Synthesis of 1,2-Di-*O*-hexadecanoyl-3-*O*-(β-D-galactopyranosyl)-L-glycerol (a 'Galactosyl Diglyceride') and 1,2-Di-*O*-octadecanoyl-3-*O*-(6-*O*-octadecanoyl-β-D-galactopyranosyl)-L-glycerol

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

1,2-Di-*O*-benzyl-L-glycerol (prepared by a new method *via* 3-*O*-allyl-1,2-di-*O*-benzyl-L-glycerol) was glycosylated to give 1,2-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glycerol. This compound was converted into the crystalline 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol, which was used as an intermediate for the synthesis of the title compounds. 3-*O*-(2,3,4-Tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol is also a potential intermediate for the syntheses of 'polygalactosyl diglycerides' which are the serologically active components of the glycolipids of *Mycoplasma pneumoniae*. 1,2-Bis-*O*-(2-methylallyl)-L-glycerol was prepared and attempts were made to condense this, in β-linkage, with 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride [prepared by a new route from allyl 6-*O*-(but-2-enyl)-α-D-galactopyranoside] in order to provide a more direct route to 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol. 3-*O*-(α-D-Galactopyranosyl)-L-glycerol was also prepared by a new route *via* 1,2-*O*-isopropylidene-3-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl]-L-glycerol. The migration of an isopropylidene group under the conditions of our α-glycoside synthesis is suspected.

'GALACTOSYL DIGLYCERIDES' [1,2-di-*O*-acyl-3-*O*-(β-D-galactopyranosyl)-L-glycerols] are major constituents of plant lipids [1] and are also present in mammalian brain [2] and in micro-organisms.[3] Acylated derivatives of galactosyl diglycerides [1,2-di-*O*-acyl-3-*O*-(6-*O*-acyl-β-D-galactopyranosyl)-L-glycerols] have also been isolated from plants.[4] Galactosyl derivatives of galactosyl diglycerides in which 1, 2, or 3 galactopyranose residues are joined to the 6-position of adjacent galactose residues have also been identified [1,5] in plant lipids and are known as di-, tri-, and tetra-galactosyl diglycerides, respectively. In the digalactosyl diglyceride the terminal galactose is joined in α-linkage [1] and this is probably the case in the trigalactosyl diglyceride.[5a] Digalactosyl diglycerides are present in mammalian brain [2c] and di-, tri-, and tetra-galactosyl diglycerides are also present in micro-organisms [3b,c] although their structures have not been fully established. The tri- and tetra-galactosyl diglycerides from *Mycoplasma pneumoniae* glycolipids and from spinach glycolipids show similar serological activity [3c,6] indicating a similarity in structure. The polygalactosyl diglycerides isolated from *Bifidobacterium bifidum* var. pennsylvanicus have a different structure in which the galactose residues are joined to the 2-position of adjacent galactose molecules by β-linkages.[7]

Two syntheses [8,9] of galactosyl diglycerides and a partial synthesis of a galactosyl diglyceride [10] and an acylated galactosyl diglyceride [4b] have been described. We describe here a new route for the synthesis of galactosyl diglycerides and 6-*O*-acylgalactosyl diglycerides involving use of intermediates which should be suitable for the syntheses of the di-, tri-, and tetra-galactosyl diglycerides required for comparison with the serologically active glycolipids of *Mycoplasma pneumoniae*.

We initially considered a route for the synthesis of galactosyl diglycerides involving the condensation of 1,2-bis-*O*-(2-methylallyl)-L-glycerol (6) with 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride [11a] (18) under some of the conditions which Kronzer and Schuerch [12] have shown to be suitable for the preparation of β-methyl glycosides from glycosyl chlorides containing non-participating groups in the 2-position. Such a condensation would give the β-galactoside (19) which could be converted into 1,2-bis-*O*-(2-methylallyl)-3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (20), since we have shown [13] that the but-2-enyl group can be removed (by potassium t-butoxide in dimethyl sulphoxide) without affecting the 2-methylallyl group. Compound (20) would be a suitable intermediate for the syntheses of galactosyl diglycerides and 6-*O*-acylgalactosyl diglycerides and for the syntheses of the polygalactosyl diglycerides since further suitably protected galactosyl residues could be added in α-linkage to the free hydroxy-group of compound (20), by the method which we have described recently,[11] by using the galactosyl chloride (18). Ultimately the 2-methylallyl groups could be removed,[13] the glycerol system acylated with long-chain fatty acids, and the benzyl groups removed by hydrogenolysis to give the corresponding polygalactosyl diglycerides.

[1] H. E. Carter, R. A. Hendry, and N. Z. Stanacev, *J. Lipid Res.*, 1961, **2**, 223; A. Rosenberg, *Science*, 1967, **157**, 1191; P. S. Sastry, *Adv. Lipid Res.*, 1974, **12**, 251.

[2] (*a*) J. M. Steim, *Biochim. Biophys. Acta*, 1967, **144**, 118; (*b*) M. G. Rumsby, *J. Chromatog.*, 1969, **42**, 237; (*c*) T. Inoue, D. S. Deshmukh, and R. A. Pieringer, *J. Biol. Chem.*, 1971, **246**, 5688, 5695.

[3] (*a*) N. Shaw, *Bacteriol. Rev.*, 1970, **34**, 365; M. Yribarren, E. Vilkas, J. Rozanis, *Chem. Phys. Lipids*, 1974, **12**, 172; (*b*) P. Plackett, B. P. Marmion, E. J. Shaw, and R. M. Lemcke, *Austral. J. Exp. Biol. Med.*, 1969, **47**, 171; (*c*) G. E. Kenny and R. M. Newton, *Ann. New York Acad. Sci.*, 1973, **225**, 54.

[4] (*a*) D. V. Mhyre, *Canad. J. Chem.*, 1968, **46**, 3071; (*b*) E. Heinz and A. P. Tulloch, *Z. physiol. Chem.*, 1969, **350**, 493.

[5] (*a*) T. Galliard, *Biochem. J.*, 1969, **115**, 335; (*b*) D. E. Webster and S. B. Chang, *Plant Physiol.*, 1969, **44**, 1523.

[6] S. Razin, I. Kahane, and J. Kovartovsky, in 'Pathogenic Mycoplasmas,' CIBA Foundation Symposium, 1972, p. 93.

[7] J. H. Veerkamp, *Biochim. Biophys. Acta*, 1972, **273**, 359; 1974, **348**, 23.

[8] H. P. Wehrli and Y. Pomeranz, *Chem. Phys. Lipids*, 1969, **3**, 357.

[9] A. I. Bashkatova, G. V. Smirnova, V. N. Volynskaya, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1973, **9**, 1393 [*J. Org. Chem.* (*U.S.S.R.*), 1973, **9**, 1422].

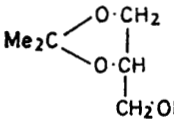
[10] E. Heinz, *Biochim. Biophys. Acta*, 1971, **231**, 537.

[11] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1974, (*a*) p. 1835; (*b*) p. 1446.

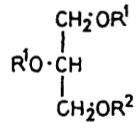
[12] F. J. Kronzer and C. Schuerch, *Carbohydrate Res.*, 1973, **27**, 379.

[13] P. A. Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1973, 1858.

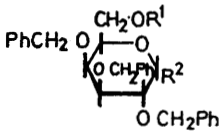
To investigate this route we have prepared 1,2-bis-*O*-(2-methylallyl)-L-glycerol (6) and developed a more convenient route than that used previously [11a] for the



(1) R = H
(2) R = $CH_2{\cdot}CH{:}CH_2$



(3) R^1 = H, R^2 = $CH_2{\cdot}CH{:}CH_2$
(4) R^1 = H, R^2 = CH:CHMe
(5) R^1 = $CH_2CMe{:}CH_2$, R^2 = CH:CHMe
(6) R^1 = $CH_2{\cdot}CMe{:}CH_2$, R^2 = H
(7) R^1 = $CH_2{\cdot}CMe{:}CH_2$, R^2 = CPh_3
(8) R^1 = $CH{:}CMe_2$, R^2 = CPh_3
(9) R^1 = H, R^2 = CPh_3
(10) R^1 = CH_2Ph, R^2 = $CH_2{\cdot}CH{:}CH_2$
(11) R^1 = CH_2Ph, R^2 = CH:CHMe
(12) R^1 = CH_2Ph, R^2 = H
(13) R^1 = CH_2Ph, R^2 = CPh_3



(14) R^1 = $CH_2{\cdot}CH{:}CHMe$, R^2 = $O{\cdot}CH_2{\cdot}CH{:}CH_2$
(15) R^1 = H, R^2 = $O{\cdot}CH{:}CHMe$
(16) R^1 = $CH_2CH{:}CHMe$, R^2 = $O{\cdot}CH{:}CHMe$
(17) R^1 = $CH_2CH{:}CHMe$, R^2 = OH
(18) R^1 = $CH_2CH{:}CHMe$, R^2 = Cl

preparation of 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride (18). This new route is also applicable to the synthesis of the corresponding 6-*O*-allyl, 6-*O*-(2-methylallyl), and 6-*O*-acyl derivatives.

1,2-*O*-Isopropylidene-L-glycerol [14] (1) was converted into the allyl ether (2) which, on acidic hydrolysis, gave 3-*O*-allyl-L-glycerol (3), characterised as the crystalline bis-*p*-nitrobenzoate. Compound (3) was converted into 3-*O*-(prop-1-enyl)-L-glycerol (4) by the action of potassium t-butoxide in dimethyl sulphoxide (as described previously [15] for the preparation of the racemic compound) and this was converted into 1,2-bis-*O*-(2-methylallyl)-3-*O*-(prop-1-enyl)-L-glycerol (5). Acidic hydrolysis removed the prop-1-enyl group to give 1,2-bis-*O*-(2-methylallyl)-L-glycerol (6). For characterisation, compound (6) was converted into the trityl ether (7), which on subsequent isomerisation [13] gave 1,2-bis-*O*-(2-methylprop-1-enyl)-3-*O*-trityl-L-glycerol (8). Compound (8) was then treated with mercury(II) chloride in the presence of mercury(II) oxide,[16b] which removed the 2-methylprop-1-enyl groups to give 3-*O*-trityl-L-glycerol (9), identical with material prepared previously.[17]

Allyl 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranoside (14) [11a] was treated with potassium t-butoxide in dimethyl sulphoxide, which removed [18] the but-2-enyl group and isomerised [16] the allyl group to give prop-1-enyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (15). This was treated with ' crotyl bromide ' and sodium hydride [18] to give the but-2-enyl ether (16) which was hydrolysed with mercury(II) chloride [16b] to give 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-D-galactopyranose (17); this gave a crystalline *p*-nitrobenzoate identical with that reported.[11a] Compound (17) was converted into the chloride (18) as described previously.[11a] Condensation of the chloride (18) with the bis-*O*-(2-methylallyl)-L-glycerol (6) under some of the conditions described by Kronzer and Schuerch [12] for the preparation of β-methyl glycosides from glycosyl halides containing non-participating groups in the 2-position have not yet proved successful, but we are continuing to investigate these methods since they are of potential value in our proposed [11b] general oligosaccharide synthesis.

Stevens and his co-workers [19] have also shown that glycosyl chlorides containing non-participating groups in the 2-position react rapidly with large excesses of methanol in the presence of silver carbonate or mercury(II) cyanide to give β-methyl glycosides stereospecifically. We have similarly observed that when 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride [11b] was reacted with an excess of methanol (*ca.* 10 mol. equiv.) in the presence of silver carbonate, the β-methyl glycoside was formed very rapidly and stereospecifically (the α- and β-anomers of methyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside can be resolved by t.l.c.[11b]). However when 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride was treated with a similar excess of 1,2-bis-*O*-(2-methylallyl)-L-glycerol (6) and silver carbonate no reaction was observed in the same time. The interaction of alcohols with silver carbonate has been studied in some detail [20] and from these results one could postulate that the unsaturated centres of the 2-methylallyl groups in compound (6) interact with the silver to prevent the proper orientation of the hydroxy-group for glycoside formation by some of the mechanisms previously described.[21] However under the same conditions no reaction was observed with isobutyl alcohol,

[14] L. Le Cocq and C. E. Ballou, *Biochemistry*, 1964, **3**, 976.
[15] J. Cunningham and R. Gigg, *J. Chem. Soc.*, 1965, 2968.
[16] (*a*) J. Gigg and R. Gigg, *J. Chem. Soc.* (*C*), 1966, 82; (*b*) R. Gigg and C. D. Warren, *ibid.*, 1968, 1903.
[17] J. Gigg and R. Gigg, *J. Chem. Soc.* (*C*), 1967, 431.
[18] P. A. Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1972, 1535.
[19] C. L. Stevens, G. H. Ransford, J. Němec, J. M. Cahoon, and P. M. Pillai, *J. Org. Chem.*, 1974, **39**, 298.
[20] F. J. Kakis, M. Fetizon, N. Douchkine, M. Golfier, P. Mourgues, and T. Prange, *J. Org. Chem.*, 1974, **39**, 523.
[21] G. Wulff and G. Röhle, *Angew. Chem. Internat. Edn.*, 1974, **13**, 157.

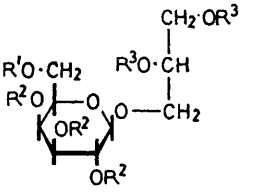
indicating that a steric factor was more likely responsible for the lack of reactivity. Since mercury(II) cyanide cannot be used in glycoside syntheses involving glycosyl halides or aglycones containing allyl groups [because the mercury(II) halide formed would react with the allyl groups] a condensation of compounds (6) and (18) in the presence of silver cyanide was attempted. A glycoside fraction was obtained in low yield and the anomeric configuration was not investigated.

1,2-*O*-Isopropylidene-L-glycerol (1) is not a suitable compound for the synthesis of glycosides by the Koenigs–Knorr procedure because of the migration of the isopropylidene group leading to racemisation of the glycerol unit under these conditions,[8,22,23] but 1,2-*O*-isopropylidene-L-glycerol has been used successfully [9] for the synthesis of a galactosyl diglyceride by the orthoester method. A synthesis [8] of the same galactosyl diglyceride by glycosidation of 2,2'-*O*-methylenebis-(1-*O*-palmitoyl-L-glycerol) under Koenigs–Knorr conditions led to a product whose m.p. (56°) was considerably different from that (141—142°) of the material prepared [9] by the orthoester method and from that (152—154°) of the same material prepared [10] by partial synthesis from a natural galactosyl diglyceride. Both the methods described [8,9] for the total synthesis of galactosyl diglycerides have used 1,2-di-*O*-acyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glycerol as an intermediate, and this has necesitated the preferential removal of the acetyl groups by hydrazine.

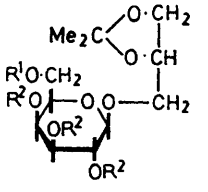
We have therefore developed a different route to galactosyl diglycerides *via* the crystalline 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (25) which has also allowed us to prepare the naturally occurring 6-*O*-acylgalactosyl diglyceride (27). Compound (25) should also be a suitable intermediate for the synthesis of the polygalactosyl diglycerides and is also a useful intermediate for characterising the products obtained by the condensation of the galactosyl chloride (18) and 1,2-bis-*O*-(2-methylallyl)-L-glycerol (6), since after removal of the allyl groups from the condensation product the β-glycoside (25) or its α-anomer (39) will be obtained directly.

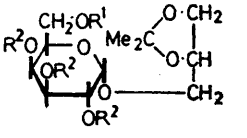
1,2-Di-*O*-benzyl-L-glycerol, which was required for this synthetic route, has been prepared previously [24] from 1,2,5,6-tetra-*O*-benzyl-D-mannitol, but we have developed a new route *via* 3-*O*-allyl-L-glycerol (3). The allyl ether (3) was converted into 3-*O*-allyl-1,2-di-*O*-benzyl-L-glycerol (10) and the allyl group was isomerised [16a] (as described previously [25] for the racemic compound) to give the prop-1-enyl ether (11). Acidic hydrolysis of compound (11) gave 1,2-di-*O*-benzyl-L-glycerol (12), which was characterised by conversion into the known [24] crystalline trityl derivative (13).

Tetra-*O*-acetyl-α-D-galactopyranosyl bromide was condensed with 1,2-di-*O*-benzyl-L-glycerol (12) by using mercury(II) cyanide in benzene–nitromethane (1 : 1) [11b] to give the β-glycoside (21). The benzyl groups were removed by hydrogenolysis to give 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glycerol (22). Acetylation of compound (22) gave the hexa-acetate (23) (which



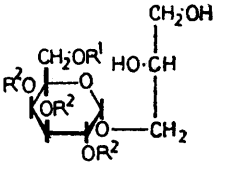
(19) $R^1 = CH_2{\cdot}CH{:}\,CHMe$, $R^2 = CH_2Ph$, $R^3 = CH_2{\cdot}CMe{:}\,CH_2$
(20) $R^1 = H$, $R^2 = CH_2Ph$, $R^3 = CH_2{\cdot}CMe{:}\,CH_2$
(21) $R^1 = R^2 = Ac$, $R^3 = CH_2Ph$
(22) $R^1 = R^2 = Ac$, $R^3 = H$
(23) $R^1 = R^2 = R^3 = Ac$
(24) $R^1 = R^2 = Ac$, $R^3 = O_2S{\cdot}C_6H_4Me\text{-}p$
(25) $R^1 = R^3 = H$, $R^2 = CH_2Ph$
(26) $R^1 = R^3 = CO{\cdot}[CH_2]_{16}Me$, $R^2 = CH_2Ph$
(27) $R^1 = R^3 = CO{\cdot}[CH_2]_{16}Me$, $R^2 = H$
(28) $R^1 = R^2 = CH_2Ph$, $R^3 = H$
(29) $R^1 = R^2 = CH_2Ph$, $R^3 = CO{\cdot}[CH_2]_{14}Me$
(30) $R^1 = R^2 = H$, $R^3 = CO{\cdot}[CH_2]_{14}Me$





(31) $R^1 = R^2 = Ac$
(32) $R^1 = R^2 = H$
(33) $R^1 = CPh_3$, $R^2 = H$
(34) $R^1 = CPh_3$, $R^2 = CH_2Ph$
(35) $R^1 = H$, $R^2 = CH_2Ph$
(36) $R^1 = R^2 = CH_2Ph$

(37) $R^1 = CH_2{\cdot}CH{:}\,CHMe$, $R^2 = CH_2Ph$
(38) $R^1 = H$, $R^2 = CH_2Ph$



(39) $R^1 = H$, $R^2 = CH_2Ph$
(40) $R^1 = R^2 = H$

has been described previously [2a,9] as a crystalline compound) as a syrup. Compound (22) was therefore converted into the di-*O*-tosyl derivative (24), which was treated with zinc and sodium iodide in refluxing acetone to give allyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside. This was deacetylated to give the known [26] crystalline

[22] B. Wickberg, *Acta Chem. Scand.*, 1958, **12**, 1187.
[23] N. K. Kochetkov, O. S. Chizhov, and A. F. Bochkov, in 'MTP International Review of Science, Organic Chemistry Series One,' ed. D. H. Hey, vol. 7, ed. G. O. Aspinall, Butterworths, London, 1973, p. 147.
[24] H. F. G. Beving, H. B. Boren, and P. J. Garegg, *Acta Chem. Scand.*, 1967, **21**, 2083.
[25] J. Cunningham, R. Gigg, and C. D. Warren, *Tetrahedron Letters*, 1964, 1191.
[26] R. Gigg and C. D. Warren, *J. Chem. Soc.*, 1965, 2205.

allyl β-D-galactopyranoside, thus confirming the configuration of the glycosidic linkage in compound (22). The hexa-acetate (23) was also hydrolysed to give 3-*O*-(β-D-galactopyranosyl)-L-glycerol with an optical rotation similar to that reported.[9,22,27]

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glycerol (22) was converted into the isopropylidene derivative (31) by the action of 2,2-dimethoxypropane in the presence of an acid catalyst. Basic hydrolysis of the acetate (31) gave 1,2-*O*-isopropylidene-3-*O*-(β-D-galactopyranosyl)-L-glycerol (32), which was converted into the trityl ether (33). Compound (33) was benzylated to give 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-galactopyranosyl)-L-glycerol (34), which on acidic hydrolysis gave the crystalline 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (25). The 220 MHz ^{1}H n.m.r. spectrum of this compound showed a doublet at τ 5·65 ($J_{1,2}$ 7·5 Hz) for the anomeric proton, indicating [28] a β-glycosidic linkage.

In an attempt to make the α-anomer (39) of compound (25), the 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride (18) was condensed with 1,2-*O*-isopropylidene-L-glycerol (1) under the conditions which we have described [11] for 1,2-*cis*-glycoside synthesis. The glycoside fraction was treated with potassium t-butoxide in dimethyl sulphoxide to remove [18] the but-2-enyl group and was subsequently hydrolysed with acid to remove the isopropylidene group to give crude 3-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-L-glycerol (39) as a syrup. We have found that this reaction, which is stereoselective for α-glycoside formation, usually gives *ca.* 10% of β-glycoside in the product.[11] The 220 MHz ^{1}H n.m.r. spectrum of the crude product showed three doublets in the anomeric proton region. One of these corresponded to the doublet obtained for the β-anomer (25) and represented about 15% of the total. The other doublets, at τ 5·94 and 5·99, both had $J_{1,2}$ 3·5 Hz, indicating [28] α-anomers. We postulate that these represent the anomeric protons of 3-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-L-glycerol (39) and of the corresponding 1-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-L-glycerol which would be obtained if the 1,2-*O*-isopropylidene-L-glycerol had been racemised in the reaction mixture. Migrations of the isopropylidene groups in 1,2-*O*-isopropylidene-L-glycerol (1) [8,22] and in other compounds [23] have been observed previously during Koenigs–Knorr glycosidation reactions and it appears that this is also happening under the conditions of our [11] glycosidation reaction. This could be confirmed by an unambiguous synthesis of compound (39) by condensation of the galactosyl chloride (18) with 1,2-di-*O*-(2-methylallyl)-L-glycerol (6) or with 1,2-di-*O*-allyl-L-glycerol.

The crude product containing 3-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-L-glycerol (39) was hydrogenolysed to remove the benzyl groups and the product was crystallised from ethanol–methanol to give the known [22] 3-*O*-(α-D-galactopyranosyl)-L-glycerol (40) in 46% yield.

[27] (*a*) T. J. Shilhavy and W. Boos, *J. Biol. Chem.*, 1973, **248**, 6571; (*b*) H. E. Carter, R. H. McCluer, and E. D. Slifer, *J. Amer. Chem. Soc.*, 1956, **78**, 3735.

3-*O*-(2,3,4-Tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (25) was acylated with octadecanoyl chloride in pyridine and the product (26) was hydrogenolysed to give 3-*O*-(6-*O*-stearoyl-β-D-galactopyranosyl)-1,2-di-*O*-stearoyl-L-glycerol (27) with properties similar to those reported for the same material prepared [4b] by partial synthesis from natural material.

Acetonation of 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (25) by the action of acid in acetone gave the isopropylidene derivative (35), which was benzylated to give 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-L-glycerol (36). The isopropylidene group was removed by acidic hydrolysis and the diol (28) obtained was acylated with hexadecanoyl chloride in pyridine to give the ester (29). Hydrogenolysis of compound (29) gave 1,2-di-*O*-palmitoyl-3-*O*-(β-D-galactopyranosyl)-L-glycerol (30) with properties similar to those reported for the same material prepared by synthesis [9] (orthoester method) and by partial synthesis [10] from natural material.

Since compound (25) is protected by benzyl groups, which must be removed by hydrogenolysis, the direct preparation of galactosyl diglycerides containing unsaturated fatty acids such as occur in the natural plant lipids is not possible. However compound (25) can be converted into derivatives identical with those obtained in the work on partial synthesis [4b,10] and this will allow the introduction of unsaturated fatty acids into the synthetic galactosyl diglycerides. The hydrogenation of the unsaturated polygalactosyl diglycerides obtained from plant sources gives [3c] stable products with no decreased activity in serological tests and thus synthetic saturated polygalactosyl diglycerides will be suitable for serological work involving the antibodies directed against *Mycoplasma pneumoniae* glycolipid antigens which appear [3c] to be more saturated than the related plant glycolipids.

EXPERIMENTAL

Solvents were evaporated off under reduced pressure. Optical rotations were measured at 22—25° with a Bendix Automatic Polarimeter. T.l.c. was carried out on microscope slides coated with silica gel G. Light petroleum had b.p. 40—60° unless otherwise stated. Potassium t-butoxide was obtained from Courtorch Chemicals Ltd., Esgairgynddu, Carmarthenshire.

3-O-*Allyl*-1,2-*Bis*-O-p-*nitrobenzoyl*-L-*glycerol*.— 1,2-*O*-Isopropylidene-L-glycerol [14] (9·7 g) was converted into 3-*O*-allyl-1,2-*O*-isopropylidene-L-glycerol (2) (13·3 g) as described [15] for the racemic compound. The crude product was treated with methanol (190 ml) and N-sulphuric acid (10 ml) at reflux for 15 min.; t.l.c. (toluene–acetone, 2 : 1) then showed complete conversion of the isopropylidene derivative (2) (R_F 0·8) into a single product (R_F 0·3). The mixture was cooled and neutralised with an excess of barium carbonate, filtered, and evaporated to give crude 3-*O*-allyl-L-glycerol (3) (8·8 g). A portion of this was treated with *p*-nitrobenzoyl chloride in pyridine to give 3-O-*allyl*-1,2-*di*-O-p-*nitrobenzoyl*-L-*glycerol*, m.p. 84·5—86·5° (from ethanol),

[28] C. G. Hellerqvist, O. Larm, and B. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 743; J. P. Kamerling, M. J. A. de Bie, and J. F. G. Vliegenthart, *Tetrahedron*, 1972, **28**, 3037.

$[\alpha]_D$ +42° (*c* 1 in $CHCl_3$) (Found: C, 55·5; H, 4·5; N, 6·2. $C_{20}H_{18}N_2O_9$ requires C, 55·8; H, 4·2; N, 6·5%).

1,2-*Bis*-O-(2-*methylallyl*)-L-*glycerol* (6).—Crude 3-*O*-allyl-L-glycerol (3) (23 g) was converted into 3-*O*-(prop-1-enyl)-L-glycerol (4) as described [15] for the racemic compound. The product was treated with 2-methylallyl chloride (40 ml) and sodium hydride (10 g) in tetrahydrofuran at reflux for 4 h; t.l.c. (ether–light petroleum, 1 : 2) then showed complete conversion of compound (4) (R_F 0) into a single product (5) (R_F 0·8). The product (27·5 g) was isolated in the usual way and treated with methanol (200 ml) and 0·1N-hydochloric acid at reflux for 30 min; t.l.c. (as above) then showed conversion of the starting material (5) into the product (6) (R_F 0·1). An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. The product (23 g) was extracted with chloroform and chromatographed on alumina. After elution of non-polar impurities with ether–light petroleum (1 : 1) the pure *product* (6) (12 g) was eluted with ether. For analysis a portion was distilled; b.p. 102—106° at 2 mmHg, α_D −6·8° (pure liquid) (Found: C, 65·5; H, 10·3. $C_{11}H_{20}O_3$ requires C, 66·0; H, 10·1%).

For characterisation, a portion of the product was converted into the trityl ether (7) in the usual way. Compound (7) (contaminated with some triphenylmethanol) was treated with potassium t-butoxide in dimethyl sulphoxide at 60° to isomerise [13] the 2-methylallyl groups and the course of the reaction was followed by t.l.c. (ether–light petroleum, 1 : 9), which showed conversion of the trityl ether (7) (R_F 0·45) into a major product (R_F 0·75). The product was isolated in the usual way [13] and chromatographed on alumina. Elution with ether–light petroleum (1 : 2) gave 1,2-bis-*O*-(2-methylprop-1-enyl)-3-*O*-trityl-L-glycerol (8). Compound (8) (1 g) was treated with mercury(II) chloride and mercury(II) oxide in acetone–water (9 : 1) [16b] to remove the 2-methylprop-1-enyl groups and the crude product was chromatographed on alumina. Elution with ether–methanol (19 : 1) removed some less polar impurity (probably formed by cyclisation of a mono 2-methylprop-1-enyl ether of 3-*O*-trityl-L-glycerol by the action of mercury chloride as described previously [16b] for the removal of a monoprop-1-enyl ether of a glycol) and elution with methanol–water (4 : 1) gave 3-*O*-trityl-L-glycerol (0·3 g, 40%), m.p. 98—99° [from ethyl acetate–light petroleum (b.p. 60—80°)], $[\alpha]_D$ +16·6° (*c* 1 in pyridine) {lit.,[17] m.p. 97—99°, $[\alpha]_D^{23}$ +15° (*c* 8 in pyridine)}.

1,2-*Di*-O-*benzyl*-L-*glycerol* (12).[24]—Crude 3-*O*-allyl-L-glycerol (3) (8·8 g) was treated with benzyl chloride (20 ml) and sodium hydride (5 g) in tetrahydrofuran at reflux for 6 h; t.l.c. (ether–light petroleum, 1 : 2) then showed complete conversion of compound (3) (R_F 0) into a single product (R_F 0·6). The product (10) was isolated in the usual way and treated with potassium t-butoxide in dimethyl sulphoxide [16a] at 60° until t.l.c. (as above) indicated complete conversion of the allyl ether (10) into the prop-1-enyl ether (11) (R_F 0·8). The product was isolated in the usual way [16a] and treated with mercury(II) chloride and mercury(II) oxide to remove [16b] the prop-1-enyl group. The product was isolated in the usual way [16b] and chromatographed on alumina. After elution of some non-polar impurities (from the benzylation reaction) with ether–light petroleum (2 : 1), the 1,2-di-*O*-benzyl-L-glycerol (12) (13·4 g) was eluted with ether–methanol (49 : 1) and obtained as a syrup. For characterisation it was converted into the trityl ether (13), m.p. 84—85·5° (from ethanol), $[\alpha]_D$ +8·0° (*c* 1 in $CHCl_3$) {lit.,[24] m.p. 84—84·5°, $[\alpha]_D$ +9·2° (*c* 1 in $CHCl_3$)}.

2,3,4-*Tri*-O-*benzyl*-6-O-(*but*-2-*enyl*)-1-O-p-*nitrobenzoyl*-β-D-*galactopyranoside*.[11a]—Crude allyl 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranoside [11a] (14) (10 g) was treated with an excess of potassium t-butoxide in dimethyl sulphoxide at 50° and the reaction was followed by t.l.c. (ether–light petroleum, 1 : 1). The but-2-enyl group was rapidly removed [18] from the starting material (R_F 0·8) to give allyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (R_F 0·3) and this was more slowly isomerised [16] to prop-1-enyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (15) (R_F 0·4). When the isomerisation was complete the product (9·7 g) was isolated in the usual way and chromatographed on alumina. Ether–light petroleum (2 : 1) first eluted non-polar contaminants and then the pure prop-1-enyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (15) (6 g). Compound (15) was treated with an excess of ' crotyl bromide ' and sodium hydride [18] in benzene at reflux until t.l.c. (as above) showed complete conversion of compound (15) into the but-2-enyl ether (16) (R_F 0·9). The product was isolated in the usual way and treated with mercury(II) chloride to remove [16b] the prop-1-enyl group and give 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-D-galactopyranose [11a] (17) as a syrup. This was converted into a crystalline *p*-nitrobenzoate identical with the material described previously.[11a]

Allyl β-D-*Galactopyranoside*.[26]—Mercury(II) cyanide (3 g) was added to a solution of 1,2-di-*O*-benzyl-L-glycerol (1·5 g) (12) and 2,3,4,6-tetra-*O*-acetylgalactopyranosyl bromide (4·5 g) in dry benzene–nitromethane (1 : 1; 50 ml) and the mixture was stirred at 40° for 12 h; t.l.c. (ether–light petroleum, 2 : 1) then showed complete conversion of the dibenzyl ether (12) (R_F 0·4) into a major product (R_F 0·3). The mixture was diluted with benzene, washed with saturated aqueous sodium hydrogen carbonate and water, and dried ($MgSO_4$). The crude product (5·2 g) was chromatographed on neutral alumina; elution with ether–methanol (199 : 1) gave 1,2-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glycerol (21) (3·3 g) as a syrup. Compound (21) (1·7 g), in methanol (20 ml), was treated with hydrogen in the presence of palladium–charcoal (pre-treated with glacial acetic acid and washed with methanol) until t.l.c. (chloroform–methanol, 9 : 1) indicated complete conversion of compound (21) (R_F 1) into a major product (R_F 0·6). This was isolated in the usual way to give the crude 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glycerol (22) (1·2 g) as a syrup which was chromatographed on neutral alumina. Elution with ether–methanol (32 : 1) removed some contaminants; elution with ether–methanol (4 : 1) gave the pure compound (22) as a syrup (0·8 g). A portion of compound (22) was acetylated with acetic anhydride in pyridine to give the hexa-acetate (23) [2a,9] as a syrup which could not be induced to crystallise. Compound (22) (0·2 g) was deacetylated with sodium methoxide in methanol to give 3-*O*-(β-D-galactopyranosyl)-L-glycerol (100 mg) as a syrup, $[\alpha]_D$ −3·7° (*c* 5·1 in H_2O) {lit.,[22] $[\alpha]_D$ −7° (*c* 2 in H_2O); lit.,[27a] $[\alpha]_D$ −4·8° (*c* 10 in H_2O); lit.,[27b] $[\alpha]_D^{27}$ +3·77°; lit.,[9] $[\alpha]_D^{20}$ −7·9° (*c* 0·5 in H_2O)}. A portion of the 3-*O*-(β-D-galactopyranosyl)-L-glycerol was hydrolysed with N-hydrochloric acid at 100° for 1 h. Paper chromatography (Whatman No. 3; butanol–acetic acid–water, 4 : 1 : 5) showed galactose (R_F 0·22) and glycerol (R_F 0·47) running concurrently with authentic standards (detected by spraying with aqueous 0·5% sodium periodate followed by 1% potassium permanganate in aqueous 2% sodium carbonate).

Pure 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-

glycerol (22) (0·3 g) was converted into the ditosylate (24) in the usual way. A solution of the tosylate (24) (0·5 g) in dry acetone (20 ml) containing sodium iodide (0·85 g) and zinc dust (0·66 g) was refluxed under nitrogen for 18 h; t.l.c. (toluene–acetone, 9 : 1) then showed complete conversion of the tosylate (24) (R_F 0·3) into a single product (R_F 0·4). The mixture was filtered and the solvent was evaporated off; the residue was extracted with chloroform and the solution was washed with water and dried ($MgSO_4$). The crude product (0·26 g) was deacetylated with sodium methoxide in methanol and the crude product (0·15 g) was recrystallised from ethyl acetate to give allyl β-D-galactopyranoside (44 mg), m.p. 105—106°, $[\alpha]_D$ −24·1° (*c* 1 in methanol) {lit.,[26] m.p. 103—104°, $[\alpha]_D$ −20° (*c* 1 in methanol)}.

3-O-(2,3,4-*Tri*-O-*benzyl*-β-D-*galactopyranosyl*)-L-*glycerol* (25).— 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-L-glycerol (22) (1·2 g), 2,2-dimethoxypropane (5 ml) and toluene-*p*-sulphonic acid (50 mg) in dry acetone (50 ml) were kept at 20° for 1 h; t.l.c. (chloroform–methanol, (9 : 1) then showed complete conversion of compound (22) (R_F 0·6) into the isopropylidene derivative (31) (R_F 0·9). An excess of sodium hydrogen carbonate was added, the solvents were evaporated off, and the product was extracted with chloroform. Compound (31) was deacetylated with sodium methoxide in methanol to give 1,2-*O*-isopropylidene-3-*O*-(β-D-galactopyranosyl)-L-glycerol (32) (0·97 g) as a syrup (R_F 0·1 in chloroform–methanol, 9 : 1) and this was converted into the trityl ether (33) (R_F 0·4). The trityl ether (33) was treated with an excess of benzyl chloride and sodium hydride in dimethylformamide at 50°; t.l.c. (toluene–acetone, 4 : 1) showed conversion into the tribenzyl ether (34) (R_F 0·8), which was isolated in the usual way and chromatographed on alumina. Elution with ether–light petroleum (1 : 2) removed some non-polar contaminants; elution with ether–light petroleum (2 : 1) gave 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-galactopyranosyl)-L-glycerol (34) (0·93 g) as a syrup. Compound (34) in dry acetone (10 ml) containing toluene-*p*-sulphonic acid (10 mg) was heated under reflux for 10 min; t.l.c. (toluene–acetone, 2 : 1) then showed a trace of starting material (34) (R_F 0·8) and a major product (R_F 0·5). An excess of sodium hydrogen carbonate was added and the solvent was evaporated off. The crude product (1 g) was extracted with chloroform and was chromatographed on neutral silica gel (B.D.H. 60—120 mesh). Elution with toluene–acetone (19 : 1) removed the triphenylmethanol; elution with toluene–acetone (5 : 1) gave the pure 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (35) as a syrup. Compound (35) in N-hydrochloric acid–methanol (1 : 9; 5 ml) was heated under reflux for 10 min; t.l.c. (toluene–acetone, 2 : 1) then showed complete conversion of compound (35) (R_F 0·5) into a single product (R_F 0·1). An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. The residue was extracted with chloroform and the product (0·35 g) was recrystallised from ethyl acetate–light petroleum (b.p. 60—80°) to give 3-O-(2,3,4-*tri*-O-*benzyl*-β-D-*galactopyranosyl*)-L-*glycerol* (25) (0·26 g), m.p. 131—133°, $[\alpha]_D$ −13·6° (*c* 0·9 in $CHCl_3$) (Found: C, 68·6; H, 7·2. $C_{30}H_{36}O_8$ requires C, 68·7; H, 6·9%), τ (220 MHz; P.C.M.U., Harwell) 2·7 (15H, 3 Ph), 5—5·5 (6H, 3 PhCH_2), 5·65 (1H, anomeric, d, $J_{1,2}$ 7·5 Hz), 6·1—6·7 (11H, carbohydrate and glycerol CH), and 6·9 (3H, 3 OH).

1,2-*Di*-O-*stearoyl*-3-O-(6-O-*stearoyl*-β-D-*galactopyranosyl*)-L-*glycerol* (27).—3-*O*-(2,3,4-Tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (25) (200 mg) in dry pyridine (5 ml) and dry dichloromethane (5 ml) was treated with stearoyl chloride (0·8 ml), and the solution was kept at 20° for 12 h. Water (0·2 ml) was added and the solution was kept at 20° for 1 h. The product (26) (contaminated with stearic acid) was isolated in the usual way and chromatographed on neutral alumina. Elution with ether–toluene (1 : 1) gave the pure stearoyl ester (26) (330 mg), which was dissolved in ethyl acetate–methanol (3 : 1) and treated with hydrogen over palladium–charcoal; t.l.c. (chloroform–methanol, 9 : 1) then showed a single product (R_F 0·6). The product was isolated in the usual way and recrystallised from ethyl acetate–light petroleum (b.p. 60—80°) to give compound (27), m.p. 80—81° (with a transition at 73°), $[\alpha]_D$ −1·4° (*c* 1 in $CHCl_3$) {lit.,[4b] m.p. 79° (transition 70—72°), $[\alpha]_D^{25}$ −0·97° (*c* 5·69 in $CHCl_3$)}.

1,2-*Di*-O-*palmitoyl*-3-O-(β-D-*galactopyranosyl*)-L-*glycerol* (30).—The crystalline compound (25) (157 mg) and toluene-*p*-sulphonic acid (5 mg) were stirred in dry acetone (10 ml) at 20° for 1·5 h; t.l.c. (toluene–acetone, 2 : 1) then showed complete conversion of compound (25) (R_F 0·1) into 1,2-*O*-isopropylidene-3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (35) (R_F 0·5). The product was isolated in the usual way and treated with an excess of benzyl chloride and sodium hydride in benzene at reflux for 1 h; t.l.c. (as above) then showed complete conversion of compound (35) into a single product (R_F 0·95). The product was isolated in the usual way and chromatographed on alumina. Elution with ether–light petroleum (2 : 1) removed non-polar impurities; elution with ether gave 1,2-*O*-isopropylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (36) (120 mg) as a syrup. Compound (36) was treated with N-hydrochloric acid–dioxan (1 : 9; 5 ml) at 70° for 10 min; t.l.c. (as above) then showed a single product (R_F 0·3), which was isolated in the usual way to give 3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (28) (113 mg) as a syrup. Compound (28) was treated with palmitoyl chloride in pyridine and the crude product (containing palmitic acid) was chromatographed on neutral alumina. Elution with ether–toluene (1 : 1) gave 1,2-di-*O*-palmitoyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-L-glycerol (29) (150 mg). Compound (29) (73 mg) in ethyl acetate–methanol (1 : 1; 10 ml) was treated with hydrogen over palladium–charcoal and the crude product (27 mg) was recrystallised from methanol to give 1,2-di-*O*-palmitoyl-3-*O*-(β-D-galactopyranosyl)-L-glycerol (30), m.p. 149—150°, $[\alpha]_D$ −4·0° (*c* 0·5 in $CHCl_3$) {lit.,[9] m.p. 141—142°, $[\alpha]_D^{20}$ −6·53° (*c* 0·7 in $CHCl_3$); lit.,[10] m.p. 152—154°, $[\alpha]_D^{21}$ −2·04° (*c* 4·7 in pyridine)}.

3-O-(α-D-*Galactopyranosyl*)-L-*glycerol* [22] (40).—2,3,4-Tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride (18) (1·17 g), 1,2-*O*-isopropylidene-L-glycerol (1) (0·59 g), dry tetraethylammonium chloride (0·38 g), dry triethylamine (0·62 ml), and dry dichloromethane (2·4 ml) were sealed in an ampoule under vacuum and kept at 80° for 16 h; t.l.c. (ether–light petroleum, 1 : 1) then showed the absence of chloride (18) (R_F 0·9) and the presence of a major product (R_F 0·6), 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-D-galactopyranose (17) (R_F 0·4), and 1,2-*O*-isopropylidene-L-glycerol (R_F 0·2). The mixture was diluted with dichloromethane and washed with sodium hydrogen carbonate solution and water and dried ($MgSO_4$). The crude product (1·38 g) was chromatographed on alumina. Elution with ether gave the glycoside fraction (0·96 g), which was treated with potassium t-butoxide (1 g) in dry dimethyl sulphoxide (20 ml) at 40° for 3 h; t.l.c. (ether–light petroleum, 2 : 1) then showed the

absence of starting material (R_F 0·8) and the presence of a single product (R_F 0·1), which was isolated in the usual way. The product (0·8 g) in N-hydrochloric acid–dioxan (1 : 9; 10 ml) was kept at 60° for 10 min; t.l.c. (toluene–acetone, 2 : 1) showed complete conversion of the starting material (R_F 0·6) into a single product (R_F 0·25). An excess of sodium hydrogen carbonate was added and the solvents were evaporated off. The crude product was extracted with chloroform and obtained as a syrup (0·52 g). The 220 MHz ^{1}H n.m.r. spectrum (P.C.M.U., Harwell) showed three doublets in the anomeric proton region: (i) τ 5·65 ($J_{1,2}$ 7·5 Hz) (*ca.* 15% of the total) corresponding to the doublet for the anomeric proton of the β-glycoside (25), (ii) τ 5·94 ($J_{1,2}$ 3·5 Hz) (*ca.* 30%), and (iii) τ 5·99 ($J_{1,2}$ 3·5 Hz) (*ca.* 55%). The other resonances were similar to those described for the β-glycoside (25). A portion (0·3 g) of the product, in methanol, was treated with hydrogen over palladium–charcoal to give a crude product (0·14 g), which was recrystallised from ethanol–methanol to give pure 3-*O*-(α-D-galactopyranosyl)-L-glycerol (70 mg, 46%), m.p. 150—151·5°, $[\alpha]_D$ +147·8° (*c* 0·5 in H_2O) {lit.,[22] m.p. 150—152° $[\alpha]_D^{20}$ +155° (*c* 1·8 in H_2O)}.

We thank Mr. R. Conant and Mr. N. Schunmann for technical assistance.

[4/2069 *Received*, 7th *October*, 1974]