Sugar Phosphates. Part I. Derivatives of Glucose 4 : 6-(Hydrogen Phosphate).

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Phosphoryl migration in the sugar phosphates is discussed in the light of recent evidence for the steric conformation of sugars. The possible participation of 6-membered cyclic phosphates as intermediates in these migrations is considered.

Cyclic phosphates of methyl and phenyl glucoside have been prepared and shown to be derivatives of glucose 4 : 6-(hydrogen phosphate) (III; R = H). Hydrogenolysis of phenyl β-D-glucoside 4 : 6-(phenyl phosphate) (IV) gave glucose 4 : 6-(hydrogen phosphate). The action of periodate on some of these compounds has been examined.

The *cyclo*hexylamine salt of glucose 4 : 6-(hydrogen phosphate) undergoes an Amadori rearrangement to the ketose derivative (IX) which, for steric reasons, is unable to assume a furanose configuration.

The structure of some other cyclic phosphates is discussed.

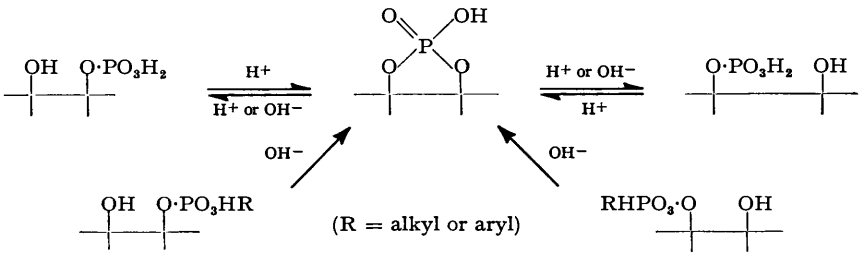
The great importance of phosphorylated intermediates in biological processes was first recognised in the field of carbohydrate metabolism. Since the first isolation of a sugar phosphate from natural sources these substances have received fairly extensive chemical and biochemical study. An excellent review of work up to 1951 is given by Leloir (*Fortschr. Chem. Org. Naturstoffe*, 1951, **8**, 47). The object of the present investigations is to study the preparation and properties of sugar phosphates, with specific reference to the configuration and conformation of the substituent groups. In particular it is proposed to examine certain group migrations and rearrangements which are believed to occur in these compounds, and to interpret the findings in the light of modern ideas of the structure of the furanose and pyranose rings (Reeves, *J. Amer. Chem. Soc.*, 1950, **72**, 1499).

Both direct and indirect evidence for migration of the phosphoryl group in sugar phosphates has been available for many years. For example, Levene and Raymond (*J. Biol. Chem.*, 1934, **107**, 75) found that attempts to synthesise xylose 3-phosphate invariably led to the formation of the 5-phosphate; also, Tankó and Robison (*Biochem. J.*, 1935, **29**, 961) suggested that migration of the phosphate group from position 6 in fructose 6-phosphate (and fructose 1 : 6-diphosphate) might explain certain changes in optical rotation observed in samples which had been subjected to acid treatment (Macfarlane and Robison, *Enzymologia*, 1937, **4**, 125; Tankó, *Acta Physiol. Acad. Sci. Hung.*, 1952, **3**, 15). Similar indirect evidence of phosphoryl migration was obtained during the mild acid hydrolysis of trehalose phosphate to glucose 6-phosphate (Tankó and Robison, *loc. cit.*; Robison and Morgan, *Biochem. J.*, 1928, **22**, 1277).

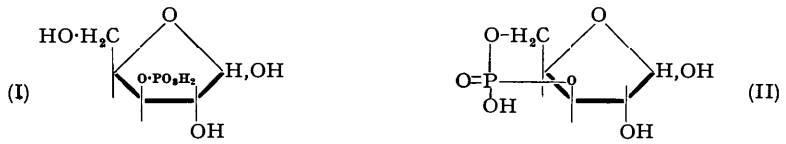
The importance of this type of migration has been largely overlooked until recently. The work of Chargaff (*J. Biol. Chem.*, 1942, **145**, 455) and of Baer and Kates (*ibid.*, 1950, **185**, 615) on the glycerophosphates and of Brown and Todd (*J.*, 1952, 52) and Cohn (*J. Amer. Chem. Soc.*, 1950, **72**, 2811) on the *a* and *b* nucleotides has demonstrated the very ready migration of phosphate groups in certain circumstances. It is clear from this work that migration is intramolecular and occurs by way of an intermediate cyclic phosphate. These isomerisations are summarised in the following scheme, in which no attempt has been made to formulate transition states or the mechanism by which the group R is eliminated.

In the glycerophosphates and nucleotides, the cyclic intermediate possess a five-membered phosphate ring. Nucleoside cyclic phosphates have been synthesised (Brown, Magrath, and Todd, *J.*, 1952, 2708) and it has been shown that they are readily hydrolysed by acids and alkalis to mixtures of the 2'- and 3'-monophosphates. Similar cyclic phosphates are formed by mild alkaline treatment of nucleotide coenzymes in which a hydroxyl group lies adjacent to a pyrophosphoric ester residue. Examples are the formation of

riboflavin-4′ : 5′ hydrogen phosphate from flavin–adenine dinucleotide (Forrest and Todd, *J.*, 1950, 3295) and glucose 1 : 2-(hydrogen phosphate) from uridine-diphosphate–glucose (Paladini and Leloir, *Biochem. J.*, 1952, **51**, 426).



The observation by Levene and Raymond (*loc. cit.*) that migration of a phosphate group from position 3 to position 5 occurred during an attempted synthesis of xylose 3-phosphate (I) has been explained by postulating a cyclic intermediate, xylose 3 : 5-(hydrogen phosphate) (II). Until recently, authentic 6-membered phosphate rings such as that shown in



(II) were not known. It is now established that cyclic 2′ : 4′-(hydrogen phosphates) of pantothenic acid and pantetheine are produced by the action of alkali on the pyrophosphate linkage in coenzyme A (Baddiley and Thain, *J.*, 1952, 3783). These substances, which have been synthesised (*idem*, *J.*, 1951, 3421), are known to contain a 6-membered phosphate ring. A synthetic cyclic phosphate of chloramphenicol, also containing a 6-membered ring, has been described recently (Mosher, Reinhart, and Prosser, *J. Amer. Chem. Soc.*, 1953, **75**, 4899). The cyclic phosphates of pantothenic acid and its derivatives appeared to be much more stable than the nucleoside cyclic phosphates and it became of interest to examine the stability of 6-membered cyclic phosphates in the sugars, with a view to their possible rôle as intermediates in the aforementioned migrations.

The existence of a 6-membered phosphate ring in sugar derivatives will depend very much on the size of the lactol ring and on the steric relation of the esterified hydroxyl groups. For example, although 3 → 5 phosphate migration occurs readily in xylofuranose derivatives (Levene and Raymond, *loc. cit.*) it has been observed in ribofuranose derivatives. This is probably due to the larger distance between the 3- and the 5-hydroxyl group which lie on opposite sides of the planar ribofuranose ring. It has a parallel in the ready formation of 3 : 5-cyclic acetal derivatives in the xylose series, in contrast to the behaviour of ribose and arabinose. The situation in the aldohexopyranosides is rather different. Here the chair conformation of the pyranose ring allows both glucose and galactose to form 4 : 6-benzylidene derivatives. Two fused chair rings are present in both these derivatives.

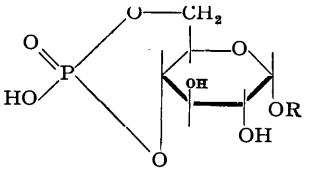
From the above considerations it seemed probable that aldohexopyranosides should form stable 4 : 6-cyclic phosphates. The possible participation of such esters in phosphoryl migration warranted their preparation and study. When methyl α-D-glucoside was allowed to react with phenyl phosphorodichloridate in pyridine the only recognisable product was a crystalline neutral ester, produced in 10—20% yield. A phenyl group was removed by catalytic hydrogenolysis, yielding a monobasic phosphoric ester of methyl α-D-glucoside, isolated as its crystalline *cyclo*hexylamine salt. Its sodium salt slowly consumed one mol. of periodate, showing the presence of an α-glycol system. It follows that the monobasic ester is methyl α-D-glucoside 4 : 6-(hydrogen phosphate) (III; R = Me) and the neutral ester is methyl α-D-glucoside 4 : 6-(phenyl phosphate).

From phenyl β-D-glucoside and phenyl phosphorodichloridate a 40% yield of phenyl β-D-glucoside 4 : 6-(phenyl phosphate) (IV) was obtained. On hydrogenolysis in the presence of platinum this neutral ester yielded mainly glucose 4 : 6-(hydrogen phosphate) (III; R = H), isolated as its barium salt. The structure of this product was confirmed by its behaviour on paper chromatography and by periodate oxidation.

On paper it moved faster than glucose 6-phosphate in both propanol–ammonia–water and butanol–acetic acid–water and gave a positive test for reducing sugar with the *p*-anisidine spray (Hough, Jones, and Wadman, *J.*, 1950, 1702). It consumed two mols. of periodate fairly rapidly.

When the total reaction mixture resulting from the hydrogenation of (IV) was examined by paper chromatography the presence of an acidic by-product was noted. This moved

(III)



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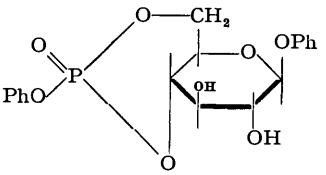

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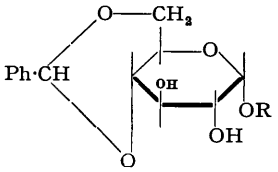
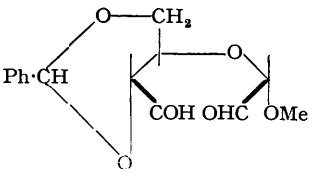


(VI)

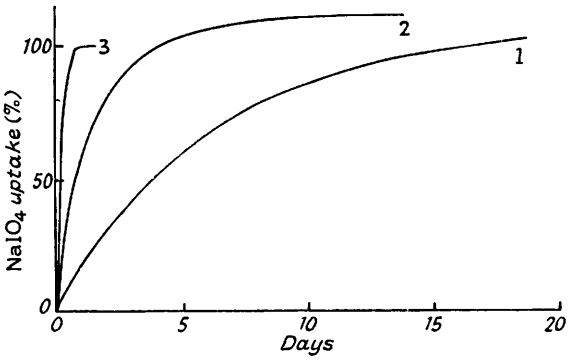


faster than glucose 4 : 6-(hydrogen phosphate) and did not possess reducing properties. When the hydrogenation was carried out with different samples of catalyst the proportion of the fast-moving substance varied and it was sometimes possible to isolate it as a crystalline *cyclo*hexylamine salt. The above properties, together with the analysis of the *cyclo*hexylamine salt, indicate that the by-product is *cyclo*hexyl β-D-glucoside 4 : 6-(hydrogen phosphate).

Marked differences were observed in the rate of periodate oxidation of the various cyclic phosphates. These differences probably arose through steric factors, and it was of some interest to compare the effects of the several possible contributory steric influences. Consequently, the rates of oxidation of several 4 : 6-cyclic phosphates of glucose were compared with those of 4 : 6-*O*-benzylidene derivatives of glucose. The three steric factors affecting periodate oxidation in the benzylidene and cyclic phosphate series are: the presence of a substituent at position 1; the presence of a 4 : 6-ring; the presence of a substituent on this ring. The combined effect of these three factors is seen in methyl α-D-glucoside 4 : 6-(phenyl phosphate) which is unaffected even by prolonged treatment with periodate. This is comparable with 7-(4 : 6-*O*-benzylidene-β-D-glucopyranosyl)theophylline which is also stable towards periodate (Harvey, Michalski, and Todd, *J.*, 1951, 2271). Both these substances bear substituents at position 1 and contain 4 : 6-rings with an attached phenyl group. After removal of the phenyl group oxidation may proceed, although rather slowly. This is shown in the case of methyl α-D-glucoside 4 : 6-(hydrogen phosphate) (see Figure). The rate of oxidation is greater in glucose 4 : 6-(hydrogen phosphate) itself. The effect of substitution at $C_{(1)}$ is also shown in the case of methyl 4 : 6-*O*-benzylidene-α-D-glucoside (V; R = Me) which is oxidised more slowly than is 4 : 6-*O*-benzylideneglucose (V; R = H). It is interesting that the dialdehyde (VI) obtained from methyl 4 : 6-*O*-benzylidene-α-D-glucoside crystallises during the oxidation.

Attempts to prepare the *cyclo*hexylamine salt of glucose 4 : 6-(hydrogen phosphate) were unsuccessful. When a solution of the free acid was neutralised with *cyclo*hexylamine and the solvent removed at a low temperature, a crystalline residue was obtained. This was shown by paper chromatography to consist of the original cyclic phosphate and another

substance, which contained both phosphorus and nitrogen and reduced methylene-blue in the cold under slightly alkaline conditions. Complete conversion into the strongly reducing substance was effected by heat. Analysis of the crystalline product indicated that 1 mol. of *cyclo*hexylamine had combined with 1 mol. of glucose 4 : 6-(hydrogen phosphate). It reduced methylene-blue and Fehling's and Benedict's solutions rapidly in the cold, and was very unstable in alkaline solution. These properties agree well with those described for substituted 1-amino-1-deoxyketoses, formed by the Amadori rearrangement of aldosylamines. We conclude that the initially formed *cyclo*hexylamine salt rearranges to an unstable glycosyl*cyclo*hexylamine (VII) which readily changes into 1-deoxy-1-*cyclo*hexylamino*keto*fructose 4 : 6-(hydrogen phosphate) (VIII). Strong support for this sequence is afforded by Helferich and Porck's observation (*Annalen*, 1953, **582**, 233) that the oxalate

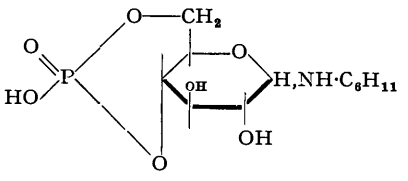


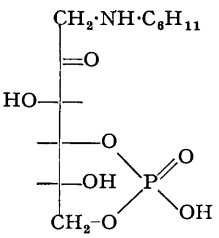
Oxidation of cyclic phosphates of glucose with periodate.
1, *Methyl* α-D-*glucoside* 4 : 6-(*hydrogen phosphate*) *at* pH 7.
2, *Methyl* 4 : 6-O-*benzylidene*-α-D-*glucoside at* pH 7.
3, *Glucose* 4 : 6-(*hydrogen phosphate*), no buffer.

of *N*-(4 : 6-*O*-benzylidene-D-glucopyranosyl)benzylamine readily undergoes an Amadori rearrangement to the corresponding 1-deoxy-1-benzylaminofructose derivative.

Although in the past it has been considered that Amadori rearrangements are confined to glycosyl derivatives of aromatic amines it is now known that this is incorrect. A number of aliphatic amines has been shown to undergo this rearrangement under suitable conditions

(VII)





(VIII)

(Hodge and Rist, *J. Amer. Chem. Soc.*, 1952, **74**, 1494; 1953, **75**, 316). It will be seen that the ketose derivative (IX) is remarkable in that, although it bears an unsubstituted hydroxyl group at position 5 and a carbonyl group at position 2, it is unable to form a furanose ring. This follows from a study of models of fructofuranose, in which the 4- and the 6-hydroxyl group lie on opposite sides of the flat ring, and hence cannot also participate in a 6-membered cyclic phosphate ring. The ultra-violet spectrum of (VIII) confirms the presence of a carbonyl group, showing a maximum at 287 mμ similar in position and intensity to those observed with penta- and tetra-*O*-acetyl*keto*fructose (Bredereck, Hoschele, and Huber, *Ber.*, 1953, **86**, 1271) and derivatives of *aldehydo*glucose (Hudson, Wolfrom, and Lowry, *J.*, 1933, 1179).

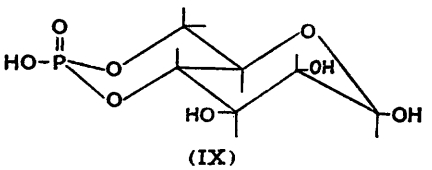
The *keto*fructose derivative (VIII) and *cyclo*hexyl β-D-glucoside 4 : 6-(hydrogen phosphate) were prepared most conveniently by heating the mixture resulting from direct hydrogenation of phenyl β-D-glucoside 4 : 6-(phenyl phosphate), after neutralisation with

*cyclo*hexylamine. The *cyclo*hexylamine salt of the *cyclo*hexyl glycoside was separated from (VIII) by means of its greater solubility in alcohol.

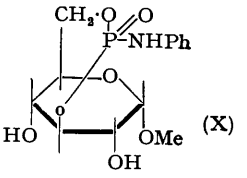
From the experiments reported in this paper it is apparent that glucopyranosides, by virtue of the equatorial conformation of hydroxyl and hydroxymethyl groups at positions 4 and 5, can form stable 4 : 6-cyclic phosphates. Furthermore, these are formed in preference to the more strained 7-membered 3 : 6-(hydrogen phosphates), even though in the latter case the 3- and the 6-hydroxyl group are *cis* to each other.

A more precise representation than (III; R = H) for glucose 4 : 6-(hydrogen phosphate) is shown in formula (IX).

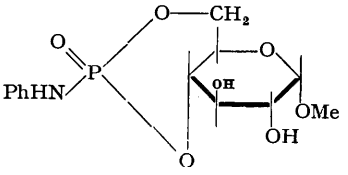
Full characterisation of the products of hydrolysis of hexopyranose 4 : 6-(hydrogen phosphates) would greatly assist the development of schemes relating to the migration of phosphoryl groups in the sugars. This problem is under investigation.



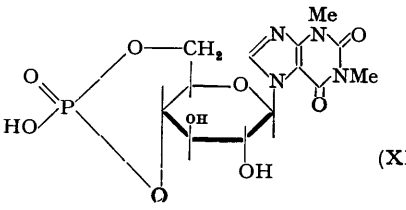
(IX)



(X)



(XI)



(XII)

Methyl α-D-glucoside yields a neutral ester by reaction with *N*-phenylphosphoramidodichloridate ($Ph{\cdot}NH{\cdot}POCl_2$) (Zeile and Kruckenberg, *Ber.*, 1942, **75**, 1127). This was formulated as methyl α-D-glucoside 3 : 6-(*N*-phenylphosphoramidate) (X). However, no evidence was presented for the presence of a 7-membered ring in the molecule. We consider that, by analogy with the compounds described in this paper, this compound is methyl α-D-glucoside 4 : 6-(*N*-phenylphosphoramidate) (XI).

A cyclic phosphate of 7-β-D-glucosyltheophylline has been obtained by the action of phosphoryl chloride on this purine glycoside (Fischer, *Ber.*, 1914, **47**, 3193). Although no structure was assigned to this compound by Fischer, it has been suggested (Kenner, *Fortschr. Chem. Org. Naturstoffe*, 1951, **8**, 96) that positions 3′ and 6′ are sterically suitable for esterification. Here again we consider the 4 : 6-(hydrogen phosphate) formulation (XII) more likely. The structure of this substance will be discussed more fully later.

Experimental

Methyl α-D-Glucoside 4 : 6-(Phenyl Phosphate).—Phenyl phosphorodichloridate (22 g.) was added with stirring at room temperature to methyl α-D-glucoside (20 g.) in pyridine (250 c.c.). The solution was kept overnight at room temperature and water (50 c.c.) was added. After 2 hr. solvent was removed *in vacuo* below 40°, chloroform and water were added, and solvents were removed again *in vacuo*. The residue was dissolved in alcohol, and water was added to opalescence (*ca.* 2 pts.). The resulting solution was passed through a column of Amberlite IR-4B (hydroxyl form) resin, and the effluent was concentrated under reduced pressure. Any oil which separated during the evaporation was removed by decantation when the volume had been reduced to about 150 c.c. The solution was then evaporated to dryness and the residue was crystallised from hot water or 0·01N-hydrochloric acid. The cyclic

phosphate (3—7 g.) had m. p. 196—197°, $[\alpha]_D^{20}$ +100·8° (*c*, 1·23 in EtOH) (Found: C, 46·7; H, 5·4. $C_{13}H_{17}O_8P$ requires C, 47·0; H, 5·1%).

Methyl α-D-*Glucoside* 4 : 6-(*Hydrogen Phosphate*).—The above phenyl ester was shaken in alcohol with hydrogen at room temperature and pressure in the presence of Adams platinum. The catalyst was filtered off and *cyclo*hexylamine was added to the solution to give pH about 8. Most of the solvent was removed *in vacuo* and ether was added until slight turbidity was produced. The solution was set aside at 0° overnight and the crystalline product was filtered off. The cyclo*hexylamine* salt (50—70%) had m. p. 228—230° (decomp.), $[\alpha]_D^{20}$ +83·1° (*c*, 1·13 in H_2O), after recrystallisation from alcohol–ether (Found: C, 44·2; H, 7·6; N, 4·0; P, 8·4. $C_{13}H_{26}O_8NP$ requires C, 43·9; H, 7·3; N, 3·9; P, 8·7%).

Phenyl β-D-*Glucoside* 4 : 6-(*Phenyl Phosphate*).—Phenyl phosphorodichloridate (25 g.) in dry pyridine (100 c.c.) was added with stirring to phenyl β-D-glucoside (35 g.) in pyridine (500 c.c.), the temperature rising to 35—40°. The mixture was kept at room temperature overnight, water (100 c.c.) was added, and the resulting solution was kept at room temperature for 2 hr., then evaporated to dryness *in vacuo*. The residue was triturated with several lots of water until only a faint smell of pyridine could be detected. The resulting solid was filtered off and recrystallised from acetic acid (250 c.c.). The crystalline *phosphate* (23·5 g.), m. p. 132—134°, resolidifying and melting at 193—194°, contained 0·5 mol. of acetic acid (Found: C, 53·8; H, 5·1; $C_{18}H_{19}O_8P,0{\cdot}5CH_3{\cdot}CO_2H$ requires C, 53·8; H, 5·0%). The acetic acid was removed by dissolving the substance in the minimum amount of hot alcohol and adding hot water until the solution became saturated at the b. p. On cooling, the phosphate crystallised (20—22 g.), m. p. 193—194°, $[\alpha]_D^{20}$ −86·3° (*c*, 1·04 in EtOH) (Found: C, 54·5; H, 4·9. $C_{18}H_{19}O_8P$ requires C, 54·8; H, 4·8%).

Glucose 4 : 6-(*Hydrogen Phosphate*).—Phenyl β-D-glucoside 4 : 6-(phenyl phosphate) (3 g.) in alcohol (100 c.c.) was reduced with hydrogen at room temperature and pressure in the presence of platinum (from 0·5 g. of platinum oxide). After filtration the acidic solution was neutralised (pH 7) with barium hydroxide solution. The precipitate which formed redissolved on standing or on addition of a little water. Alcohol (4 vols.) was added, the mixture was kept overnight at 0°, and the precipitate was removed by centrifugation. The *barium* salt (1·2 g.) was washed with alcohol, then acetone, and dried in the air. When prepared in this manner it is usually a dry white powder. However, occasionally it appeared to be hygroscopic. In this case it was allowed to remain in the air overnight and then triturated with acetone and filtered. A dry, stable product was thus obtained, which had $[\alpha]_D^{20}$ +16·1° (*c*, 3·4% in H_2O) (Found: C, 19·5; H, 4·3; P, 8·0. $C_6H_{10}O_8PBa_{\frac{1}{2}},3H_2O$ requires C, 19·8; H, 4·3; P, 8·5%).

Periodate Oxidations.—Samples of the sugar derivatives (25—250 mg.) were dissolved in suitable buffers (phosphate buffer at pH 7; acetate buffer at pH 3·6) or in water and oxidised at room temperature in the dark with an excess (20—100%) of sodium periodate. Approximately 0·01N-solutions were employed for samples of less than 100 mg., and 0·1N-solutions for the larger ones. Aliquot portions were removed periodically and periodate was determined by the usual methods.

Oxidation of Methyl 4 : 6-*O-Benzylidene*-α-D-*glucoside*. The glucoside (2·8 g.) was dissolved in hot water (750 c.c.). After cooling, sodium periodate (2·14 g.) was added and the solution was set aside for a week at 20° in the dark. The crystalline solid was filtered off, washed with water, and dried. The *dialdehyde* (2·9 g.) had m. p. 142° (Found: C, 53·2; H, 6·3. $C_{14}H_{16}O_6,2H_2O$ requires C, 53·2; H, 6·3%).

1-*Deoxy*-1-cyclo*hexylamino*keto*fructose* 4 : 6-(*Hydrogen Phosphate*).—Phenyl β-D-glucoside 4 : 6-(phenyl phosphate) (3 g.) in alcohol (100 c.c.) was reduced with hydrogen as above in the presence of Adams platinum oxide (0·3 g.). Catalyst was removed and the solution was neutralised with *cyclo*hexylamine to pH 6, refluxed under nitrogen for 5 hr., and kept overnight at room temperature. The crystalline deposit (800 mg.) was filtered off, dissolved in a little water (charcoal), and precipitated by acetone. The keto*fructose* derivative (600 mg.) had m. p. 160—165° (decomp.), $[\alpha]_D^{20}$ −39·8° (*c*, 1·41 in H_2O) (Found: C, 43·9; H, 7·4; N, 4·2; P, 9·0. $C_{12}H_{22}O_7NP$ requires C, 44·6; H, 6·8; N, 4·4; P, 9·6%).

cyclo*Hexyl* β-D-*Glucoside* 4 : 6-(*Hydrogen Phosphate*).—The alcoholic mother-liquors from the above preparations were concentrated *in vacuo* to a small volume, then set aside. The crystalline precipitate which formed was filtered off, dissolved in water (charcoal), and reprecipitated with acetone. The cyclo*hexylamine* salt of the phosphate (200 mg.) formed needles, m. p. 246—248° (decomp.) (Found: C, 51·2; H, 8·4; N, 3·5. $C_{18}H_{34}O_8NP$ requires C, 51·1; H, 8·0; N, 3·3%).

Paper Chromatography of Phosphates.—Ascending chromatograms on Whatman No. 4 paper (previously washed with 2N-acetic acid, then water, and dried) were run without temperature

control. The solvent systems were : A, butanol–acetic acid–water (4 : 1 : 5); B, *n*-propanol–ammonia–water (6 : 3 : 1). Results are tabulated.

	R_F in solvent : A	B
Glucose 4 : 6-(hydrogen phosphate)	0·14	0·29
Methyl α-D-glucoside 4 : 6-(hydrogen phosphate)	0·40	0·47
*cyclo*Hexyl β-D-glucoside 4 : 6-(hydrogen phosphate)	0·56	0·71
1-Deoxy-1-*cyclo*hexylamino*keto*fructose 4 : 6-(hydrogen phosphate)	0·53	Streak
Glucose	0·31	0·51

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