501. *Studies on Phosphorylation. Part IX.* The Preparation of Phosphates of Carbohydrate Derivatives by the Epoxide Route.*

By W. E. Harvey, J. J. Michalski, and A. R. Todd.

The preparation of phosphate esters by addition of dibenzyl hydrogen phosphate to ethylene oxide derivatives (Part V; *J.*, 1949, 815) has been extended to epoxides of the carbohydrate series to assess the potentialities of the method for use in the field of nucleotide synthesis. 4 : 6-Benzylidene α-methyl-2 : 3-anhydro-D-allopyranoside (II) reacts with dibenzyl hydrogen phosphate much less readily than does 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) and gives a mixture of benzylidene methylhexoside dibenzyl phosphates. Removal of the benzyl and benzylidene groups gives a mixture of α-methyl-D-altropyranoside-2 phosphate (isolated as barium salt) and α-methyl-D-glucopyranoside-3 phosphate in which the former predominates. 7-β-(4 : 6-Benzylidene 2 : 3-anhydro-D-allopyranosyl)theophylline (VIII) reacted with dibenzyl hydrogen phosphate when heated in dioxan solution; in this case the product isolated appeared to be 7-β-D-glucopyranosyltheophylline-3′ phosphate (IX).

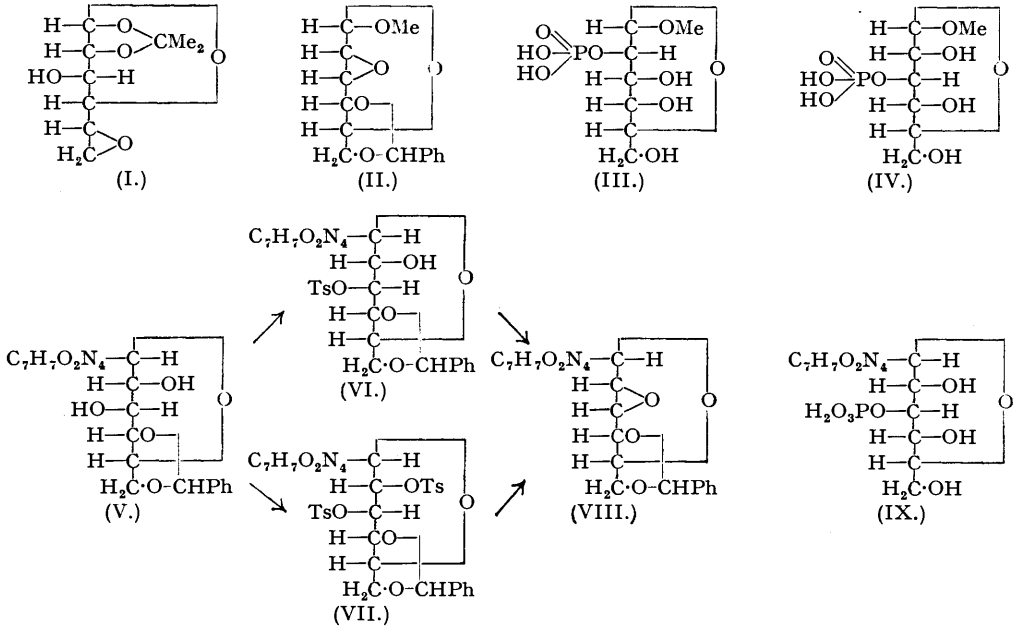
In further model experiments, 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (X; R = H, R′ = $PhCH_2$) condensed with *cyclo*hexene oxide to give (1 : 2 : 3 : 4-tetra-acetyl-β-D-glucopyranose-6) benzyl 2-hydroxy*cyclo*hexyl phosphate (XI; R = $PhCH_2$) from which the acid ester (XI; R = H) was obtained by hydrogenation. In similar fashion (X; R = H, R′ = $PhCH_2$) condensed with (I) to give (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6)(1 : 2-*iso*propylidene α-D-glucofuranose-6) benzyl phosphate (XII; R = $PhCH_2$), yielding (XII; R = H) on hydrogenation. (1 : 2 : 3 : 4-Tetra-acetyl β-D-glucopyranose-6) (2 : 3 : 4 : 6-tetra-acetyl β-D-glucopyranose-1) hydrogen phosphate (XIII) has also been prepared by starting from the silver salt of (X; R = H, R′ = $PhCH_2$) and α-acetobromoglucose.

The first recorded synthesis of an ester of phosphoric acid from a 1 : 2-epoxide is due to Bailly (*Ann. Chim.*, 1916, **6**, 133), who showed that disodium α-glyceryl phosphate is formed from glycide alcohol and disodium hydrogen phosphate in aqueous solution at room temperature. Later, Zetsche and Aeschlimann (*Helv. Chim. Acta*, 1926, **9**, 708) showed that syrupy phosphoric acid reacted readily with glycide alcohol and epichlorohydrin giving, respectively, α-glyceryl phosphate and two chlorohydroxypropyl phosphates, and further that α-glyceryl phosphate reacted with epichlorohydrin to give a mixture of diesters. Eidebenz and Depner (*Arch. Pharm.*, 1942, **280**, 227) obtained either a mono- or a di-ester by varying the molar ratio of phosphoric acid which was allowed to react with epi-iodohydrin, and an American patent specification (Adams and Shoemaker, 1945; U.S.P. 2,372,244) describes the reaction of phosphoric and phosphorous acid with ethylene oxides to give phosphates and phosphites and indicates that the sulphur and nitrogen analogues of the oxides behave in a similar way. In Part I of this series (Atherton, Openshaw, and Todd, *J.*, 1945, 382) Bailly's observation was extended to the preparation of 2-hydroxyethyl phosphate and 2-hydroxypropyl phosphate by interaction of disodium hydrogen phosphate with ethylene and propylene oxides. Since then, Lampson and Lardy (*J. Biol. Chem.*, 1949, **181**, 697), apparently unaware of the earlier literature, have described the preparation of 2-hydroxypropyl phosphate from propylene oxide and dipotassium hydrogen phosphate and have also made glucose-6 phosphate by a similar method starting from 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (*ibid.*, p. 693). As indicated briefly in Part I (*loc. cit.*), we were interested in the possible application of the method to carbohydrate epoxide derivatives as a possible route to the synthesis of compounds of the dinucleotide type. Baddiley, Clark, Michalski, and Todd (Part V; *J.*, 1949, 815) showed that dibenzyl and substituted dibenzyl hydrogen phosphates react with *cyclo*hexene oxide to yield the expected triesters of phosphoric acid, and it was clear that if the method could be applied to nucleoside epoxides, then not only could dibenzyl and thence monobenzyl nucleoside phosphates be

* Part VIII, preceding paper.

prepared but the latter might be brought into reaction with a second nucleoside epoxide to produce a dinucleotide. The method could not, of course, apply to nucleotides derived from the naturally occurring ribonucleosides since the latter are D-ribofuranose derivatives with a *cis*-arrangement of hydroxyls, and the opening of an epoxide would inevitably yield a *trans*-arrangement of groups, but it could be of value in other cases.

Although 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) reacts with dipotassium or disodium hydrogen phosphate (Lampson and Lardy, *loc. cit.*), the latter salt failed to react in aqueous dioxan solution with 4 : 6-benzylidene α-methyl-2 : 3-anhydro-D-allopyranoside (II) even when heated at 150° for 36 hours. When dibenzyl hydrogen phosphate and 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose were heated in carbon tetrachloride solution under reflux for 24 hours, a neutral product was obtained which, although it could not be crystallised, was evidently 1 : 2-*iso*propylidene α-D-glucofuranose-6 dibenzyl phosphate, since hydrogenolysis followed by removal of the *iso*propylidene group yielded the known glucose-6 phosphate.



Monodebenzylation of the neutral ester gave an acidic material believed to be the corresponding monobenzyl ester, which, however, could not be crystallised either as the free acid or as its *cyclo*hexylamine salt. 4 : 6-Benzylidene α-methyl-2 : 3-anhydro-D-allopyranoside (II) also reacted with dibenzyl hydrogen phosphate in carbon tetrachloride solution, although much less readily than (I), 7 days' heating being required to complete the reaction. The product was a colourless neutral resin which, after chromatography on alumina, gave analytical values in good agreement with those required for a benzylidene methylhexoside dibenzyl phosphate. It is well established that when the epoxide ring of a 2 : 3-anhydro-sugar is opened by acid or alkaline reagents the entering group may become attached to either of the carbon atoms forming part of the three-membered ring, a Walden inversion occurring at the point of attachment. Thus, Newth, Overend, and Wiggins (*J.*, 1947, 10) showed that treatment of (II) with hydrochloric or hydrobromic acid gave an α-methyl-2-deoxy-2-halogeno-D-altroside and an α-methyl-3-deoxy-3-halogeno-α-D-glucoside, the latter compound predominating in each case. It was, therefore, to be expected that the neutral resin obtained from (II) on reaction with dibenzyl hydrogen phosphate would be a mixture of 4 : 6-benzylidene α-methyl-D-altropyranoside-2 dibenzyl phosphate and 4 : 6-benzylidene α-methyl-D-glucopyranoside-3 dibenzyl phosphate. Benzyl and benzylidene groups were removed from the product by hydrogenolysis to give a mixture of methylglycoside phosphates. Chromatography of the mixture as ammonium salts on acid-washed paper showed the absence of inorganic phosphate and the presence of two organic phosphorus-containing compounds, one of which was decomposed by treatment with

periodate, the other being unaffected. This is in accordance with the expected behaviour of a mixture of methylaltroside-2 phosphate (III) and methylglucoside-3 phosphate (IV), since only the former contains a free 1 : 2-glycol system. Measurement of the amount of periodate consumed by the mixture indicated that it contained about four times as much of the altroside-2 phosphate as the glucoside-3 phosphate, a finding which is surprising in view of the above-mentioned observations of Newth, Overend, and Wiggins (*loc. cit.*). Acid hydroysis of the mixed phosphates yielded a mixture of two sugars identified as altrose and glucose. An attempt was made to separate the mixture of phosphates by using the chromatopile technique; although the separation was not complete, it was possible to isolate the major component, α-methyl-D-altropyranoside-2 phosphate (III) as its amorphous barium salt.

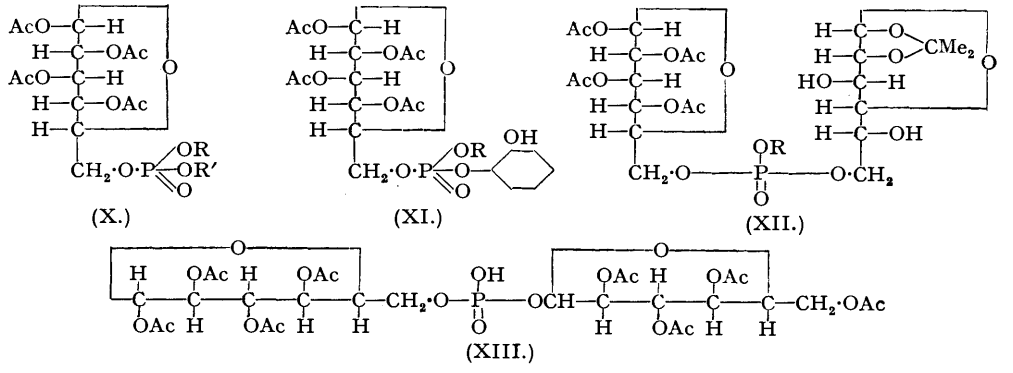
The results obtained in the foregoing experiments were considered sufficiently encouraging to warrant application of the method to the preparation of a nucleotide. Condensation of 7-β-D-glucopyranosyltheophylline with benzaldehyde in presence of zinc chloride gave a derivative which, by analogy with the behaviour of α-methyl-D-glucopyranoside and on the basis of the reactions described below, is formulated as 7-β-(4 : 6-benzylidene D-glucopyranosyl)-theophylline (V). A remarkable feature of (V) is that it is not oxidised by periodate under the usual conditions; the reason for this is not clear, but it may be due to the combined steric effect of the large theophylline and benzylidene residues. Simultaneous toluene-*p*-sulphonylation of both hydroxy-groups in (V) proved difficult, although dimethanesulphonylation could be fairly readily effected. Even under drastic conditions, the main product was a mixture of a monotoluene-*p*-sulphonyl derivative with some of the required 7-β-(4 : 6-benzylidene 2 : 3-di-toluene-*p*-sulphonyl D-glucopyranosyl)theophylline (VII) which could be separated with difficulty by chromatography. No attempt was made to prepare (VII) on a large scale since the monoester could be obtained readily under much milder reaction conditions and it gave with sodium methoxide an anhydro-compound identical with that prepared from (VII). The monotoluene-*p*-sulphonyl derivative must therefore be 7-β-(4 : 6-benzylidene 3-toluene-*p*-sulphonyl D-glucopyranosyl)theophylline (VI) and the anhydro-compound must be 7-β-(4 : 6-benzylidene 2 : 3-anhydro-D-allopyranosyl)theophylline (VIII), since there is no recorded instance of a 2 : 3-toluene-*p*-sulphonyl glucose derivative yielding a 2 : 3-anhydro-sugar with the mannose configuration and since, in forming an anhydro-sugar from a monotoluene-*p*-sulphonyl derivative, a Walden inversion occurs at the position originally occupied by the toluene-*p*-sulphonyl group.

The anhydro-glycoside (VIII) in dioxan solution was subjected to prolonged heating with dibenzyl hydrogen phosphate at 100°. The crystalline product which separated from the solution was soluble in water and strongly acidic. Analysis and potentiometric titration indicated that both benzyl and benzylidene groups had been lost, presumably by hydrolysis due to small amounts of water in the dioxan used. Paper chromatography showed that the product was homogeneous and it did not react with periodate under conditions known to be satisfactory for the oxidation of natural nucleotides. This product is therefore formulated as 7-β-D-glucopyranosyltheophylline-3′ phosphate (IX). No other phosphate was detected although in view of the small scale on which the reaction was carried out the possibility that some isomeric 2′-phosphate is also produced cannot be excluded.

As a further extension of these studies, and to provide suitable model experiments for eventual dinucleotide syntheses, it was decided to attempt the preparation of esters of phosphoric acid containing two different carbohydrate residues. No compounds of this nature had hitherto been prepared, but it seemed that some examples might be obtained from the reaction of 2 : 3-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) with sugar phosphates. Accordingly, 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 dibenzyl phosphate (X; R = R′ = $PhCH_2$) was prepared from 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose and dibenzyl chlorophosphonate in the usual manner, and from it the crystalline 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (X; R = H, R′ = $PhCH_2$) was readily prepared by reaction with 4-methyl-morpholine (cf. Baddiley, Clark, Michalski, and Todd, *loc. cit.*) or lithium chloride (cf. Clark and Todd, *J.*, 1950, 2030) and characterised as its lithium and *cyclo*hexylamine salts. Complete removal of the benzyl groups from (X; R = R′ = $PhCH_2$) by hydrogenolysis gave 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 phosphate (X; R = R′ = H), identical with the material described by Lardy and Fischer (*J. Biol. Chem.*, 1946, **164**, 513).

Before condensation of the monobenzyl phosphate (X; R = H, R′ = $PhCH_2$) with (I) was attempted, its reaction with *cyclo*hexene oxide was examined. In chloroform solution reaction proceeded rather slowly but it furnished (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) benzyl 2-hydroxy*cyclo*hexyl phosphate (XI; R = $PhCH_2$) in fair yield as a crystalline solid. Removal

of the benzyl group by hydrogenation in dioxan solution gave the resinous 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 2′-hydroxy*cyclo*hexyl hydrogen phosphate (XI; R = H) characterised as its crystalline *cyclo*hexylamine salt. A solution of equimolecular quantities of (X; R = H, R′ = $PhCH_2$) and 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) in chloroform was now heated under reflux for 45 minutes then set aside at room temperature for a day. The crystalline reaction product was stable to periodate and gave analytical values indicating that it was the expected (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) (1 : 2-*iso*-propylidene α-D-glucofuranose-6) benzyl phosphate (XII; R = $PhCH_2$). Catalytic hydrogenation of this product gave (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) (1 : 2-*iso*-



(X.) (XI.) (XII.)

(XIII.)

propylidene α-D-glucofuranose-6) hydrogen phosphate (XII; R = H), characterised as its *cyclo*hexylamine salt, and the same product was also obtained by reaction of (X; R = R′ = H) with (I) in dioxan solution. As another example of a diester of phosphoric acid containing two sugar residues (1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6) (2 : 3 : 4 : 6-tetra-acetyl β-D-gluco-pyranose-1) hydrogen phosphate (XIII) was prepared as its crystalline *cyclo*hexylamine salt by treating the silver salt of (X; R = H, R′ = $PhCH_2$) with acetobromoglucose and hydrogenating the neutral reaction product.

The general conclusion drawn from the work described in this paper is that the epoxide route offers a feasible route to dicarbohydrate esters of phosphoric acid and to compounds of the nucleotide type. It is, however, severely limited in its application by the accessibility of appropriate anhydro-compounds, the tendency to formation of more than one product from other than 5 : 6-anhydro-sugar derivatives, and by the stereochemical considerations referred to earlier.

Experimental.

Reaction of 1 : 2-iso*Propylidene* 5 : 6-*Anhydro*-α-D-*glucofuranose* (I) *with Dibenzyl Hydrogen Phosphate.*—The anhydro-sugar (I) (1 g., 1 mol.) (Ohle and Vargha, *Ber.*, 1929, **62**, 2435) and dibenzyl hydrogen phosphate (1·38 g., 1 mol.) (prepared according to Clark and Todd, *J.*, 1950, 2023) were dissolved in carbon tetrachloride (75 c.c.), then heated to boiling, and *ca.* one-third of the solvent distilled off to remove traces of water. The residual liquid was now heated under reflux for 24 hours, moisture being excluded. The solution, now only faintly acidic, was washed with aqueous sodium hydrogen carbonate and then water, dried, and evaporated. The almost colourless gum so obtained was dissolved in chloroform and chromatographed on neutral alumina. A small amount of oily material was washed through with chloroform, and the main bulk of product was eluted with chloroform–methanol (50 : 1). Evaporation of the eluate gave 1 : 2-iso*propylidene* α-D-*glucofuranose*-6 *dibenzyl phosphate* as a colourless glass which could not be crystallised (Found : C, 55·0; H, 6·4. $C_{23}H_{29}O_9P,H_2O$ requires C, 55·4; H, 6·3%). Hydrolysis with aqueous-alcoholic sulphuric acid (N.) at room temperature yielded the known glucose-6 phosphate, isolated as its barium salt ($[\alpha]_D^{18}$ = +17·3°; *c*, 2·55 in water).

In a second experiment the crude dibenzyl ester was hydrogenated in ethanol (100 c.c.) by means of a palladised charcoal catalyst (0·5 g.; 10%). When absorption of hydrogen ceased the filtered solution was evaporated under reduced pressure and the gummy residue was dissolved in water and neutralised with barium hydroxide, and the solution saturated with carbon dioxide, heated to boiling, and concentrated under reduced pressure to small volume (3—4 c.c.). The solution was filtered and poured into ethanol (200 c.c.), and the precipitated amorphous barium salt (0·7 g.) collected. It had $[\alpha]_D^{18}$ = +3·8° (*c*, 4·98 in water) and, as it only reduced Fehling's solution very slightly, it probably consisted mainly of barium 1 : 2-*iso*propylidene α-D-glucofuranose-6 phosphate. Without further purification the *iso*propylidene group was removed by hydrolysis, giving glucose-6 phosphate.

When the above dibenzyl ester (2·4 g.) was heated with 4-methylmorpholine (25 c.c.) at 100° for 2 hours an acidic amorphous material was obtained which gave analytical values suggesting that it was

the expected 1 : 2-iso*propylidene* α-D-*glucofuranose*-6 *benzyl hydrogen phosphate* (Found : C, 48·0; H, 6·4. $C_{16}H_{23}O_9P,\frac{1}{2}H_2O$ requires C, 48·1; H, 6·1%). This product gave a hygroscopic *cyclo*hexylamine salt which could not be crystallised.

Reaction of 4 : 6-*Benzylidene* α-*Methyl*-2 : 3-*anhydro*-D-*allopyranoside* (II) *with Dibenzyl Hydrogen Phosphate*.—A solution of the anhydro-glycoside (II) (5·3 g., 1 mol.) (Rosenfeld, Richtmeyer, and Hudson, *J. Amer. Chem. Soc.*, 1948, **70**, 2201) and dibenzyl hydrogen phosphate (5·6 g., 1 mol.) in carbon tetrachloride (250 c.c.) was distilled until about 50 c.c. of distillate had been collected, then heated under reflux for 7 days with exclusion of moisture. At intervals aliquots (2 c.c.) were removed and titrated with N/10-sodium hydroxide; it was observed that about 50% of the original acid was used up in 24 hours and about 95% in 7 days. The cooled solution was filtered, washed first with dilute sodium hydroxide then with water, dried, and evaporated. The residue (10 g.), a colourless glass, defied all attempts at crystallisation. A small sample (43 mg.) was dissolved in benzene–chloroform (20 c.c.; 1 : 1) and chromatographed on neutral alumina (3 g.). Elution with chloroform–methanol (100 : 1) yielded a colourless glass which had the correct analysis for a mixture of benzylidene methylhexoside dibenzyl phosphates (Found : C, 62·1; H, 5·8. Calc. for $C_{28}H_{31}O_9P$: C, 62·0; H, 5·8%).

The crude product obtained as above (6·3 g.) was dissolved in ethanol (200 c.c.) and hydrogenated at room temperature and atmospheric pressure by aid of a palladised charcoal catalyst (0·5 g.; 10%). Hydrogenation was slow and ceased after the hydrogen uptake corresponded to 3·5 mols. Catalyst was removed by filtration, the filtrate evaporated under reduced pressure, and the residual syrup dissolved in water (150 c.c.). Saturated aqueous barium hydroxide was added until the mixture was just alkaline to phenolphthalein, the mixture was saturated with carbon dioxide, then boiled, filtered, and concentrated to small bulk (50 c.c.) under reduced pressure. After removal of a small precipitate by centrifugation the solution was poured into acetone (300 c.c.). After several hours the precipitated mixture of barium α-methyl-D-altroside-2 phosphate (III) and barium α-methyl-D-glucoside-3 phosphate (IV) was collected. The mixture was purified by dissolution in water and reprecipitation with acetone giving a white amorphous powder (3·55 g.) (Found : C, 20·9; H, 4·0; P, 7·2. Calc. for $C_7H_{13}O_9PBa$: C, 20·5; H, 3·2; P, 7·6%).

A portion of the crude initial reaction product (1·3 g.) was hydrogenated as before and the resulting gum was heated for 6 hours under reflux with dilute sulphuric acid (25 c.c.; 2N.) then diluted to 100 c.c. with water, and barium carbonate added gradually until the solution was neutral. The mixture was heated to boiling and filtered from precipitated barium salts. The filtered solution was passed successively through short columns of Zeocarb 215 and Deacidite B to remove inorganic material and then concentrated to small bulk (10 c.c.). Paper chromatography of this solution by means of a collidine–water system with ammoniacal silver nitrate as spraying agent revealed the presence of two sugars which were identified as glucose and altrose by comparison with authentic samples run simultaneously. The altrose used was prepared from a sample of 4 : 6-benzylidene α-methyl-D-altroside kindly supplied by Prof. M. Stacey, to whom our thanks are due. The R_F value for altrose in collidine–water was 0·53 (corrected to 20° by using glucose, R_F 0·39, as standard). A small sample of the solution of altrose and glucose obtained as above was evaporated, and the residual gum taken up in a few drops of water and heated on the water-bath for 2 hours with phenylhydrazine acetate. The crystalline precipitate, recrystallised several times from aqueous ethanol, gave altrose phenylosazone, m. p. 178—179°.

Periodate Oxidation of Mixed Phosphates.—The above mixture of barium salts of methylaltroside-2 phosphate and methylglucoside-3 phosphate (97·5 mg.) was dissolved in water (3 c.c.), sodium sulphate (50 mg. in 1 c.c. water) added, and barium sulphate removed by centrifugation, the precipitate being washed thoroughly with hot water. The combined filtrate and washings were now treated with sodium metaperiodate solution (5 c.c.; 0·245M.), the volume was made up to 25 c.c., and the solution set aside at room temperature and the course of the oxidation followed in the usual way. The uptake of periodate was at first rapid up to 0·8 mol./mol. Subsequently a very slow further uptake occurred, the total absorption after 24 hours being 0·9 mol./mol. This result indicates that the original mixture contained approx. 80% of barium α-methyl-D-altroside-2 phosphate and 20% of barium α-methyl-D-glucoside-3 phosphate.

Barium α-*Methyl*-D-*altropyranoside*-2 *Phosphate* (III).—Dilute sulphuric acid (*ca.* 4 c.c.; N/2) was added to a solution of the mixed barium methylglycoside phosphates (400 mg.) prepared as described above so as to remove barium ions as sulphate. The barium-free solution was neutralised with dilute ammonia solution and a sample was chromatographed on acid-washed paper by Hanes and Isherwood's method (*Nature*, 1949, **164**, 1107), propanol–ammonia–water (6 : 3 : 1) being used as solvent system. Treatment of the chromatogram with the phosphate reagent described by these authors showed the presence of two main spots corresponding to the two sugar phosphates. Of these, that with the lower R_F value, 0·25, was evidently α-methyl-D-altroside-2 phosphate, since it was considerably more intense and it was absent if the mixture was treated with periodate before chromatography. The other spot, R_F 0·32, was stable to periodate and evidently was due to α-methyl-D-glucoside-3 phosphate. Although the separation of the two phosphates in this way was quite clear-cut, the low R_F values suggested that separation and isolation on any scale on a cellulose column would be very laborious. Recourse was therefore had to separation by using the chromatopile described by Mitchell and Haskins (*Science*, 1949, **110**, 278). In setting up the chromatopile the filter-papers were washed first with acetic acid (2N.) then with water until free from acid and dried at 60° before use. The mixed phosphates (from 350 mg. of the mixed barium salts) in the form of ammonium salts were applied on 13 papers, the chromatopile allowed to come to equilibrium in the presence of solvent (propanol–ammonia–water; 6 : 3 : 1) for 72 hours, and then run for 28 hours, by which time the solvent front had almost reached the bottom of the pile. The papers were now dried at 60°, and the position of the phosphates determined by spraying each fifteenth paper with phosphate reagent. All phosphate-containing material was found concentrated in the first 300 papers which were divided into groups of 15 sheets, each group being separately extracted with

water in a small Soxhlet and the extract concentrated to small bulk (2—3 c.c.). Small test paper chromatograms on each extract showed that a number of the fractions contained only the altrose derivative but none contained only the glucose derivative. The fractions (papers 81—170) containing only the altrose compound were combined and passed through a short column of Zeocarb 215 which was washed with water until the washings were no longer acidic. The combined washings (20 c.c.) were made alkaline (phenolphthalein) with barium hydroxide solution, saturated with carbon dioxide, boiled, and filtered. The filtrate was concentrated to small bulk (2 c.c.) under reduced pressure and poured into acetone (100 c.c.). *Barium α-methyl-*D*-altropyranoside-2 phosphate* separated as a white powder (90 mg.). It was purified by precipitation from aqueous solution with acetone and then had $[\alpha]_D^{12} = +34°$ (*c*, 1·03 in water), unchanged by further treatment (Found : C, 19·6; H, 4·1. $C_7H_{13}O_9PBa,H_2O$ requires C, 19·7; H, 3·5%). On periodate oxidation the substance consumed 1·06 mols. of oxidant per mol. in 24 hours, and paper chromatography of the corresponding ammonium salt showed that it was homogeneous and free from inorganic phosphate.

7-*β*-(4 : 6-*Benzylidene* D-*Glucopyranosyl*)*theophylline* (V).—A mixture of 7-*β*-D-glucopyranosyltheophylline (10 g., 1 mol.; Fischer and Helferich, *Ber.*, 1914, **47**, 217), anhydrous zinc chloride (20 g., 5 mols.), and freshly distilled benzaldehyde (50 c.c.) was shaken for 18 hours. The viscous mixture was now shaken vigorously with an equal volume of water, and the benzaldehyde phase separated, washed twice with water, and set aside for 2—3 hours. The crystalline product (10 g., m. p. *ca.* 245°) which separated was collected and recrystallised several times from aqueous ethanol or aqueous Cellosolve, giving 7-*β*-(4 : 6-*benzylidene* D-*glucopyranosyl*)*theophylline* as colourless hexagonal plates, m. p. 272—273° (Found : C, 55·5; H, 4·9; N, 12·8. $C_{20}H_{22}O_7N_4$ requires C, 55·8; H, 5·1; N, 13·0%). The substance was unaffected by aqueous periodate at room temperature for 24 hours either in aqueous suspension or dissolved in ethanol or dioxan.

*Toluene-*p*-sulphonylation of* 7-*β*-(4 : 6-*Benzylidene* D-*Glucopyranosyl*)*theophylline*.—A solution of the above benzylidene glucoside (0·5 g., 1 mol.) and toluene-*p*-sulphonyl chloride (0·66 g., 3 mols.) in dry pyridine (15 c.c.) was kept at 40° for 3 days then heated to 100° for 2 hours. The cooled brownish solution was poured into a well-stirred mixture of crushed ice and water (200 c.c.). After 2 hours' further stirring, the precipitate (0·6 g.) was collected, washed with water, and recrystallised from aqueous Cellosolve, giving 7-*β*-(4 : 6-*benzylidene* 3-*toluene-*p*-sulphonyl* D-*glucopyranosyl*)*theophylline* (VI) as colourless plates, m. p. 232—233° (decomp.) (Found : C, 55·5; H, 4·8; N, 9·5; S, 5·3. $C_{27}H_{28}O_9N_4S$ requires C, 55·4; H, 4·8; N, 9·6; S, 5·5%). A sample of this material was recovered unchanged after 6 hours' heating with sodium iodide in acetone in a sealed tube at 110°.

The Cellosolve mother-liquors from the crystallisation of the monotoluene-*p*-sulphonyl compound were combined and evaporated, and the residue (200 mg.) dissolved in benzene (75 c.c.) and put on a column of neutral alumina (6 g.). Elution with benzene–acetone (10 : 1) yielded 7-*β*-(4 : 6-*benzylidene* 2 : 3-*ditoluene-*p*-sulphonyl* D-*glucopyranosyl*)*theophylline* (VII) which crystallised from aqueous acetone as colourless woolly needles, m. p. 242° (decomp.) (Found : C, 55·5; H, 4·8; N, 7·9. $C_{34}H_{34}O_{11}N_4S_2$ requires C, 55·3; H, 4·6; N, 7·6%).

7-*β*-(4 : 6-*Benzylidene* 2 : 3-*Dimethanesulphonyl* D-*Glucopyranosyl*)*theophylline*.—A mixture of the above benzylidene glucoside (2 g., 1 mol.), methanesulphonyl chloride (1·6 g., 3 mols.), and dry pyridine (50 c.c.) was set aside at 40° for 18 hours then poured into a mixture of ice and water (500 c.c.), and stirring continued for 2 hours. The precipitated solid (1 g.) was collected, washed with water, and recrystallised from aqueous dioxan. The *dimethanesulphonyl* derivative was thus obtained as colourless needles, m. p. 233—234° (decomp.) (Found : C, 45·0; H, 4·2; N, 9·5. $C_{22}H_{26}O_{11}N_4S_2$ requires C, 45·0; H, 4·5; N, 9·6%).

7-*β*-(4 : 6-*Benzylidene* 2 : 3-*Anhydro-*D*-allopyranosyl*)*theophylline* (VIII).—(1) Methanolic sodium methoxide (from 0·2 g. of sodium in 5 c.c. of methanol) was added to a solution of the above 2 : 3-di-toluene-*p*-sulphonyl compound (VII) (0·2 g.) in chloroform (10 c.c.), and the mixture set aside for 4½ days, during which time a white precipitate formed. Water (15 c.c.) was added, the mixture was thoroughly shaken, and the chloroform layer separated, washed again with water until the washings were neutral (3 × 8 c.c.), then dried and evaporated under reduced pressure. The residue of 7-*β*-(4 : 6-*benzylidene* 2 : 3-*anhydro-*D*-allopyranosyl*)*theophylline* crystallised on addition of ether; recrystallised from aqueous ethanol, the anhydro-compound formed long colourless needles, m. p. 225—226° (Found : C, 58·2; H, 4·7; N, 13·5. $C_{20}H_{20}O_6N_4$ requires C, 58·3; H, 4·9; N, 13·6%).

(2) The above preparation was repeated, 7-*β*-(4 : 6-benzylidene 3-toluene-*p*-sulphonyl D-glucopyranosyl)theophylline (VI) (0·2 g.) being used in place of the ditoluene-*p*-sulphonyl compound. The product was again 7-*β*-(4 : 6-benzylidene 2 : 3-anhydro-D-allopyranosyl)theophylline, m. p. and mixed m. p. 225—226° (Found : C, 58·3; H, 4·7%).

7-*β*-D-*Glucopyranosyltheophylline-3′ Phosphate* (IX).—A mixture of the above anhydro-alloside (VIII) (0·2 g., 1 mol.), dibenzyl hydrogen phosphate (0·135 g., 1 mol.), and dioxan (7 c.c.) was heated in a sealed tube at 100° for 8 days. The crystalline product which had separated (80 mg.; m. p. 210—211°) was collected and recrystallised from water containing a small amount of acetone, giving 7-*β*-D-*glucopyranosyltheophylline-3′ phosphate* as colourless needles, m. p. 218° (decomp.), both benzyl and benzylidene groups having apparently been removed during the reaction (Found : C, 37·5; H, 5·3. $C_{13}H_{19}O_{10}N_4P$ requires C, 37·0; H, 4·5%). Potentiometric titration showed the presence of a secondary phosphoryl dissociation p*K* 6·0, and paper chromatography, propanol–ammonia–water or 5% aqueous disodium hydrogen phosphate–*iso*amyl alcohol being used, showed only one spot. Treatment of the paper chromatogram with periodate (Buchanan, Dekker, and Long, *J.*, 1950, 3162) showed the absence of a 1 : 2-glycol grouping. The dioxan mother-liquors from the original reaction smelt strongly of benzaldehyde but did not seem to contain any other glycoside phosphate; they were not, however, investigated in detail.

1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6 *Dibenzyl Phosphate* (X; R = R′ = $PhCH_2$).—To an ice-cold solution of dibenzyl chlorophosphonate (from 20 g. of dibenzyl phosphite) in 2 : 6-lutidine (15 c.c.) a solution of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucose (10 g.) (*Org. Synth.*, **22**, 57) in 2 : 6-lutidine (40 c.c.) was added dropwise with vigorous stirring during 45 minutes. The temperature was allowed to rise slowly to 15° during 1½ hours, and stirring continued at this temperature for a further 1½ hours. The stirred mixture was now cooled in ice, water (10 c.c.) was added dropwise, and after 45 minutes' stirring more water (100 c.c.) was added to dissolve the 2 : 6-lutidine hydrochloride and the solution was extracted with ice-cold chloroform (3 × 100 c.c.). The combined extracts were washed thoroughly with cold hydrochloric acid (3 × 100 c.c.; 3N.), then with sodium hydrogen carbonate, and finally with ice-water, dried, and evaporated. Ether (40 c.c.) was added to the residual oil, and the amorphous precipitate was filtered off and washed with a little ether. The combined filtrate and washings were evaporated, the residual syrup was taken up in methanol (35 c.c.), and water added until the mixture was opalescent. Set aside in the refrigerator overnight (more rapidly on being seeded), hydrated crystals of 1 : 2 : 3 : 4-*tetra-acetyl* β-D-*glucopyranose*-6 *dibenzyl phosphate* separated (6·3 g.); a further quantity (1·1 g.) was obtained by evaporating the mother-liquor, dissolving the residue in benzene (100 c.c.), shaking the solution with neutral alumina (10 g.), then evaporating, and crystallising the residue as before from aqueous methanol. Recrystallised from aqueous methanol, the ester formed colourless hydrated needles, m. p. 89—90° (Found : C, 54·0; H, 5·5; loss at 105°/0·1 mm., 2·6. $C_{28}H_{33}O_{12}P,H_2O$ requires C, 53·7; H, 5·6; H_2O, 2·9%). The ester had $[\alpha]_D^{26}$ +24·4° (*c*, 1·8 in methanol). The monohydrate slowly lost water *in vacuo* over phosphoric oxide at room temperature; the anhydrous ester could be recrystallised from dry ether and then had m. p. 105—107°.

A sample of the ester (1·22 g.) was hydrogenated in methanolic solution by aid of a palladium oxide catalyst. Catalyst was removed by filtration, the filtrate evaporated, and the residue (0·86 g.) recrystallised from methanol–light petroleum, giving 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 phosphate, m. p. 126—127° undepressed in admixture with a sample prepared by hydrogenating 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 diphenyl phosphate (Lardy and Fischer, *loc. cit.*).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6) *Benzyl Hydrogen Phosphate* (X; R = H, R′ = $PhCH_2$).—A solution of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 dibenzyl phosphate (1 g.) and lithium chloride (0·35 g.) in ethoxyethanol (20 c.c.) was heated on a boiling-water bath for 2 hours. During the heating, crystalline material separated and a strong odour of benzyl chloride developed. The mixture was cooled, and the crystalline *lithium* salt (0·73 g., 88%) collected, washed with ethoxyethanol, and recrystallised from methylated spirit, from which it formed colourless needles, m. p. 270° (decomp.) (Found : C, 48·5; H, 5·0. $C_{21}H_{26}O_{13}PLi$ requires C, 48·1; H, 5·0%).

The above lithium salt (2·5 g.) was dissolved in water (50 c.c.), cooled to 0°, and acidified with ice-cold hydrochloric acid (6 c.c.). The oil which separated immediately crystallised on being shaken and was then collected and washed with a little ice-water. The yield of product was 1·68 g. and a further 0·5 g. was obtained from the mother-liquor by chloroform extraction. Recrystallised by slow addition of light petroleum (b. p. 60—80°) to its solution in cold ethoxyethanol, 1 : 2 : 3 : 4-*tetra-acetyl* β-D-*gluco-pyranose*-6 *benzyl hydrogen phosphate* forms colourless needles, m. p. 132—134° (Found : C, 48·4; H, 5·4. $C_{21}H_{27}O_{13}P$ requires C, 48·7; H, 5·3%). The monobenzyl ester is rather unstable and decomposes when its solution in ethoxyethanol is boiled or when it is heated for a time at 110°. It can also be prepared in a yield of 50—60% by heating the dibenzyl ester with 4-methylmorpholine for 2 hours at 100°; in this case it is most conveniently isolated as its cyclo*hexylamine* salt, which separates from water as fine colourless needles, m. p. 208—209° (Found : C, 52·4; H, 6·6; N, 2·3. $C_{27}H_{40}O_{13}NP$ requires C, 52·5; H, 6·5; N, 2·3%).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6) *Benzyl* 2-*Hydroxy*cyclo*hexyl Phosphate* (XI; R = $PhCH_2$).—The *cyclo*hexylamine salt of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (0·5 g.) was dissolved in water (70 c.c.), and the solution cooled to 0°, acidified with ice-cold sulphuric acid (2 c.c.; N.), and rapidly extracted with ice-cold chloroform (3 × 25 c.c.). The extract was dried and evaporated and the free monobenzyl phosphate so obtained was dissolved in dry alcohol-free chloroform (10 c.c.). To this solution freshly distilled *cyclo*hexene oxide (0·2 g.) was added, and the mixture set aside for 5 days with exclusion of moisture. The solution was now washed with dilute aqueous sodium carbonate, then with water, and dried, and solvent and unchanged *cyclo*hexene oxide removed by evaporation under reduced pressure. The partially crystalline residue was taken up as far as possible in benzene, the solution filtered, and the filtrate evaporated, leaving a residue (0·47 g.) which was dissolved in hot methanol. Water was added until turbidity appeared; the solution was clarified again by addition of a few drops of methanol, boiled with charcoal, filtered hot, and set aside. On cooling the *ester* (XI; R = $PhCH_2$) separated as colourless needles (0·38 g.), m. p. 76—77° (Found : C, 52·4; H, 6·3. $C_{27}H_{37}O_{14}P$ requires C, 52·6; H, 6·1%).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6) 2-*Hydroxy*cyclo*hexyl Hydrogen Phosphate* (XI; R = H).—The neutral ester (XI; R = $PhCH_2$) (0·175 g.) was dissolved in purified dioxan (20 c.c.) and hydrogenated in presence of a palladium oxide catalyst at room temperature. Hydrogenation was complete in 2 hours, the catalyst was filtered off, and the filtrate evaporated under reduced pressure at room temperature. The resinous acidic residue was dissolved in acetone (5 c.c.), and a solution of *cyclo*-hexylamine (50 mg.) in light petroleum (b. p. 40—60°) added. The gelatinous precipitate was collected and washed with acetone. It was now dissolved in water (5 c.c.), and the solution boiled with charcoal, filtered, and again evaporated to dryness under reduced pressure. The residual cyclo*hexylamine* salt was recrystallised from ethanol–acetone and formed colourless needles (0·12 g., 70%), m. p. 204—205° (decomp.); the salt was readily soluble in water, methanol, and ethanol and insoluble in ether, acetone, and light petroleum (Found : C, 50·2; H, 7·4; N, 2·6. $C_{28}H_{44}O_{14}NP$ requires C, 49·9; H, 7·1; N, 2·2%).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6)(1 : 2-iso*Propylidene* α-D-*Glucofuranose*-6) *Benzyl*

Phosphate (XII; R = $PhCH_2$).—The *cyclo*hexylamine salt of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl hydrogen phosphate (X; R = H, R′ = $PhCH_2$) (0·75 g.) was converted into the free acid as before, and the latter dissolved in dry alcohol-free chloroform (5 c.c.). 1 : 2-*iso*Propylidene 5 : 6-anhydro-α-D-glucofuranose (I) (0·4 g.) was added, and the solution heated under reflux for 45 minutes then set aside at room temperature for 24 hours, moisture being excluded. The chloroform solution was now washed with aqueous sodium hydrogen carbonate then water, dried, and evaporated under reduced pressure at room temperature. The syrupy residue was dissolved in ethanol (15 c.c.), and the solution filtered from a small precipitate and concentrated to small bulk (3—4 c.c.). The crude *ester* was now recrystallised from methanol and yielded fine colourless needles (0·38 g.), m. p. 169—170° (Found: C, 49·8; H, 5·8; P, 4·0. $C_{30}H_{41}O_{18}P$ requires C, 50·0; H, 5·7; P, 4·3%). The preparation was repeated with the following variations: (*a*) use of half the amount of anhydro-sugar (yield, 0·21 g.); (*b*) use of double the amount of anhydro-sugar (yield, 0·4 g.); (*c*) quantities as before but the solution refluxed for 3 hours (decomposition occurred and no crystalline product was isolated).

The product was soluble in chloroform, methanol, dioxan, and benzene and insoluble in water and light petroleum. In aqueous dioxan solution, the compound was stable to sodium metaperiodate; after 24 hours at room temperature the uptake of oxidant was only 0·04 mol./mol.

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6)(1 : 2-iso*Propylidene* α-D-*Glucofuranose*-6) *Hydrogen Phosphate* (XII; R = H).—(*a*) The above benzyl ester (135 mg.) was hydrogenated in dioxan solution (15 c.c.) by use of a palladium oxide catalyst (50 mg.) at room temperature and pressure. Hydrogenation was complete in 40 minutes, the hydrogen uptake (4·5 c.c.) being slightly more than the theoretical for 1 mol. Catalyst was removed by filtration, the filtrate evaporated under reduced pressure, and the crystalline residue of the *acid ester* (XII; R = H) (0·114 g.) washed thoroughly first with dioxan–light petroleum then with light petroleum alone, and dried at room temperature over phosphoric oxide and paraffin wax (Found: C, 43·3; H, 5·9. $C_{23}H_{35}O_{18}P$ requires C, 43·8; H, 5·6%). The product had a rather indefinite m. p., 132—135°, which was not affected by reprecipitation from dioxan solution with light petroleum; it was rather unstable, decomposed slowly when kept, and could not be recrystallised from boiling solvents.

When the product (100 mg.) was dissolved in dioxan (10 c.c.) and a solution of *cyclo*hexylamine (50 mg.) in dioxan (5 c.c.) added, a gelatinous precipitate was formed. This was collected and recrystallised from ethanol, giving the cyclo*hexylamine* salt (102 mg.) as colourless needles, m. p. 217° (decomp.) (Found: C, 47·7; H, 6·9; N, 2·0. $C_{29}H_{48}O_{18}NP$ requires C, 47·7; H, 6·6; N, 1·9%).

(*b*) A solution of 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 phosphate (X; R = R′ = H) (535 mg.) and 1 : 2-*iso*propylidene 5 : 6-anhydro-α-D-glucofuranose (I) (265 mg.) in anhydrous dioxan was set aside for 7 days at room temperature. The slightly turbid solution was filtered and evaporated under reduced pressure. The oily residue was dissolved in ice-cold chloroform (50 c.c.), the solution extracted with ice-cold aqueous sodium hydrogen carbonate (50 c.c. of 1%), and the extract acidified with ice-cold sulphuric acid (10 c.c.; N.) and rapidly extracted with ice-cold chloroform (240 c.c.). The chloroform extract was washed with water, dried (Na_2SO_4) for 30 minutes, and evaporated. The residue was dissolved in dioxan (10 c.c.), and *cyclo*hexylamine (100 mg.) in dioxan (5 c.c.) added. The precipitated crude *cyclo*hexylamine salt (453 mg.) was thrice recrystallised from ethanol and then formed colourless needles (134 mg.), m. p. 216—217°, undepressed in admixture with the *cyclo*hexylamine salt prepared as described under (*a*).

(1 : 2 : 3 : 4-*Tetra-acetyl* β-D-*Glucopyranose*-6)(2 : 3 : 4 : 6-*Tetra-acetyl* β-D-*Glucopyranose*-1) *Hydrogen Phosphate* (XIII).—A mixture of α-acetobromoglucose (0·4 g.), silver 1 : 2 : 3 : 4-tetra-acetyl β-D-glucopyranose-6 benzyl phosphate (X; R = H, R′ = $PhCH_2$) (1·2 g.), and dry benzene (30 c.c.) was warmed to 50° for 30 minutes in a flask fitted with a reflux condenser and an efficient mechanical stirrer. The mixture was now refluxed for 40 minutes with continued vigorous stirring. The dark-coloured solution was filtered from silver bromide, boiled for a few minutes with charcoal, then evaporated to dryness under reduced pressure. The syrupy residue could not be crystallised and so was hydrogenated directly in dioxan solution with a palladium oxide catalyst (100 mg.) to remove the benzyl group; hydrogen uptake (28 c.c.) was complete in 1½ hours. The filtered solution was evaporated at room temperature under reduced pressure, the residue taken up in chloroform (25 c.c.), and the solution extracted with ice-cold sodium hydrogen carbonate (30 c.c.; 1%). The aqueous layer was separated, acidified with ice-cold sulphuric acid (5 c.c.; N.), and quickly extracted with cold chloroform (2 × 30 c.c.). The chloroform extract was washed with ice-water, dried (Na_2SO_4) for 30 minutes, and evaporated under reduced pressure at room temperature. The yellowish syrupy residue was dissolved in dioxan (5 c.c.), and a solution of *cyclo*hexylamine (50 mg.) in light petroleum (10 c.c.; b. p. 60—80°) added. The precipitated salt was purified by dissolution in acetone–methanol (5 c.c.; 1 : 1) and addition of light petroleum (b. p. 40—60°) to turbidity; filtration and addition of more light petroleum then reprecipitated it. After three such treatments, the cyclo*hexylamine* salt of (1 : 2 : 3 : 4-*tetra-acetyl* β-D-*glucopyranose*-6)(2 : 3 : 4 : 6-*tetra-acetyl* β-D-*glucopyranose*-1) *hydrogen phosphate* was obtained as a white microcrystalline powder (120 mg.), m. p. 204—206° (decomp.) (Found: C, 47·3; H, 6·5; N, 1·8. $C_{34}H_{52}O_{22}NP$ requires C, 47·6; H, 6·1; N, 1·6%).

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