conversion of benzyl bromide into benzyl methyl ether and the product was isolated in the usual way.

The crude product (20) (3.6 g) was treated with potassium t-butoxide (4 g) in dry dimethyl sulphoxide (50 ml) at 50 °C with stirring for 5 h after which time t.l.c. [ether–light petroleum (1 : 1)] showed complete conversion of the but-2-enyl ether (20) (R_F 0.9) into an alcohol (R_F 0.5). The t.l.c. did not distinguish between the allyl ether (21) and the prop-1-enyl ether (22) and therefore a portion of the product was treated with 1N hydrochloric acid–acetone (1 : 9) at reflux for 15 min, when t.l.c. (as above) showed complete conversion of the product (R_F 0.5) into a new product (R_F 0.1) indicating that the product (R_F 0.5) was entirely in the form of the prop-1-enyl ether (22). The product (22) was isolated in the usual way and the solvents were evaporated off in the presence of a portion of potassium carbonate to ensure the survival of the prop-1-enyl group. The crude product (22) (3.3 g) was chromatographed on basic alumina; elution with ether removed by-products (methyl benzyl ether), and elution with ether–methanol (99 : 1) gave the pure product (22) (2.1 g, 79%).

A solution of compound (22) (1.94 g) and hexadecanoyl chloride (1.25 g) [prepared from hexadecanoic acid (B.D.H. Ltd., ' not less than 98% by g.l.c.')] in dry pyridine (25 ml) was stirred at 20 °C for 4 h after which time t.l.c. [ether–light petroleum (1 : 1)] showed complete conversion of the alcohol (22) (R_F 0.5) into the palmitate (23) (R_F 0.9). Water (2 ml) was added and the solution was stirred at 20 °C for 4 h to convert any anhydride into the acid. More water (100 ml) was then added, the product was extracted with ether, the

extract was washed with saturated potassium chloride solution, dried ($MgSO_4$), and evaporated to dryness. Toluene was evaporated from the residue to remove the last traces of pyridine.

The crude product (23) (containing hexadecanoic acid) was dissolved in acetone (100 ml), water (5 ml), and mercury(II) chloride (3 g) were added, and the solution was stirred at 20 °C for 15 min. T.l.c. (as above) then showed complete conversion of the prop-1-enyl ether (23) (R_F 0.9) into the alcohol (24) (R_F 0.6) together with hexadecanoic acid (R_F 0—0.4, detected only after strong heating of the t.l.c. plate). The acetone was evaporated off and ether was added to the residue. A saturated solution of potassium iodide was added to convert all the mercury derivatives into water-soluble salts,[15b] and the ether extract was washed with saturated potassium chloride solution and dried ($MgSO_4$). The ether extract was evaporated, and benzene was evaporated from the residue before chromatography on neutral alumina (Merck, Art. 107 7, washed with methyl formate–methanol and methanol and reactivated by heating at 150 °C). Elution with ether–light petroleum (1 : 2) removed some non-polar by-products and elution with ether gave the alcohol (24) free from hexadecanoic acid. Recrystallisation from light petroleum gave the *alcohol* (24) (1.5 g) as a waxy solid, m.p. 47—49 °C, $[\alpha]_D$ +4.1° (*c* 2 in $CHCl_3$) (Found: C, 75.6; H, 10.3. $C_{62}H_{98}O_9$ requires C, 75.4; H, 10.0%).

A portion of the product (130 mg) was heated under reflux in dioxan (9 ml) and 1N hydrochloric acid (1 ml) for 14 h, after which time t.l.c. [ether–light petroleum (1 : 1)] showed conversion of compound (24) (R_F 0.6) into other products. Water (50 ml) was added and the products (130 mg) were extracted with ether. These were taken up in light petroleum (5 ml) and the solution was set aside at 20 °C for three days and the crystals (15 mg) which separated were filtered off. These co-chromatographed with the 2,4,6-tri-*O*-benzyl-D-galactopyranose prepared previously [14] and had m.p. and mixed m.p. 120—122 °C (lit.,[14] m.p. 123—124 °C).

3-O-(β-D-*Galactopyranosyl 3-sulphate*)-2-O-*hexadecanoyl*-1-O-*hexadecyl*-L-*glycerol*, *' Seminolipid '* (26).—Pyridine–sulphur trioxide complex (900 mg) was added to a solution of the alcohol (24) (450 mg) in dry pyridine (10 ml) and the mixture was stirred at 50 °C for 3 h after which time t.l.c. [ether–light petroleum (1 : 1)] showed complete conversion of compound (24) (R_F 0.6) into a product (R_F 0). Ether (100 ml), water (50 ml), and 2N hydrochloric acid (75 ml) were added and the ether layer was separated, washed with saturated sodium chloride solution and saturated sodium hydrogencarbonate solution, and dried (Na_2SO_4). The extract was evaporated to give the sodium salt of the sulphate (25) (440 mg) [t.l.c. on silica gel (Merck No. 5721) in chloroform–methanol–water (65 : 25 : 4) showed the product with R_F 0.75]. Palladium–charcoal (Fluka, 10%, 500 mg) and glacial acetic acid (25 ml) were added to the product and the mixture was stirred under hydrogen at 20 °C and the course of the reaction was followed by t.l.c. (as above). Samples after 2, 4, and 7 h showed progressive conversion of compound (25) (R_F 0.75) into debenzylated products (R_F 0.5, 0.45 and 0.4). The product (R_F 0.4) co-chromatographed with an authentic sample of ' seminolipid ' obtained from Professor T. Yamakawa. A 24-h sample showed predominantly the product (R_F 0.4) and after 40 h the mixture was filtered through Celite and the filtrate was evaporated to give the product (106 mg). The catalyst was washed with chloroform–methanol–water (65 : 25 : 4) and the filtrate was evaporated to give a further quantity of the product (166 mg) (combined yield 272 mg, 90%). T.l.c. (as above) showed that both fractions were essentially pure and the products co-chromatographed with authentic ' seminolipid '. The recovered product was recrystallised from ethyl acetate and methanol to give *' seminolipid '* (26), which softened from 165 °C forming a meniscus at 170 °C $[\alpha]_D$ +2.4° [*c* 2 in chloroform–methanol (1 : 1)] (Found: C, 60.0; H, 9.9; S, 3.8. $C_{41}H_{79}NaO_{12}S$ requires C, 60.1; H, 9.7; S, 3.9%) {lit.,[5] $[\alpha]_D$ 0° [*c* 0.39 in chloroform–methanol (1 : 1) for ' seminolipid ' from human testes and $[\alpha]_D$ 0 ± 0.5° [*c* 1 in chloroform–methanol (1 : 1)] for ' seminolipid ' from boar testes}.

3-O-(β-D-*Galactopyranosyl*)-2-O-*hexadecanoyl*-1-O-*hexadecyl*-L-*glycerol*, *' Desulphato-seminolipid '* (27).—Compound (24) (200 mg) and palladium–charcoal (200 mg, Fluka, 10%) in glacial acetic acid (15 ml) were stirred under hydrogen at 20 °C for 24 h. The mixture was filtered through Celite and the catalyst was washed with chloroform–methanol–water (65 : 25 : 4). The combined filtrates were evaporated and the crystalline product was recrystallised from methanol to give *compound* (27), softening at 80 °C and forming a meniscus at 139—141 °C, $[\alpha]_D$ −4.2° [*c* 1 in chloroform–methanol (1 : 1)] (Found C, 68.2; H, 11.6. $C_{41}H_{80}O_9$ requires C, 68.7; H, 11.25%) [*cf.* m.p. 149—150 °C, $[\alpha]_D$ −4° (*c* 0.5 in chloroform) for 3-*O*-(β-D-galactopyranosyl)-1,2-di-*O*-hexadecanoyl-L-glycerol [12]].

We thank Dr J. Feeney for the ^1H n.m.r. spectrum, Dr J. Turvey for samples of galactose sulphates, Professor T. Yamakawa for a sample of ' seminolipid ', and Mr R. Conant for technical assistance.

[8/577 *Received*, 29*th March*, 1978]

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