80. *Phosphorylated Sugars. Part III.*[1] *The Formation of Five-, Six-, and Seven-membered Cyclic Phosphates of* 1,2-O-*Isopropylidene-*D-*glucofuranose, and the Synthesis of Two New Cyclic Phosphates of* D-*Glucose.*

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Syntheses of a seven-membered cyclic phosphate, D-glucose 3,6-(hydrogen phosphate) (VI), and of a new six-membered cyclic phosphate of D-glucose, the 3,5-(hydrogen phosphate) (XI), are described. Six- and seven-membered cyclic phosphates of 1,2-*O*-isopropylidene-D-glucofuranose and a new monophosphate of this sugar derivative have been synthesised. Cyclisation of the monophosphates of 1,2-*O*-isopropylidene-D-glucofuranose with dicyclohexylcarbodi-imide has been studied and a novel reaction of a six-membered cyclic phosphate with this reagent has been reported. Previous findings [2] on the formation of five- and six-membered cyclic phosphates and on the reactions of the former with dicyclohexylcarbodi-imide have been confirmed for the monophosphates of 1,2-*O*-isopropylidene-D-glucofuranose. Part of this work has been published in a preliminary note.[3]

In view of the importance of cyclic phosphates during the degradation of natural polyhydroxylated substances, it seemed of interest to see whether, besides the well-known five- and six-membered cyclic esters, larger phosphate-containing rings could be formed in the sugar series. Although Zeile and Kruckenberg [4] assigned a formula containing a seven-membered cyclic phosphate ring to the neutral ester which they obtained by reaction of *N*-phenylphosphoramidodichloridate with methyl α-D-glucoside, they presented no evidence to prove the structure postulated. Kenner [5] suggested that the cyclic phosphate of 7-β-D-glucopyranosyltheophylline described by Fischer [6] might be a 3,6-cyclic phosphate. For both compounds the structure of the six-membered 4,6-phosphates was later suggested [7] in view of the formation of 4,6-phosphates from both α-methyl and β-phenyl glucosides in reaction with phenyl phosphorodichloridate. The only authentic seven-membered

[1] Part II, *J.*, 1960, 3762.
[2] Khorana, Tener, Wright, and Moffatt, *J. Amer. Chem. Soc.*, 1957, **79**, 430.
[3] Szabó and Szabó, *Compt. rend.*, 1959, **249**, 1243.
[4] Zeile and Kruckenberg, *Ber.*, 1942, **75**, 1127.
[5] Kenner, *Fortschr. Chem. org. Naturstoffe*, 1951, **8**, 96.
[6] Fischer, *Ber.*, 1914, **47**, 3193.
[7] Baddiley, Buchanan, and Szabó, *J.*, 1954, 3826.

phosphorus-containing ring is the butane-1,4-diol cyclic phosphate prepared by Khorana *et al.*[2] by cyclisation of butane-1,4-diol monophosphate with dicyclohexylcarbodi-imide in aqueous pyridine. Cherbuliez and his co-workers[8] later also prepared this cyclic phosphate by treating butane-1,4-diol monophosphate with polyphosphoric acid.

1,2-*O*-Isopropylidene-D-glucofuranose 3,6-(hydrogen phosphate) (III) containing a seven-membered phosphate ring has now been prepared in three different ways, the starting material for the syntheses being in each case 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate (I). This compound was prepared by Bell[9] by nitration of 6-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose,[10] to give the 3,5-dinitrate, and treatment of the latter with dimethylamine. We found that good yields of 6-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose could not be obtained by acetylation of the glucose–boric acid complex[11] with acetic anhydride and sodium acetate.[10] The yield was much improved if the acetylation was carried out with acetic anhydride in the presence of pyridine and the product formed extracted with 1,1,2,2-tetrachloroethane instead of with chloroform. Although nitric oxides were used for nitrating the 6-acetyl-monoisopropylidene glucose in the original preparation,[9] it was here found convenient to use a modification of Honeyman and Morgan's method of nitrating sugars,[12] as described in the Experimental part. It was also found that better yields of 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate were obtained if, after the 6-acetyl-isopropylene glucose 3,5-dinitrate had reacted with dimethylamine for 48 hr. and the excess of base and the solvents had been removed,[9] the residue was taken up in benzene and the nitrated derivative exhaustively extracted into water.

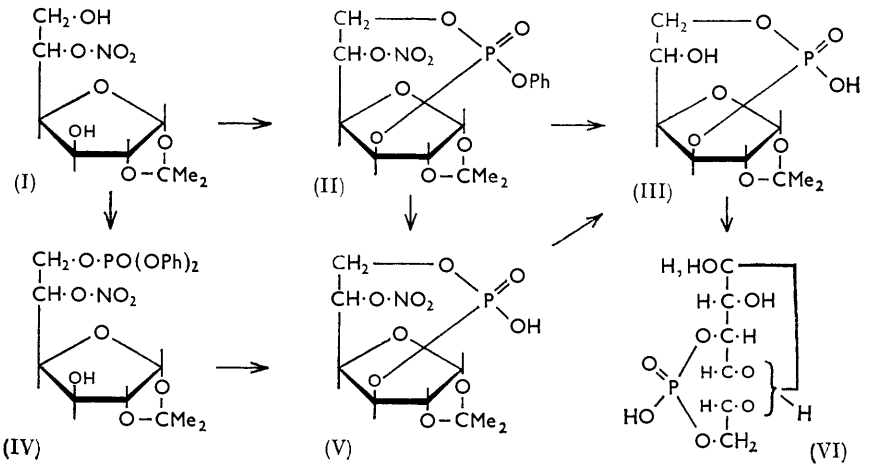
1,2-*O*-Isopropylidene-D-glucofuranose 5-nitrate was phosphorylated in the first place with monophenyl phosphorodichloridate, and the resulting oily 5-nitrate 3,6-(phenyl phosphate) (II) was treated with potassium hydroxide in aqueous dioxan to remove the phenyl group. The resulting 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate 3,6-(hydrogen phosphate) (V) was isolated as its crystalline cyclohexylammonium salt.

In the second instance, 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate was phosphorylated with diphenyl phosphorochloridate, and the crystalline 6-(diphenyl phosphate) (IV) thus obtained was treated with potassium hydroxide: an excellent yield of isopropylideneglucose 5-nitrate 3,6-(hydrogen phosphate) (V) was obtained, cyclisation and loss of the phenyl group occurring simultaneously. This is in contrast to results of Khorana *et al.*[2] who found that alkaline treatment of butane-1,4-diol 4-(diphenyl phosphate) yielded tetrahydrofuran and diphenyl phosphate ion; if a similar reaction had occurred, a 3,6-anhydro-compound, in this case 3,6-anhydro-1,2-*O*-isopropylidene-D-glucose 5-nitrate, should have been formed. This compound could not, however, be detected in the reaction mixture. It is also of interest that, while 1,2-*O*-isopropylidene-3-*O*-methyl-D-glucose 6-(diphenyl phosphate) is extremely unstable,[13] yielding, by reaction of the tertiary phosphate ester with the vicinal hydroxyl group, the 5,6-(hydrogen phosphate), the monoisopropylideneglucose 5-nitrate 6-(diphenyl phosphate), which does not possess a vicinal hydroxyl group, is stable and gives a cyclic phosphate only on treatment with alkali.

When the above-mentioned oily 1,2-*O*-isopropylidene-D-glucose 5-nitrate 3,6-(phenyl phosphate) (II) was hydrogenated in the presence of Adams platinum catalyst, about three mols. of hydrogen were absorbed and the crystalline ammonium salt of 1,2-*O*-isopropylidene-D-glucofuranose 3,6-(hydrogen phosphate) (III) was obtained. The fact that the same compound was isolated when the reduction proceeded in the presence of acetic acid shows that the elimination of phenol was not determined by the increase in alkalinity due to the formation of ammonia by reduction of the nitrate group.

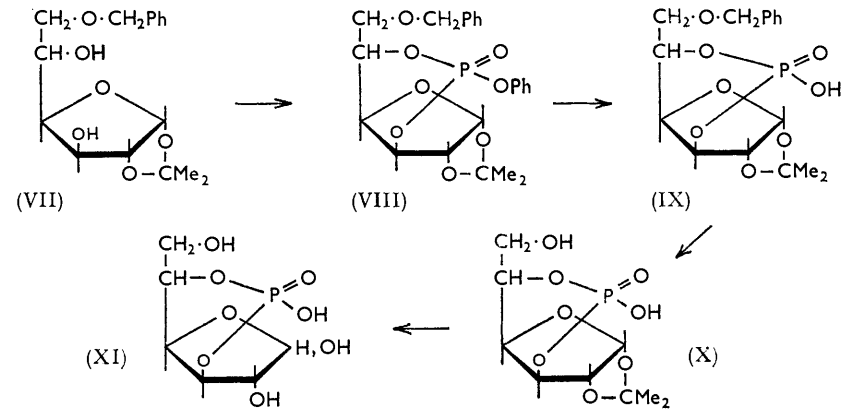
[8] Cherbuliez, Probst, and Rabinowitz, *Helv. Chim. Acta*, 1959, **42**, 1377; 1960, **43**, 464.
[9] Bell, *J.*, 1936, 1553.
[10] Bell, *J.*, 1936, 859; Freudenberg and Hüll, *Ber.*, 1941, **74**, 237.
[11] Vargha, *Ber.*, 1933, **66**, 704.
[12] Honeyman and Morgan, *Chem. and Ind.*, 1953, 1035.
[13] Lewak, Derache, and Szabó, *Compt. rend.*, 1959, **248**, 1837.

Monoisopropylideneglucose 3,6-(hydrogen phosphate) was also obtained after hydrogenation of 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate 3,6-(hydrogen phosphate) in the presence of a palladium catalyst and one equivalent of acetic acid.



The compound obtained by any of these routes was formulated as 1,2-*O*-isopropylidene-D-glucofuranose 3,6-(hydrogen phosphate) for the following reasons: electrometric titration and elementary analysis established that it was a cyclic phosphate of 1,2-*O*-isopropylidene-D-glucofuranose and it has been shown that this cyclic phosphate was neither the 3,5- nor the 5,6-cyclic phosphate, the only two other cyclic phosphates which could be formed from 1,2-*O*-isopropylidene-D-glucofuranose.

The 1,2-*O*-isopropylidene D-glucofuranose 3,5-(hydrogen phosphate) (X) mentioned above has been synthesised as follows. 6-*O*-Benzyl-1,2-*O*-isopropylidene-D-glucofuranose 3,5-(phenyl phosphate) (VIII), obtained by phosphorylation of 6-*O*-benzyl-monoisopropylideneglucofuranose [14] (VII) with monophenyl phosphorodichloridate, was treated with potassium hydroxide in aqueous dioxan to yield 6-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose 3,5-(hydrogen phosphate) (IX). This compound was isolated as its crystalline cyclohexylammonium salt and its structure proved by elementary analysis and



electrometric titration. It yielded 1,2-*O*-isopropylidene-D-glucofuranose 3,5-(hydrogen phosphate), also isolated as the cyclohexylammonium salt, when the benzyl group was removed by hydrogenation in the presence of a palladium catalyst.

[14] Ohle and Tessmar, *Ber.*, 1938, **71**, 1843.

The possibility that the compound formulated as the 3,6-cyclic phosphate was the 5,6-cyclic phosphate was excluded by the fact that, when the 5,6-(phenyl phosphate) resulting from the phosphorylation of 3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose [15] was submitted to alkaline hydrolysis under the same conditions as were used for the hydrolyses of the 3,6- and 3,5-(phenyl phosphate), the much less stable five-membered phosphate ring was hydrolysed and after hydrogenolysis a mixture containing presumably 1,2-*O*-isopropylidene-D-glucofuranose 5- and 6-phosphates was obtained. In addition, the compound designated as the 3,6-phosphate did not react with dicyclohexylcarbodi-imide to give *N*-phosphorylureas whereas, according to Khorana and his co-workers [2] and also according to our experience, this is a general reaction of five-membered cyclic phosphates. Indeed, as will be seen below, the cyclic phosphates obtained by treatment of the 1,2-*O*-isopropylidene-D-glucofuranose 5- and 6-monophosphate underwent this reaction with the carbodi-imide.

Mild acid-hydrolysis of the 1,2-*O*-isopropylidene-D-glucofuranose 3,5- and 3,6-cyclic phosphate removed the isopropylidene groups and D-glucose 3,5- (XI) and 3,6-phosphate (VI) were isolated as their barium salts. This 3,5-cyclic phosphate is a new six-membered cyclic phosphate of D-glucose. The only other example to our knowledge is D-glucose 4,6-(hydrogen phosphate) which has been synthesised by Baddiley *et al.*[7] and which Khorana and his collaborators [2] obtained by treatment of D-glucose 6-phosphate with dicyclohexylcarbodi-imide. The 3,6-cyclic phosphate is the first example of a seven-membered cyclic phosphate of any sugar. No attempt was made to prepare the cyclohexylammonium salt of these cyclic phosphates in view of the rearrangement which readily occurred during the attempted isolation of D-glucose 4,6-(hydrogen phosphate) in this form.[7] D-Glucose 3,5- and 3,6-cyclic phosphate have different mobilities on paper chromatograms. Rapid consumption of only one equivalent of periodate by both compounds proved that the sugar was substituted on the 3-hydroxyl group. However, whereas with the 3,5-phosphate no further periodate uptake was noticed, the 3,6-phosphate slowly underwent " overoxidation," the final quantity of periodate consumed being five equivalents. This phenomenon, together with the behaviour of other sugar phosphates towards periodate, will be discussed in a future publication.

The 1,2-*O*-isopropylidene-D-glucofuranose 3,5- and 3,6-cyclic phosphate described above offered an interesting possibility of studying the cyclisation of the monophosphates of 1,2-*O*-isopropylidene-D-glucofuranose, the more so as they were easily distinguishable from each other by paper chromatography.

Khorana and his co-workers [2] have shown that, when the structure of a monophosphate is such that by cyclisation it could give theoretically either a five- or a six-membered cyclic phosphate, the formation of a five-membered ring is favoured. It is not known, however, which phosphate would be formed by the cyclisation of a monophosphate theoretically capable of giving either a six- or a seven-membered cyclic phosphate, as in the case of 1,2-*O*-isopropylidene-D-glucofuranose 3-phosphate. We therefore prepared this compound by the method of Brown, Hayes, and Todd,[16] isolating the ester as its crystalline cyclohexylammonium salt instead of its barium salt. This ester was treated with the carbodi-imide in the manner described by Khorana and his collaborators [2] and the reaction was followed by paper chromatography (for details see the Table). A cyclic phosphate having the same mobility as 1,2-*O*-isopropylidene-D-glucofuranose 3,5-cyclic phosphate was first formed. With increasing reaction time, this substance gradually disappeared and another cyclic phosphate having the same mobility as 1,2-*O*-isopropylidene-D-glucofuranose 3,6-cyclic phosphate appeared. It thus seemed that a six-membered cyclic phosphate was the first product of the reaction and that this phosphate was subsequently transformed into a seven-membered cyclic phosphate.

[15] Meyer and Reichstein, *Helv. Chim. Acta*, 1946, **29**, 152.
[16] Brown, Hayes, and Todd, *Chem. Ber.*, 1957, **90**, 936.

Confirmation of this series of reactions was obtained by treating 1,2-*O*-isopropylidene-D-glucofuranose 3,5-cyclic phosphate with dicyclohexylcarbodi-imide under the same conditions. The 3,5-cyclic phosphate gradually disappeared and another phosphate having the same mobility as the 1,2-*O*-isopropylidene-D-glucofuranose 3,6-cyclic phosphate was formed. In addition, when 1,2-*O*-isopropylidene D-glucofuranose 3,6-(hydrogen phosphate) was treated with the carbodi-imide, no change was observed. 1,2-*O*-Isopropylidene-D-glucofuranose 3-phosphate was then treated on a larger scale with dicyclohexylcarbodi-imide and, when paper chromatography showed that the first reaction product had been completely converted into the second, the latter was isolated as its crystalline cyclohexylammonium salt. It was shown, by elementary analysis, melting point and mixed melting point, electrometric titration, and optical rotation to be 1,2-*O*-isopropylidene-D-glucofuranose 3,6-(hydrogen phosphate).

To complete this study, the cyclisation of 1,2-*O*-isopropylidene-D-glucofuranose 5- and 6-phosphate with dicyclohexylcarbodi-imide was examined.

The 5-phosphate was synthesised by phosphorylation of 3,6-di-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose [17] with diphenyl phosphorochloridate. The phenyl groups were removed from the crystalline diphenyl phosphate by catalytic hydrogenation, and the acetyl groups by alkaline hydrolysis, and the 1,2-*O*-isopropylidene-D-glucofuranose 5-phosphate was isolated as its crystalline monocyclohexylammonium salt.

A synthesis of 1,2-*O*-isopropylidene-D-glucofuranose 6-phosphate, isolated as its barium salt, by direct phosphorylation of 1,2-*O*-isopropylidene D-glucofuranose with phosphorus oxychloride has been described by Percival and Percival.[18] This ester has now been prepared by phosphorylation of 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-D-glucofuranose [19] with diphenyl phosphorochloridate and subsequent removal of the phenyl and benzylidene groups by hydrogenolysis; it was isolated as its crystalline dicyclohexylammonium salt.

Theoretically, two cyclic phosphates could be formed from the 5-phosphate: the five-membered 5,6-phosphate and the six-membered 3,5-phosphate. The 6-phosphate could also give two cyclic phosphates: a five-membered 5,6-phosphate, and a seven-membered 3,6-phosphate. According to Khorana and his co-workers,[2] the 5-phosphate should give the five-membered cyclic phosphate only, and this phosphate should react further with the carbodi-imide to form *N*-phosphorylureas; it is not known which phosphate would be preferred in the case of the 6-phosphate.

When the 5-phosphate was treated with dicyclohexylcarbodi-imide, a cyclic phosphate was first formed and this then reacted with more carbodi-imide to give *N*-phosphorylureas. The latter reaction, however, did not go to completion and did not therefore permit the conclusion that the 5,6-phosphate was the only cyclic phosphate formed. Moreover, it is our experience that five-membered cyclic phosphates are not quantitatively converted into *N*-phosphorylureas by this carbodi-imide. For example, when ethylene glycol cyclic phosphate (kindly presented by Dr. Jean Lecocq) was treated with the carbodi-imide in the usual conditions, *N*-phosphorylureas were formed, but considerable quantities of starting material remained even after long reaction periods (50 hr.). Thus it seems that the fact that *N*-phosphorylureas are formed proves that a five-membered cyclic phosphate is present but does not exclude the presence also of other cyclic phosphates. That only the 5,6-cyclic phosphate was formed in the reaction of the 5-phosphate with dicyclohexylcarbodi-imide was concluded from the following evidence: (1) The cyclic phosphate formed is not the 3,6-phosphate as the two compounds have different R_F values. (2) Although the cyclic phosphate formed has an R_F similar to that of the 3,5-phosphate, it cannot be the six-membered cyclic phosphate as it has been shown that the latter reacts with the carbodi-imide to give the 3,6-cyclic phosphate which was never detected during the experiments even after prolonged (53 hr.) reaction times.

[17] Freudenberg and von Oertzen, *Annalen*, 1951, **574**, 37.
[18] Percival and Percival, *J.*, 1945, 874.
[19] Brigl and Grüner, *Ber.*, 1932, **65**, 1428.

When treated with dicyclohexylcarbodi-imide, 1,2-*O*-isopropylidene-D-glucofuranose 6-phosphate behaved in the same way as the 5-phosphate. It was therefore concluded that again only the 5,6-cyclic phosphate was formed. It thus seems that a five-membered ring is formed in preference to a six- or to a seven-membered ring, and that the five-membered ring of 1,2-*O*-isopropylidene-D-glucofuranose 5,6-phosphate does not undergo transesterification analogous to that undergone by the six-membered 3,5-cyclic phosphate.

During treatment of 1,2-*O*-isopropylidene-D-glucofuranose 5-phosphate (or 6-phosphate) with the carbodi-imide, it was sometimes observed that a small amount of a substance having the same R_F as the 6-phosphate (or 5-phosphate) was formed. This observation is comparable with results of Pizer and Ballou [20] who observed the formation of some myoinositol 1-phosphate (or 2-phosphate) during the treatment of myoinositol 2-phosphate (or 1-phosphate) with dicyclohexylcarbodi-imide.

Experimental

Chloroform and ether solutions were dried over anhydrous sodium sulphate before removal of the solvent. Unless otherwise stated, evaporations were conducted under reduced pressure and specific rotations were determined for aqueous solutions.

At the end of the time specified for the phosphorylations, the solutions were cooled, a little water was added to decompose the excess of the phosphorylating agent, and the solution was left for 2 hr. before being worked up as described.

6-O-*Acetyl*-1,2-O-*isopropylidene*-D-*glucofuranose*.—Glucose (150 g.) was treated with acetone, boric acid, and concentrated sulphuric acid as described by Vargha.[11] To the crude product, isolated by removal of the unchanged acetone by distillation after neutralisation, acetic anhydride (210 ml.) and anhydrous pyridine (80 ml.) were added and the whole was heated on the water-bath. An exothermic reaction took place. It was allowed to proceed until the mixture became deep brown and was then controlled by cooling in tap-water. After remaining for 16 hr. at room temperature, the mixture was diluted with water (1·2 l.), and the aqueous solution was extracted 15 times with 1,1,2,2-tetrachloroethane (100 ml. each time). The organic phase was washed with a saturated solution of sodium hydrogen carbonate until it was neutral and then with water, dried (Na_2SO_4), and decolorised with Norite. The solvent was removed and the residue which crystallised spontaneously was recrystallised from ethyl acetate (*ca.* 150 ml.). The 6-acetate (60 g.) had m. p. 147—148° (lit.,[10] 145—146°).

6-O-*Acetyl*-1,2-O-*isopropylidene*-D-*glucofuranose* 3,5-*Dinitrate*.—A mixture of nitric acid (*d* 1·52; 16·6 ml.) and acetic anhydride (65 ml.) prepared at −40° was added to a suspension of 6-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose (26·2 g.) in acetic anhydride (65 ml.) cooled to −50°. The temperature was kept between −50° and −10° during the addition and until all the solids were dissolved and then between −20° and −10° for 1 hr. The solution was then cooled to −50° and chloroform (250 ml.), cooled to −50°, added. The chloroform solution was poured slowly on a solution of potassium carbonate (400 g.) in iced water (1400 ml.) containing ice (200 g.), with stirring. When the chloroform layer was neutral it was separated from the aqueous phase, washed with water, and dried. The chloroform was removed and the residue crystallised from ethanol. The 6-acetate 3,5-dinitrate (34 g., 96·5%) had m. p. 81—82° (lit.,[9] 81·5—82·5°).

1,2-O-*Isopropylidene*-D-*glucofuranose* 5-*Nitrate*.—30% Ethanolic dimethylamine (67 ml.) was added to a solution of the preceding compound (34 g.) in benzene (40 ml.), the mixture being cooled in running water during the addition. After 48 hr., the solution was evaporated to dryness below 50° under nitrogen, traces of volatile material being finally removed at 100°/0·1 mm. The crude product was dissolved in benzene and the solution exhaustively extracted with water. The combined aqueous extracts were decolorised with Norite and concentrated to a small volume. The compound which crystallised was filtered off, washed with a little water, and dried over phosphorus pentoxide. It was then dissolved in benzene (200 ml.), and the solution was concentrated on the water-bath at atmospheric pressure to 80 ml. The 5-nitrate (10 g.) which crystallised when the solution was cooled had m. p. 107·5—108° (lit.,[9] 106°).

[20] Pizer and Ballou, *J. Amer. Chem. Soc.*, 1959, **81**, 915.

1,2-O-*Isopropylidene*-D-*glucofuranose* 5-*Nitrate* 6-(*Diphenyl Phosphate*).—Diphenyl phosphorochloridate (5·9 g.) was added slowly to a solution of 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate (5·3 g.) in anhydrous pyridine (60 ml.). The mixture was kept at 37° for 2 days. The solvents were removed and the residue was dissolved in chloroform. The chloroform solution was cooled, washed twice with ice-water, then with ice-cold sulphuric acid (1% v/v) until the aqueous layer remained acid and finally three times with ice-water, and dried. The chloroform was removed and the residue crystallised from 80% ethanol. The 6-(*diphenyl phosphate*) (7·5 g., 75%) had m. p. 116—117°, $[\alpha]_D^{22}$ +1·5° (*c* 1·28 in $CHCl_3$) (Found: C, 50·7; H, 4·7; N, 2·9; P, 6·1. $C_{21}H_{24}NO_{11}P$ requires C, 50·7; H, 4·8; N, 2·8; P, 6·2%).

1,2-O-*Isopropylidene*-D-*glucofuranose* 5-*Nitrate* 3,6-(*Hydrogen Phosphate*).—(*a*) Phenyl phosphorodichloridate (3·8 g.) in pyridine (10 ml.) was added to 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate (4·75 g.) in pyridine (40 ml.). The solution was kept at 37° for 48 hr. The pyridine was removed, the residue dissolved in chloroform, and the solution washed with water and dried. The chloroform was removed, and the residual oily 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate 3,6-(phenyl phosphate) (6·1 g.) dissolved in dioxan (87 ml.), and 2N-potassium hydroxide (8·7 ml.) added. The mixture was shaken until the oil which separated had dissolved. After 16 hr., the solution was neutralised with Amberlite IR-120 resin (H^+ form). The resin was filtered off and washed with water, and the combined filtrate and washings were concentrated to dryness. The residue was dissolved in water and the aqueous solution extracted three times with ether before being passed slowly through a column of Amberlite IR-120 (cyclohexylammonium form). The effluent was concentrated to dryness and the residue crystallised from 95% ethanol, to give 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate 3,6-(cyclohexylammonium phosphate) (3·0 g., 46·5%), m. p. 197—205° (decomp.), $[\alpha]_D^{24}$ +22° (*c* 1·08) (Found: C, 42·3; H, 6·3; N, 6·5. Calc. for $C_{15}H_{27}N_2O_{10}P$: C, 42·25; H, 6·3; N, 6·6%).

(*b*) 2N-Potassium hydroxide (15 ml.) was added to a solution of 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate 6-(diphenyl phosphate) (4·97 g.) in dioxan (50 ml.). The oil which was precipitated redissolved after the mixture had been shaken for 15 min., giving a slightly yellow solution which was kept for 16 hr. at room temperature. The solution was neutralised with Amberlite IR-120 (H^+ form) and, after removal of the resin, diluted with water and extracted three times with ether. The aqueous solution was then passed very slowly through an Amberlite IR-120 column (cyclohexylammonium form), and the effluent was concentrated. The crystalline residue was triturated with absolute ethanol, filtered off, and recrystallised from 95% ethanol. The 5-nitrate 3,6-(cyclohexylammonium phosphate) had m. p. 197—205° (decomp.), $[\alpha]_D^{24}$ +22° (*c* 1·08) (Found: C, 42·3; H, 6·3; N, 6·55%).

1,2-O-*Isopropylidene*-D-*glucofuranose* 3,6-(*Hydrogen Phosphate*).—(*a*) The oily 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate 3,6-(phenyl phosphate) obtained by phosphorylation of 1,2-*O*-isopropylidene-D-glucofuranose 5-nitrate (4·8 g.) (see above) was hydrogenated in ethanol (20 ml.) in the presence of Adams platinum. When the hydrogen uptake had ceased, the catalyst was filtered off and the solution concentrated. When triturated with ether, the residue crystallised. After recrystallisation from ethanol–ether, the ammonium salt (2·1 g., 39%) of the cyclic phosphate had m. p. 160—170° (decomp.), $[\alpha]_D^{25}$ +16·2° (*c* 0·83) (Found: C, 36·3; H, 6·0; N, 4·6. Calc. for $C_9H_{18}NO_8P$: C, 36·1; H, 6·0; N, 4·7%).

(*b*) 1,2-*O*-Isopropylidene-D-glucofuranose 5-nitrate 3,6-(cyclohexylammonium phosphate) (1·9 g.) was hydrogenated in water (50 ml.) containing acetic acid (0·24 ml.) in the presence of a palladium catalyst. When the hydrogen uptake had ceased, the catalyst was filtered off and the solution concentrated. After crystallisation from ethanol–ether, the cyclohexylammonium salt (1·4 g., 89·5%) had m. p. 195—200° (decomp.), $[\alpha]_D^{24}$ +13·6° (*c* 0·62) (Found: C, 47·1; H, 7·2; N, 3·8. Calc. for $C_{15}H_{28}NO_8P$: C, 47·2; H, 7·3; N, 3·7%).

(*c*) 1,2-*O*-Isopropylidene-D-glucofuranose 3-(dicyclohexylammonium phosphate) (1 g.) was dissolved in water (5 ml.) and transformed into the free acid by addition of an excess of Amberlite IR-120 resin (H^+ form). The resin was filtered off, and the solution neutralised with pyridine and evaporated to dryness. The residue was dissolved in water (5 ml.) containing pyridine (10 ml.), and dicyclohexylcarbodi-imide (3 g.) in pyridine (10 ml.) was added. The mixture was shaken at room temperature for 66 hr., then diluted with water (50 ml.), and the dicyclohexylurea filtered off. The aqueous solution was washed three times with ether, acidified with an excess of Amberlite IR-120 (H^+ form), filtered, and neutralised with cyclohexylamine. The solution was evaporated, the last traces of water being removed by repeated distillation with ethanol. The residue was dissolved in ethanol, and ether added to slight turbidity. The

cyclohexylammonium salt of the 3,6-(hydrogen phosphate) which crystallised had m. p. 195—200° (decomp.), $[\alpha]_D^{25}$ +13·4° (*c* 0·82) (Found: C, 47·2; H, 7·2; N, 3·7; P, 8·6. Calc. for $C_{15}H_{28}NO_8P$: C, 47·2; H, 7·3; N, 3·7; P, 8·1%).

D-*Glucose* 3,6-(*Hydrogen Phosphate*).—The ammonium salt (5·5 g.) or the cyclohexylammonium salt (7·0 g.) of 1,2-*O*-isopropylidene-D-glucofuranose 3,6-(hydrogen phosphate) was dissolved in water (75 ml.) and passed through a column of Amberlite IR-120 resin (H^+ form) (*ca.* 100 ml.), and the column was washed with water. The effluent (200 ml.) was heated in a boiling-water bath for 15 min., cooled, neutralised with 0·3N-barium hydroxide, and concentrated to 12 ml. Addition of ethanol precipitated an oil which crystallised on trituration. The *barium salt* (3·7 g., 58%) of the 3,6-cyclic phosphate recrystallised from water and ethanol and had $[\alpha]_D^{21}$ +25·5° (*c* 2·0) (Found: C, 21·0; H, 4·1. $C_6H_{10}O_8PBa_{\frac{1}{2}},2H_2O$ requires C, 20·8; H, 4·05%).

6-O-*Benzyl*-1,2-O-*isopropylidene*-D-*glucofuranose* 3,5-(*Hydrogen Phosphate*).—Phenyl phosphorodichloridate (2·11 g.) was added to 6-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose [14] (3·1 g.), dissolved in pyridine (35 ml.). The solution was left overnight at 37°. The pyridine was removed and the residue dissolved in ether. The ether solution was washed twice with iced water, then with ice-cold (1% v/v) sulphuric acid until the aqueous phase remained acid, and finally three times with iced water, and dried. The ether was removed, the residue (3·8 g.) dissolved in dioxan (50 ml.), and 2N-potassium hydroxide (6·5 ml.) added. The mixture was shaken and after 0·5 hr. the oil which at first separated dissolved. After 16 hr., the solution was neutralised with Amberlite IR-120 resin (H^+ form). The resin was filtered off and washed with water. The combined filtrate and washings were concentrated to dryness and the residue was dissolved in water. The solution was extracted twice with ether before being passed slowly through a column of Amberlite IR-120 resin (cyclohexylammonium form). The effluent was concentrated to dryness and the residue triturated with ether until it crystallised. After recrystallisation from ethanol–ether, 6-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose 3,5-(cyclohexylammonium phosphate) (2·4 g., 50·9%) had m. p. 185—200° (decomp.), $[\alpha]_D^{26}$ +18·7° (*c* 0·48) (Found: C, 55·85; H, 7·2; N, 3·1. Calc. for $C_{22}H_{34}NO_8P$: C, 56·05; H, 7·2; N, 3·0%).

1,2-O-*Isopropylidene*-D-*glucofuranose* 3,5-(*Hydrogen Phosphate*).—The preceding compound (1 g.) was hydrogenated in ethanol (30 ml.) in the presence of a palladium catalyst. This gave crystals which, recrystallised from ethanol–ether, afforded the cyclohexylammonium salt (0·6 g., 75%), m. p. 180—190° (decomp.), $[\alpha]_D^{24}$ +32·0° (*c* 1·14), of the cyclic phosphate (Found: C, 47·5; H, 7·3; N, 3·75. Calc. for $C_{15}H_{28}NO_8P$: C, 47·2; H, 7·3; N, 3·7%).

D-*Glucose* 3,5-(*Hydrogen Phosphate*).—The preceding cyclohexylammonium salt (7·5 g.) was dissolved in water (75 ml.) and passed through a column of Amberlite IR-120 resin (H^+ form) (100 ml.). The effluent and washings (200 ml.) were heated on a boiling-water bath for 15 min., cooled, and neutralised with barium hydroxide solution. The aqueous solution was concentrated to dryness and the residue dissolved in the minimum amount of water. Addition of ethanol precipitated the *barium salt* (3·9 g., 60·5%) of D-glucose 3,5-phosphate. It had $[\alpha]_D^{21}$ +20·7° (*c* 2·0) (Found: C, 22·2; H, 3·8. $C_6H_{10}O_8PBa_{\frac{1}{2}},H_2O$ requires C, 21·95; H, 3·7%).

3-O-*Benzyl*-1,2-O-*isopropylidene*-D-*glucofuranose* 5,6-(*Phenyl Phosphate*).—Phenyl phosphorodichloridate (3·4 g.) was added to 3-*O*-benzyl-1,2-*O*-isopropylidene-D-glucofuranose [15] (5·0 g.) in pyridine (60 ml.). The solution was kept at 37° for 48 hr. No water was added at the end of the reaction time. The pyridine was removed and the residue dissolved in cold chloroform (−15°). The chloroform layer was washed rapidly three times with ice-water and dried. After removal of the chloroform, an oily, neutral ester (6·9 g.) remained. This ester was extremely unstable. In the presence of pyridine and water, a pronounced odour of phenol developed. After treatment with potassium hydroxide (1 g. in 5 ml. of water) in dioxan (80 ml.), followed by the usual working up and hydrogenation in the presence of palladium to remove the benzyl group, the substance gave two phosphorus-containing spots on a paper chromatogram. The R_F's of these spots corresponded to those of 1,2-*O*-isopropylideneglucose 5- and 6-phosphate.

1,2-O-*Isopropylidene*-D-*glucofuranose* 3-(*Dihydrogen Phosphate*).—This ester was prepared by the method of Brown, Hayes, and Todd [16] and isolated as its dicyclohexylammonium salt which was crystallised by adding acetone to an aqueous solution of the salt. It had m. p. 155—170° (decomp.), $[\alpha]_D^{24}$ +5·5° (*c* 1·8) (Found: C, 50·5; H, 8·7; N, 5·5; P, 6·1. Calc. for $C_{21}H_{43}N_2O_9P$: C. 50·6; H, 8·6; N, 5·6; P, 6·2%).

3,6-*Di*-O-*acetyl*-1,2-O-*isopropylidene*-D-*glucofuranose* 5-(*Diphenyl Phosphate*).—Diphenyl phosphorochloridate (4·28 g.) was added slowly to a solution of 3,6-di-*O*-acetyl-1,2-*O*-isopropylidene-D-glucofuranose [17] (4·4 g.) in anhydrous pyridine (60 ml.). The solution was kept at 37° for 48 hr. The pyridine was removed below 40° and the oily residue triturated with several lots of iced water until it crystallised. After recrystallisation from ethanol, the diphenyl phosphate (5 g., 64·5%) had m. p. 94—95°, $[\alpha]_D^{22}$ −16·9° (*c* 0·53 in $CHCl_3$) (Found: C, 56·0; H, 5·4; P, 5·7. Calc. for $C_{25}H_{29}O_{11}P$: C, 56·0; H, 5·4; P, 5·8%).

1,2-O-*Isopropylidene*-D-*glucofuranose* 5-(*Dihydrogen Phosphate*).—The above diphenyl phosphate (5 g.) was hydrogenated in absolute ethanol (200 ml.) in the presence of Adams platinum. When the hydrogen uptake ceased, the catalyst was filtered off and the solution was saturated several times with ammonia. When a paper chromatogram showed that the removal of the acetyl groups was complete, the ammonia and ethanol were removed and the residue was dissolved in water. The aqueous solution was passed slowly through a column of Amberlite IR-120 resin (cyclohexylammonium form), and the effluent concentrated. The residue was dissolved in the minimum amount of water. The monocyclohexylammonium salt of the 5-phosphate which crystallised on the addition of acetone had m. p. 170—172° (decomp.), $[\alpha]_D^{24}$ +8·4° (*c* 1·3) (Found: C, 44·0; H, 7·7; N, 3·9. Calc. for $C_{15}H_{30}NO_9P,\frac{1}{2}H_2O$: C, 44·1; H, 7·6; N, 3·4%).

1,2-O-*Isopropylidene*-D-*glucofuranose* 6-(*Dihydrogen Phosphate*).—Diphenyl phosphorochloridate (9·5 g.) was added slowly to 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-D-glucofuranose [19]

Reaction of the monophosphates of 1,2-O-*isopropylidene*-D-*glucofuranose with dicyclohexylcarbodi-imide* (DCC).

Phosphate of 1,2-*O*-isopropylidene-D-glucofuranose	R_F in Solvent A			
3-Phosphate + DCC (1 hr.)	0·42+	0·72+		0·76++
(5 hr.)	—	0·73++		0·77++
(22 hr.)	—	0·73++		0·77+
(27 hr.)	—	0·73+++		—
3,5-Phosphate + DCC (4 hr.)	—	0·72+		0·77++
(29 hr.)		0·72+++		0·77+/−
3-Phosphate	0·42			
3,5-Phosphate				0·77
3,6-Phosphate		0·72		
5-Phosphate + DCC (1 hr.)	0·38+	0·49++	0·78+++	—
(7 hr.)	—	—	0·78+++	0·99+
(53 hr.)	—	—	0·78+++	0·99+
6-Phosphate + DCC (1 hr.)	0·38+	0·49+/−	0·78++	—
(7 hr.)	—	—	0·78++	0·99+
(53 hr.)	—	—	0·78++	0·99+
5-Phosphate		0·49		
6-Phosphate	0·38			
3,5-Phosphate			0·77	
3,6-Phosphate		0·72		

Phosphate of 1,2-*O*-isopropylidene-D-glucofuranose	R_F in Solvent B			
3-Phosphate + DCC (1 hr.)				
(5 hr.)	—		0·59++	0·67++
(22 hr.)	—		0·59++	0·67+
(27 hr.)				
3,5-Phosphate + DCC (4 hr.)			0·59+	0·67++
(29 hr.)			0·59+++	0·67+/−
3-Phosphate	0·16			
3,5-Phosphate				0·67
3,6-Phosphate			0·60	
5-Phosphate + DCC (1 hr.)	0·11+	0·20++	0·57+++	0·98+
(7 hr.)	—	—	0·57+++	0·98+
(53 hr.)	—	—	0·57+++	0·98+
6-Phosphate + DCC (1 hr.)	0·11+	0·20+/−	0·58++	—
(7 hr.)	—	—	0·58++	0·98+
(53 hr.)	—	—	0·58++	0·98+
5-Phosphate		0·20		
6-Phosphate	0·11			
3,5-Phosphate				0·60
3,6-Phosphate		0·53		

(10 g.) dissolved in anhydrous pyridine (20 ml.). The solution was kept at 37° for 48 hr. The pyridine was removed below 40° and the residue dissolved in chloroform. The chloroform solution was washed twice with iced water, then with ice-cold (1% v/v) sulphuric acid until the aqueous layer remained acid, and finally three times with iced water, and dried. The chloroform was removed and the residue hydrogenated in ethanol (200 ml.) in the presence of Adams platinum. The quantity of hydrogen absorbed corresponded to the quantity calculated for the hydrogenolysis of the two phenyl groups and of the benzylidene group. When the hydrogen uptake had ceased, the catalyst was filtered off and the solution neutralised with cyclohexylamine. The solution was concentrated to a small volume and ether was added to turbidity. The cyclohexylammonium salt (8·3 g., 51·2%) of 1,2-*O*-isopropylidene-D-glucofuranose 6-phosphate which crystallised overnight at 0° had, after one recrystallisation from water–acetone, m. p. 135—140° [resolidifies and remelts at 195—197° (decomp.)], $[\alpha]_D^{25}$ −6·9° (*c* 2·01) (Found: C, 50·5; H, 8·9; N, 5·6; P, 6·2. Calc. for $C_{21}H_{43}N_2O_9P$: C, 50·6; H, 8·6; N, 5·6; P, 6·2%).

Cyclisation of the Phosphates of 1,2-O-*Isopropylidene-*D-*glucofuranose with Dicyclohexylcarbodi-imide.*—The cyclohexylammonium salts of the monophosphates (20 mg.) were dissolved in water, and the solution was acidified with Amberlite IR-120 (H^+ form). The resin was filtered off and washed with water, and the combined filtrates were neutralised with pyridine and evaporated to dryness. The residues were dissolved in pyridine (0·8 ml.) containing water (0·2 ml.), and dicyclohexylcarbodi-imide (60 mg.) was added to the solutions which were shaken mechanically. At intervals, samples (0·2 ml.) of the solutions were removed and diluted with water (0·2 ml.), and the aqueous layer extracted three times with ether before being chromatographed. The solvent systems employed were: A, propan-2-ol–ammonia–water (7 : 1 : 2); B, propan-2-ol–ammonia–water (8 : 1 : 1). Ascending chromatograms were run on Whatman No. 1 paper without time- or temperature-control, appropriate control substances being chromatographed at the same time. The results are given in the Table.

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