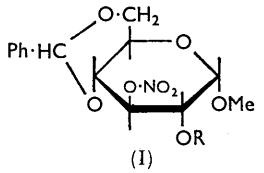
749. *Phosphorylated Sugars. Part II.*[1] *The Synthesis of Some Methyl Glycoside Phosphates.*

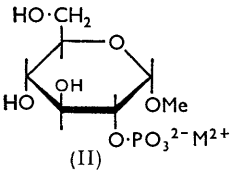
By (Mrs.) Patricia Szabó and L. Szabó.

Syntheses of the four isomeric monophosphates of methyl α-D-glucoside, and of methyl β-D-galactoside 4- and 6-monophosphate, are described. Cyclic phosphates of methyl β-D-galactoside and of methyl 2,3-di-*O*-benzyl-α-D-glucoside have been prepared. An unambiguous synthesis of methyl α-D-glucoside 4,6-(hydrogen phosphate) is given.

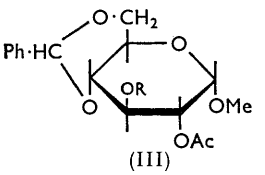
It was shown in the preceding paper that alkaline hydrolysis of methyl α-D-glucoside 4,6-(hydrogen phosphate) [2] gave a ~4 : 1 mixture of methyl α-D-glucoside 4- and 6-phosphate, and that an analogous mixture of methyl β-D-galactoside phosphates was formed similarly from methyl β-D-galactoside 4,6-(hydrogen phosphate). The chromatographic analyses of the hydrolysates required samples of methyl glucoside 2-, 3-, 4-, and 6-phosphate and of methyl β-D-galactoside 4- and 6-phosphate.



(I)



(II)



(III)

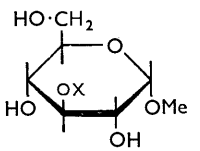
In general, the methyl glycoside monophosphates were prepared by phosphorylation of suitably substituted methyl glycosides by diphenyl phosphorochloridate in the presence of pyridine and subsequent removal of the phenyl groups (usually by hydrogenolysis) and then of the protecting groups. The phosphates were isolated as crystalline cyclohexylammonium salts.

Preparation of methyl α-D-glucoside 2-phosphate (II) started from methyl 4,6-*O*-benzylidene-α-D-glucoside 3-nitrate (I; R = H) whose preparation by Honeyman and Morgan's method [3] was improved so as to give a 90% yield. This nitrate, on phosphorylation, gave a crystalline ester [I; R = $(PhO)_2PO$] whence the phenyl and nitrate groups were removed by hydrogenolysis and the benzylidene group then by acid-hydrolysis, giving methyl α-D-glucoside 2-phosphate (II). As phosphate groups may undergo acid-catalysed migration [4] it was necessary to prove that the final acid-treatment did not cause phosphoryl migration. The phosphate produced consumed one equivalent of periodate, and so could only be a methyl glucopyranoside substituted in position 2 or 4.

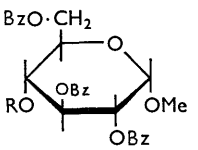
[1] Part I, preceding paper.
[2] Baddiley, Buchanan, and Szabó, *J.*, 1954, 3826.
[3] Honeyman and Morgan, *J.*, 1955, 3660.
[4] Levene and Raymond, *J. Biol. Chem.*, 1934, **107**, 75; Chargaff, *ibid.*, 1942, **144**, 455; Baer and Kates, *ibid.*, 1950, **185**, 615.

As the methyl α-D-glucoside 4-phosphate has been prepared by unequivocal methods (see below) and differs in melting point, rotation, and elution position during ion-exchange chromatography [1] from the 2-phosphate described here, no phosphate migration could have occurred and the assignment of the structure must be correct.

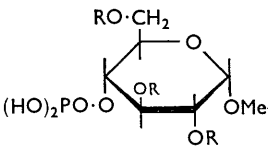
Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucoside (III; R = H) was the starting material for synthesis of methyl α-D-glucoside 3-phosphate (IV). The former had already been prepared by two different methods [5,6] neither of which is suitable for large-scale preparation. It has now been prepared by hydrogenation of the easily accessible nitrate [3] (III; R = NO_2). Phosphorylation with diphenyl phosphorochloridate gave the oily 3-(diphenyl phosphate) [III; R = $(PhO)_2PO$], from which hydrogenation removed the phenyl groups and successive alkaline and acid hydrolyses the protecting acetyl and benzylidene groups, giving the expected methyl α-D-glucopyranoside 3-phosphate. This product was unaffected by periodate even on prolonged contact, which proved that no phosphoryl migration occurred—the only structure for a methyl glucoside phosphate compatible with this behaviour is that where the 3-hydroxyl group is esterified. In addition the elution position of this compound during ion-exchange chromatography distinguishes it from the other three isomeric phosphates.[1] Harvey, Michalski, and Todd [7] obtained a mixture of methyl α-D-glucoside 3-phosphate and methyl α-D-altropyranoside 2-phosphate by treatment of methyl 2,3-anhydro-4,6-*O*-benzylidene α-D-alloside with dibenzyl hydrogen phosphate, but they were unable to isolate pure 3-phosphate.



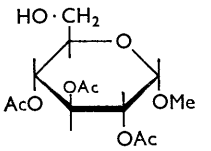
(IV) X = $PO_3^{2-}M^{2+}$



(V)



(VI)



(VII)

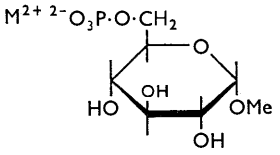
For the synthesis of methyl α-D-glucoside 4-phosphate (VI; R = H) methyl 2,3,6-tri-*O*-benzoyl-α-D-glucoside [8] (V; R = H) was phosphorylated to give the crystalline 4-diphenyl phosphate [V; R = $(PhO)_2PO$], from which the phenyl groups were removed by hydrogenation in presence of Adams catalyst, the benzoyl groups being perhydrogenated simultaneously. The crystalline cyclohexylammonium salt of methyl α-D-glucoside 2,3,6-trishexahydrobenzate 4-phosphate (VI; R = C_6H_{11}·CO) was isolated. The protecting groups were then removed by alkaline hydrolysis and methyl glucoside 4-phosphate (VI; R = H) was isolated. As monoesters of phosphoric acid are stable under mild alkaline conditions this synthesis should yield the 4-phosphate exclusively. As expected, the compound consumed one equivalent of periodate and differed in its elution position from all the other methyl α-D-glucoside phosphates.

Methyl α-D-glucoside 6-phosphate was synthesised by phosphorylation of the known [9] methyl 2,3,4,-tri-*O*-acetyl-α-D-glucoside (VII). The oily diphenyl phosphate produced was hydrogenated and then deacetylated by means of a base, and the resulting methyl α-D-glucoside 6-phosphate (VIII) was isolated. This compound consumed two equivalents of periodate in accordance with the structure proposed.

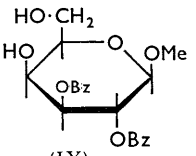
Several syntheses of methyl α-D-glucoside 6-phosphate have been described [10] but none is unequivocal inasmuch as all involve acid-treatment of a phosphate containing free hydroxyl groups. To our knowledge this is the first unequivocal synthesis of this compound.

[5] Bourne, Stacey, Tatlow, and Tatlow, *J.*, 1951, 826.
[6] Jeanloz and Jeanloz, *J. Amer. Chem. Soc.*, 1957, **79**, 2579.
[7] Harvey, Michalski, and Todd, *J.*, 1951, 2271.
[8] Bell, *J.*, 1934, 1177.
[9] Helferich, Bredereck and Schneidmüller, *Annalen*, 1927, **458**, 111.
[10] Fischer, *Ber.*, 1914, **47**, 3193; Helferich and du Mont, *Z. physiol. Chem.*, 1929, **181**, 300; Percival and Percival, *J.*, 1945, 874.

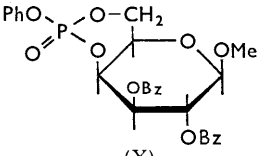
The syntheses of methyl β-D-galactoside 4- and 6-phosphate are analogous to those of the corresponding methyl α-D-glucoside phosphates and are therefore unequivocal. Incidentally we isolated crystalline methyl 2,3,4-tri-*O*-benzoyl-β-D-galactoside which previously had only been obtained as an oil.[11] Our tribenzoate gave a 6-toluene-*p*-sulphonate having the same melting point as that obtained by Müller from his oily



(VIII)



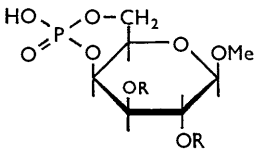
(IX)



(X)

tribenzoate. As expected the methyl galactoside 4-phosphate consumed one equivalent of periodate, and the 6-phosphate two equivalents.

The cyclic phosphates were synthesised by using phenyl phosphorodichloridate. When methyl 2,3-di-*O*-benzoyl-β-D-galactoside [12] (IX) was treated with this reagent in anhydrous pyridine the crystalline methyl 2,3-di-*O*-benzoyl-β-D-galactoside 4,6-(phenyl phosphate) (X) could be isolated. After hydrogenation which removed the phenyl group, the crystalline cyclohexylammonium salt of methyl 2,3-di-*O*-hexahydrobenzoyl-β-D-galactoside (XI; R = C_6H_{11}·CO) was obtained in good yield. The protecting groups were then removed by alkaline treatment and the methyl β-D-galactoside 4,6-(hydrogen phosphate) (XI; R = H) was isolated. This cyclic phosphate consumed the calculated amount of periodate, and electrometric titration showed the absence of secondary phosphoryl dissociation.



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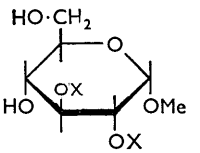
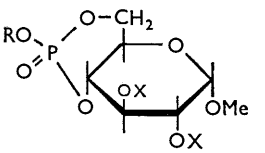


(XII) X = CH_2Ph



(XIII) X = CH_2Ph

The crystalline methyl 2,3-di-*O*-benzyl-α-D-glucoside 4,6-(hydrogen phosphate) (XIII; R = H) was obtained by phosphorylation of methyl 2,3-di-*O*-benzyl-α-D-glucoside [13] (XII) and treatment of the resulting crystalline phenyl ester (XIII; R = Ph) with three equivalents of potassium hydroxide [14] to remove the phenyl group.

This cyclic phosphate incidentally provided a new route for the synthesis of methyl α-D-glucoside 4,6-(hydrogen phosphate), the benzyl groups being easily removed by hydrogenation with a palladium catalyst. The compound prepared by this method was identical with that obtained by direct phosphorylation of methyl α-D-glucoside.[2]

Experimental

Methyl 4,6-O-*Benzylidene*-α-D-*glucoside* 2,3-*Dinitrate*.—A mixture of fuming nitric acid (16 ml.) and acetic anhydride (40 ml.), prepared at −40°, was added very slowly to a suspension of methyl 4,6-*O*-benzylidene-α-D-glucoside [15] (20 g.) in acetic anhydride (40 ml.) at −10°, the

[11] Müller, *Ber.*, 1931, **64**, 1820.
[12] Bacon, Bell, and Kosterlitz, *J.*, 1939, 1248.
[13] Bell and Lorber, *J.*, 1940, 453.
[14] Moffatt and Khorana, *J. Amer. Chem. Soc.*, 1957, **79**, 1194.
[15] Freudenberg, Toepffer, and Anderson, *Ber.*, 1928, **61**, 1750.

temperature being kept at about 0° during the addition. The nitrated mixture was kept at 0° for a further 30 min., and diluted with chloroform (100 ml.) cooled to −10°. The chloroform solution was poured slowly on ice-cold aqueous potassium carbonate (300 g. in 1 l. of water) with stirring. When the chloroform layer was neutral, it was washed with water, dried (Na_2SO_4), and evaporated *in vacuo*. The residue, on crystallisation from ethanol, gave methyl 4,6-*O*-benzylidene-α-D-glucoside 2,3-dinitrate (24 g., 90%), m. p. 124—125° (lit.,[3] 124—125°).

Methyl 4,6-O-*Benzylidene*-α-D-*glucoside* 2-(*Diphenyl Phosphate*) 3-*Nitrate*.—Diphenyl phosphorochloridate (6·3 g.) in pyridine (10 ml.) was added to methyl 4,6-*O*-benzylidene-α-D-glucoside 3-nitrate [3] (7 g.) in pyridine (50 ml.). The solution was kept at 37° for 64 hr., then cooled, and water (10 ml.) was added. After 2 hr., the solvents were removed *in vacuo* below 40° and the residue was triturated with several batches of iced water until it crystallised. Recrystallisation from ethanol gave the *diphenyl phosphate* (10·1 g., 84%), m. p. 123—124°, $[\alpha]_D^{25}$ +56° (*c* 1·00 in dioxan) (Found: C, 56·1; H, 5·0; N, 2·4. $C_{26}H_{26}O_{11}NP$ requires C, 55·8; H, 4·7; N, 2·5%).

Methyl α-D-*Glucoside* 2-(*Dihydrogen Phosphate*).—The above diphenyl ester (2 g.) was shaken in ethanol (80 ml.) containing acetic acid (0·2 ml.) with hydrogen at room temperature and pressure in the presence of Adams platinum. When the hydrogen uptake had ceased, the catalyst was filtered off and sodium hydroxide solution added to give pH 7. The solvent was removed *in vacuo* and the residue dissolved in water. The solution was passed through a column of Dowex 50 (H^+ form), and the effluent heated in a boiling-water bath for 10 min., filtered, and neutralised with cyclohexylamine. The solvent was removed *in vacuo* and the residue dissolved in the minimum amount of water. The *dicyclohexylammonium salt* (0·4 g.) which crystallised on addition of acetone had m. p. 156° (decomp.), $[\alpha]_D^{23}$ +46° (*c* 1·00 in H_2O) (Found: C, 48·3; H, 8·8; N, 6·0; P, 6·6. $C_{19}H_{41}O_9N_2P$ requires C, 48·3; H, 8·7; N, 5·9; P, 6·6%)

Methyl 2-O-*Acetyl*-4,6-O-*benzylidene*-α-D-*glucoside*.—Methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucoside 3-nitrate [3] (2 g.) in ethanol (80 ml.) containing acetic acid (0·323 g.) was shaken with hydrogen at room temperature and pressure in the presence of palladium catalyst (from 0·5 g. of palladium oxide). When the hydrogen uptake had ceased, the catalyst was filtered off and the solution neutralised with lead carbonate. The solution was filtered and the solvents were removed *in vacuo*. The residue was dissolved in ether. On addition of hexane, the methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucoside (1·2 g.) crystallised, having m. p. 133—134°, $[\alpha]_D^{24}$ +108° (*c* 1·27 in $CHCl_3$). Bourne, Stacey, Tatlow, and Tatlow [5] and Jeanloz and Jeanloz,[6] gave m. p. 133—134°, $[\alpha]_D^{19}$ +112° (*c* 0·86 in $CHCl_3$) and m. p. 133—134°, $[\alpha]_D^{26}$ +106° ± 2° (*c* 1·27 in $CHCl_3$) respectively.

Methyl α-D-*Glucoside* 3-(*Dihydrogen Phosphate*).—Diphenyl phosphorochloridate (2·98 g.) in pyridine (5 ml.) was added to methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-glucoside (3 g.) in pyridine (15 ml.). The solution was kept at 37° for 5 days, then cooled, and water (10 ml.) was added. After 2 hr., the solvents were removed *in vacuo* and the residue was dissolved in chloroform. The chloroform was washed first with iced water, then with ice-cold 1% sulphuric acid, and finally with water and dried (Na_2SO_4). The chloroform was removed *in vacuo* and the resulting syrup (4·9 g.) dissolved in acetic acid and shaken with hydrogen at room temperature and pressure in the presence of Adams platinum. When the hydrogen uptake had ceased, the catalyst was filtered off and the acetic acid removed *in vacuo* below 35°, the last traces being removed by lyophilisation. The residue was dissolved in anhydrous methanol, and the solution was saturated several times with dry ammonia gas. After 3 days, the ammonia and methanol were removed *in vacuo* and the residue was dissolved in water and passed through a column of Amberlite IR-120 (H^+ form). The effluent was heated for 10 min. on a boiling-water bath, cooled, and neutralised to pH 6 with cyclohexylamine. The solvents were removed *in vacuo* and the residue was dissolved in a little water. Addition of acetone gave the *dicyclohexylammonium salt* of *methyl* α-D-*glucoside* 3-*phosphate*, m. p. 162—166° (decomp.), $[\alpha]_D^{20}$ +70·8° (*c* 1·00 in H_2O) (from water–acetone) (Found: C, 48·3; H, 8·8; N, 6·1; P, 6·5. $C_{19}H_{41}O_9N_2P$ requires C, 48·3; H, 8·7; N, 5·9; P, 6·6%).

Methyl 2,3,6-*Tri*-O-*benzoyl*-α-D-*glucoside* 4-(*Diphenyl Phosphate*).—Diphenyl phosphorochloridate (2·3 g.) in pyridine (6 ml.) was added to methyl 2,3,6-tri-*O*-benzoyl-α-D-glucoside [8] (4 g.) in pyridine (20 ml.). The solution was kept at 37° for 48 hr., then cooled, and water (10 ml.) was added. After 1 hr., the solvents were removed *in vacuo* below 40° and the residue

was triturated with several lots of water until it crystallised. After recrystallisation from ethanol the *diphenyl phosphate* (4·5 g., 77%) had m. p. 158—159°, $[\alpha]_D^{26}$ +71·5° (*c* 1·00 in dioxan) (Found: C, 65·0; H, 4·9. $C_{40}H_{35}O_{12}P$ requires C, 65·0; H, 4·7%).

Methyl 2,3,6-*Tri*-O-*hexahydrobenzoyl*-α-D-*glucoside* 4-(*Dihydrogen Phosphate*).—The above diphenyl phosphate (10 g.) in acetic acid (200 ml.) was shaken with hydrogen at room temperature and pressure in the presence of Adams platinum. When the hydrogen uptake had ceased, the catalyst was filtered off and the acetic acid removed *in vacuo* below 30°, the last traces being removed by lyophilisation. The residue was dissolved in ethanol and cyclohexylamine added to pH 7 whereupon the *methyl* 2,3,6-*tri*-O-*hexahydrobenzoyl*-α-D-*glucoside* 4-*phosphate* (7·6 g., 80%) crystallised spontaneously as its *monocyclohexylammonium salt*, m. p. 205—207° (decomp.), $[\alpha]_D^{24}$ +62·5° (*c* 0·88 in MeOH) (Found: C, 58·0; H, 8·2; N, 1·8. $C_{34}H_{58}O_{12}NP$ requires C, 58·0; H, 8·2; N, 1·9%).

Methyl α-D-*Glucoside* 4-(*Dihydrogen Phosphate*).—The above hexahydrobenzoate (3·1 g.) was dissolved in anhydrous methanol and a sufficient quantity of a solution of sodium (1 g.) in methanol (100 ml.) to bring the pH to 12 was added. The solution was refluxed for 3 hr., cooled, and neutralised with carbon dioxide. The methanol was removed *in vacuo*, and the residue dissolved in water and passed slowly through a column of Dowex 50 (cyclohexylammonium form). The effluent was evaporated and the residue dissolved in ethanol. Addition of ether precipitated the crystalline *methyl* α-D-*glucoside* 4-(*dicyclohexylammonium phosphate*) (1·2 g.), m. p. 140—144° (decomp.), $[\alpha]_D^{23}$ +88·0° (*c* 1·00 in H_2O) (Found: C, 46·6; H, 9·0; N, 5·8. $C_{19}H_{41}O_9N_2P,H_2O$ requires C, 46·5; H, 8·8; N, 5·7%).

Methyl α-D-*Glucoside* 6-(*Dihydrogen Phosphate*).—Diphenyl phosphorochloridate (9·95 g.) in pyridine (36 ml.) was added to methyl 2,3,4-tri-*O*-acetyl-α-D-glucoside [9] (10·8 g.) in pyridine (108 ml.). The solution was kept at room temperature overnight and water (10 ml.) was added. After 1 hr., the solvents were removed *in vacuo* below 40° and the residue was dissolved in chloroform. The chloroform solution was washed twice with iced water, then with ice-cold 1% sulphuric acid until the aqueous phase remained acid, and three times with iced water and dried (Na_2SO_4). The chloroform was removed *in vacuo* and the residual syrup shaken in ethanol (200 ml.) with hydrogen at room temperature and pressure in the presence of Adams catalyst. When the hydrogen uptake had ceased, the catalyst was filtered off and the ethanolic solution saturated several times with dry ammonia gas. When a paper chromatogram showed that the removal of the acetyl groups was complete, the ammonia and ethanol were removed *in vacuo*, and the residue was dissolved in water and passed through a column of Dowex 50 (cyclohexylammonium form). The effluent was evaporated and the residue taken up in a little water. Addition of ethanol gave the *methyl* α-D-*glucoside* 6-(*dicyclohexylammonium phosphate*) (7·2 g.), m. p. 157—159° (decomp.), $[\alpha]_D^{25}$ +61° (*c* 1·00 in H_2O) (from water–ethanol) (Found: C, 48·3; H, 8·7; N, 6·1; P, 6·4. $C_{19}H_{41}O_9N_2P$ requires C, 48·3; H, 8·7; N, 5·8; P, 6·5%).

Methyl 2,3,6-*Tri*-O-*benzoyl*-β-D-*galactoside* 4-(*Diphenyl Phosphate*).—Diphenyl phosphorochloridate (4·5 g.) was added to methyl 2,3,6-tri-*O*-benzoyl-β-D-galactoside [12] (7·1 g.) in pyridine (100 ml.). The solution was kept for 5 days at 37°, then cooled, and water was added. After 1 hr., the solvents were removed *in vacuo* and the residue was triturated with several lots of iced water until it crystallised. After recrystallisation from ethanol, the *diphenyl phosphate* (6·0 g., 58%) had m. p. 126—128°, $[\alpha]_D^{24}$ +25·2° (*c* 1·00 in dioxan) (Found: C, 64·4; H, 4·7. $C_{40}H_{35}O_{12}P$ requires C, 65·0; H, 4·7%).

Methyl α-D-*Galactoside* 4-(*Dihydrogen Phosphate*).—The above diphenyl phosphate (2·0 g.) in acetic acid (40 ml.) was shaken with hydrogen at room temperature and pressure in the presence of Adams platinum. Treatment was as in the previous case but ion-exchange was on Dowex 50 (H^+ form). The effluent was neutralised with cyclohexylamine and evaporated to dryness. After recrystallisation from water–acetone, *methyl* β-D-*galactoside* 4-(*dicyclohexylammonium phosphate*) had m. p. 136—140° (decomp.), $[\alpha]_D^{20}$ −10·5° (*c* 1·00 in H_2O) (Found: C, 45·9; H, 9·0; N, 5·7; P, 6·4. $C_{19}H_{41}O_9N_2P,H_2O$ requires C, 46·5; H, 8·8; N, 5·7; P, 6·3%).

Methyl 2,3,4-*Tri*-O-*benzoyl*-β-D-*galactoside*.—Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-trityl-β-D-galactoside [11] (5 g.) was dissolved in hot acetic acid (15 ml.). The solution was cooled in salt–ice and a solution (1·8 g.) of hydrobromic acid in acetic acid (212 g. in 430 g.) added. The mixture was shaken for 1 min. and filtered quickly through a sintered-glass filter into iced water (100 ml.). The precipitate was washed several times with iced water and dissolved in chloroform. The chloroform solution was dried (Na_2SO_4) and the chloroform removed *in vacuo*. After recrystallisation from ether–light petroleum (b. p. 30—60°), the *tribenzoate* (2·4 g., 72%) had m. p. 152°,

$[\alpha]_D^{20}$ +170·2° (*c* 2·016 in EtOH) (Found: C, 66·1; H, 4·9; O, 28·8. $C_{28}H_{26}O_9$ requires C, 66·4; H, 5·1; O, 28·5%).

Methyl 2,3,4-*Tri*-O-*benzoyl*-β-D-*galactoside* 6-(*Diphenyl Phosphate*).—Diphenyl phosphorochloridate (3·25 g.) was added to the above tribenzoate (5·5 g.) in pyridine (60 ml.). The solution was kept at 37° for 18 hr., then cooled, and water (10 ml.) was added. After 2 hr., the solvents were removed *in vacuo* and the residue was triturated with several lots of iced water until it crystallised. The *diphenyl phosphate* (6·6 g., 83%), after recrystallisation from ethanol, had m. p. 133—134°, $[\alpha]_D^{25}$ +106° (*c* 1·00 in dioxan) (Found: C, 65·1; H, 4·9. $C_{40}H_{35}O_{12}P$ requires C, 65·0; H, 4·7%).

Methyl β-D-*Galactoside* 6-(*Dihydrogen Phosphate*).—The above diphenyl phosphate (2 g.) in acetic acid (40 ml.) was shaken with hydrogen at room temperature and pressure in the presence of Adams platinum. Working up as before gave an aqueous solution which was extracted three times with benzene and passed through Dowex 50 (cyclohexylammonium form). The effluent was concentrated to a small volume. Addition of ethanol gave *methyl* β-D-*galactoside* 6-(*dicyclohexylammonium phosphate*) (0·6 g., 50%), m. p. 138—144° (decomp.), $[\alpha]_D^{20}$ −11·9° (*c* 0·46 in H_2O) (Found: C, 48·5; H, 8·6; N, 6·0; P, 6·9. $C_{19}H_{41}O_9N_2P$ requires C, 48·3; H, 8·7; N, 5·9; P, 6·6%).

Methyl 2,3-*Di*-O-*benzoyl*-β-D-*galactoside* 4,6-(*Phenyl Phosphate*).—Phenyl phosphorodichloridate (2·75 g.) in pyridine (25 ml.) was added to methyl 2,3-di-*O*-benzoyl-β-D-galactoside [12] (5 g.) in pyridine (63 ml.). The solution was kept at 37° for 5 days, then cooled, and water was added. After 1 hr., the solvents were removed *in vacuo* and the residue was triturated with several batches of iced water until it solidified. The *phenyl phosphate* (1·5 g., 22·4%) crystallised from ethanol and had m. p. 233—234° (decomp.) (Found: C, 59·6; H, 4·9; P, 5·7. $C_{27}H_{25}O_{10}P$ requires C, 60·0; H, 4·6; P, 5·7%).

Methyl 2,3-*Di*-O-*hexahydrobenzoyl*-β-D-*galactoside* 4,6-(*Hydrogen Phosphate*).—The above phenyl phosphate (2·5 g.) in acetic acid (140 ml.) was shaken with hydrogen at room temperature and pressure in the presence of Adams platinum. When the hydrogen uptake had ceased, the catalyst was filtered off and the acetic acid removed *in vacuo* below 30°, the last traces being removed by lyophilisation. Ethanol was added to the residue which dissolved when the pH was brought to 6 with cyclohexylamine. The volume of ethanol was reduced *in vacuo* and ether and light petroleum (b. p. 30—60°) were added, whereupon *methyl* 2,3-*di*-O-*hexahydrobenzoyl*-β-D-*galactoside* 4,6-(*cyclohexylammonium phosphate*) (1·9 g., 71%), m. p. 220—225° (decomp.), $[\alpha]_D^{25}$ +19·9° (*c* 1·00 in EtOH), crystallised (Found: C, 56·3; H, 8·3; N, 2·8. $C_{27}H_{46}O_{10}NP$ requires C, 56·3; H, 8·0; N, 2·8%).

Methyl β-D-*Galactoside* 4,6-(*Hydrogen Phosphate*).—(*a*) The above cyclic phosphate (1 g.) was dissolved in anhydrous methanol (30 ml.) and a solution of sodium (0·5 g.) in methanol (50 ml.) was added to bring the pH to 13. The solution was refluxed for 16 hr. A paper chromatogram at this stage showed only one spot containing phosphate. The solution was neutralised with acetic acid and evaporated to dryness *in vacuo*. After crystallisation from ethanol, methyl β-D-galactoside 4,6-(cyclohexylammonium phosphate) (0·4 g., 66%) had m. p. 210—212° (decomp.).

(*b*) The cyclohexylammonium salt of methyl 2,3-di-*O*-hexahydrobenzoyl-β-D-galactoside 4,6-cyclic phosphate (2 g.) was dissolved in anhydrous methanol and saturated several times with dry ammonia. When paper chromatography showed that the removal of the hexahydrobenzoyl groups was complete the ammonia and methanol were removed *in vacuo*, and the residue in water was passed through Amberlite IR-120 (H^+ form). The effluent was neutralised with cyclohexylamine and evaporated to dryness *in vacuo*. The *cyclohexylammonium salt* of the 4,6-cyclic phosphate, after crystallisation from ethanol, had m. p. 210—212° (decomp.), $[\alpha]_D^{22}$ −21·2° (*c* 0·87 in H_2O) (Found: C, 43·8; H, 7·3; N, 4·0; P, 9·4. $C_{13}H_{26}O_8NP$ requires C, 43·9; H, 7·3; N, 3·9; P, 8·7%).

Methyl 2,3-*Di*-O-*benzyl*-α-D-*glucoside* 4,6-(*Phenyl Phosphate*).—Phenyl phosphorodichloridate (10·85 g.) in pyridine (50 ml.) was added slowly to methyl 2,3-di-*O*-benzyl-α-D-glucoside [13] (16 g.) in pyridine (100 ml.) at <40°. The solution was kept for 48 hr. at 37°, then working up as usual gave a residue which crystallised from ethanol–pentane. After recrystallisation from ethanol, the *phenyl phosphate* had m. p. 141—143°, $[\alpha]_D^{25}$ +24·0° (*c* 1·00 in $CHCl_3$), +50·5° (*c* 1·00 in dioxan) (Found: C, 63·3; H, 5·6; P, 6·3. $C_{27}H_{29}O_8P$ requires C, 63·3; H, 5·6; P, 6·0%).

Methyl 2,3-*Di*-O-*benzyl*-α-D-*glucoside* 4,6-(*Hydrogen Phosphate*).—The above phenyl phosphate (5 g.) was dissolved in dioxan (50 ml.) and a mixture of 1·6N-potassium hydroxide (20 ml.)

and water (10 ml.) was added. The oil which separated redissolved on agitation after 1·5 hr. The solution was kept at room temperature for 18 hr. and the pH brought to 5 with Dowex 50 (H^+ form). The resin was filtered off and washed with water. The dioxan was removed *in vacuo* and the aqueous solution extracted five times with ether before being passed slowly through Amberlite IR-120 (cyclohexylammonium form). The effluent was evaporated to dryness *in vacuo* and the residue triturated with ether until it crystallised. After recrystallisation from ethanol–ether, *methyl* 2,3-*di*-O-*benzyl*-α-D-*glucoside* 4,6-(*cyclohexylammonium phosphate*) (2·7 g., 52%) had m. p. 180—181° (decomp.), $[\alpha]_D^{24}$ +51·8° (*c* 1·00 in H_2O) (Found: C, 60·6; H, 7·2; N, 2·7; P, 6·0. $C_{27}H_{38}O_8NP$ requires C, 60·6; H, 7·1; N, 2·6; P, 5·8%).

Methyl α-D-*Glucoside* 4,6-(*Hydrogen Phosphate*).—The above cyclic phosphate (250 mg.) was dissolved in ethanol and the pH brought to 2—3 with Dowex 50 (H^+ form). The resin was filtered off and washed with ethanol. The combined filtrates were shaken with hydrogen at room temperature and pressure in the presence of palladium catalyst. When the hydrogen uptake had ceased, the catalyst was filtered off and the solution neutralised with cyclohexylamine and concentrated to 2 ml. Addition of ether gave the methyl α-D-glucoside 4,6-(cyclohexylammonium phosphate), m. p. 228—231° (decomp.), $[\alpha]_D^{22}$ +81·2° (*c* 1·13 in H_2O). Baddiley, Buchanan, and Szabó [2] gave m. p. 228—230° (decomp.), $[\alpha]_D^{20}$ +81·3° (*c* 1·13 in H_2O).

Periodate Oxidations.—The sugar derivatives (0·1 mmole) were oxidised at room temperature in the dark with an excess of sodium metaperiodate in aqueous medium buffered with N-sodium acetate–acetic acid (pH 3·2) (2·5 ml. of buffer per 25 ml. of solution). Aliquot portions were removed periodically and periodate was determined by the usual methods.

Paper Chromatography of Phosphates.—Ascending chromatograms on Whatman No. 1 paper were run without temperature control. The solvent systems were: A, propan-2-ol–ammonia–water (7 : 1 : 2); B, propan-1-ol–ammonia–water (7 : 1 : 2); C, butan-1-ol–acetic acid–water (4 : 1 : 5); D, propan-2-ol–concentrated hydrochloric acid–water (65 : 17·2 : 17·8); E, propan-1-ol–ammonia–water (6 : 1 : 3). Results are tabulated.

	R_F in solvent				
	A	B	C	D	E
Me α-D-glucoside 2-(dihydrogen phosphate)	0·29	0·18	0·15	—	0·48
Me α-D-glucoside 3-(dihydrogen phosphate)	0·34	0·20	0·19	—	0·50
Me α-D-glucoside 4-(dihydrogen phosphate)	0·32	0·18	0·15	—	0·45
Me α-D-glucoside 6-(dihydrogen phosphate)	0·26	0·13	—	—	0·41
Me β-D-galactoside 4-(dihydrogen phosphate)	—	0·21	0·17	—	0·45
Me β-D-galactoside 6-(dihydrogen phosphate)	—	0·11	0·09	—	0·36
Me α-D-glucoside 4,6-(hydrogen phosphate) [2]	0·64	0·47	0·16	0·90	—
Me α-D-glucoside 4,6-(hydrogen phosphate)	—	0·47	0·16	—	—
Me β-D-galactoside 4,6-(hydrogen phosphate)	0·57	0·38	0·12	0·86	—

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