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SYNTHESIS OF GLYCOSYL PHOSPHATES FROM SUGAR ORTHO ESTERS: FORMATION OF BIS(2,3,4,6-TETRA-*O*-ACETYL-β-D-GLUCOPYRANOSYL) PHOSPHATE, AND THE EFFECT OF SOLVENTS ON THE SYNTHESIS OF β-D-GLUCOPYRANOSYL PHOSPHATE

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ABSTRACT

Reaction of *exo*-3,4,6-tri-*O*-acetyl-1,2-*O*-(*tert*-butyl orthoacetyl)-α-D-glucopyranose with phosphoric acid and phosphorus pentaoxide in oxolane gave 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phosphate. Treatment of the reaction mixture with aqueous lithium hydroxide gave the expected β-D-glucopyranosyl phosphate. However, neutralization with ammonium hydroxide gave exclusively the phosphoric diester, namely, bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) phosphate. Formation of the diester was traced to the phosphorus pentaoxide in the initial reaction-mixture. A study of solvents showed that phosphorylation of the ortho ester proceeds rapidly in a variety of ethers and *N*,*N*-dimethylamides.

INTRODUCTION

β-D-Glucopyranosyl phosphate has been prepared[1] by reaction of peracetylated D-glucose orthoesters with dibenzyl hydrogenphosphate followed by removal of the protecting groups, as well as by other methods[2]. For the synthesis of β-L-fucopyranosyl phosphate, we subsequently reported for this procedure a simplification in which unsubstituted phosphoric acid was used, thus eliminating the need for debenzylation[3]. This approach has recently been extended to β-L-galactopyranosyl phosphate[4], β-D-galactopyranosyl phosphate[5], and α-D-mannopyranosyl phosphate[5]. We now report the results of a study of the reaction between 3,4,6-tri-*O*-acetyl-1,2-*O*-(*tert*-butyl orthoacetyl)-α-D-glucopyranose and unsubstituted phosphoric acid which dealt with the effects of solvents and isolation procedures on the synthesis. Under certain conditions, a phosphoric diester was an unexpected product of this reaction.

RESULTS AND DISCUSSION

A mixture of *exo*-3,4,6-tri-*O*-acetyl-1,2-*O*-(*tert*-butyl orthoacetyl)-α-D-gluco-

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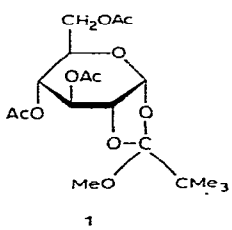
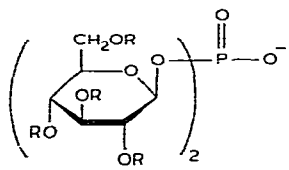
TABLE I

T.L.C. AND ELECTROPHORETIC MOBILITIES

Compound	*T.L.C.*		*Electrophoresis*
	Solvent A R_F	*Solvent B* R_F	R_D
1	0.50	—	—
2	—	0.70	0.38
3	—	0.04	0.46
4	0.0	streak	1.34
5	—	0.1	1.36
7	0.16	—	—
8	—	0.40	—
Inorganic phosphate	—	—	2.1

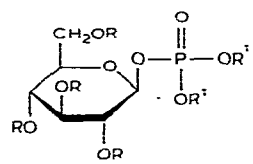


1



2 R = Ac

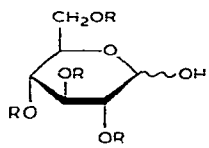
3 R = H



4 R = Ac, R' = H

5 R = H, R' = H

6 R = H, R' = $C_6H_{11}NH_3$



7 R = Ac

8 R = H

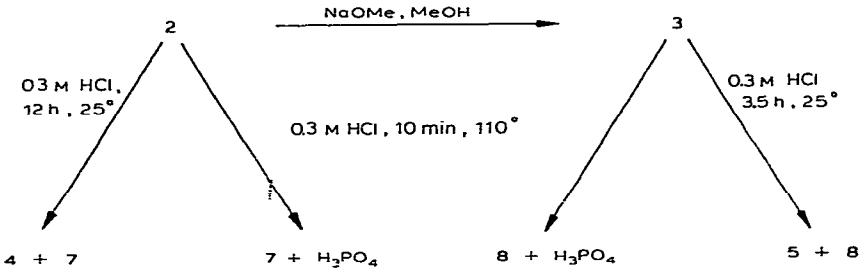
pyranose (**1**) with a five-fold excess of phosphoric acid in oxolane (tetrahydrofuran, THF), dried by the addition of phosphorus pentaoxide, showed complete disappearance of the starting material (t.l.c., solvent *A*) after 10 min at 25°, and formation of a single, intense, phosphate-containing spot, R_F 0 (see Table I). On electrophoresis, a single, phosphate-containing spot appeared at R_P 1.34, corresponding to 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl phosphate (**4**). When the solution of this compound in THF was made neutral with an excess of ammonium hydroxide, the material having R_P 1.34 was replaced by a single spot at R_P 0.38. On conversion of this ammonium salt into the potassium salt by treatment with a cation-exchange

TABLE II

^{13}C- AND ^{31}P-N.M.R.-SPECTRAL DATA

Compound	*^{13}C Chemical shift[a] (p.p.m.)*						*^{31}P[b] Chemical shift (p.p.m.)*	*Coupling constants (Hz)*		
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*		J_{PC-1}	J_{PC-2}	J_{PH}
2	97.8	74.3	74.7	70.7	75.4	64.3	4.4(t)	4.8	[c]	8.5
5[d]	99.8	77.2	78.2	72.6	78.9	63.8	−2.4(d)	4.8	6.0	7.3

[a]^1H-Decoupled spectra in D_2O, with DSS as the internal reference, at 25°; pD 4.7 (**2**) and 8.6 (**5**). [b]85% H_3PO_4 as the external reference, D_2O, pD 9.4 at 25°. [c]Not measurable, because of overlapping resonances. [d]These values are close to the literature values[6–8], when correction is made for differences in referencing; see also, ref. 9.



Scheme 1

resin, some deacetylation occurred, as shown by the appearance of minor, slower-moving spots (t.l.c., solvent *B*), but these compounds were reconverted by reacetylation with acetic anhydride into the slow-moving material (electrophoresis, R_P 0.38). Elemental analysis suggested that this compound was the phosphoric diester, namely, bis(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) phosphate (**2**). The ^{13}C-n.m.r. spectrum of the diester **2** showed only six resonances, in addition to the acetyl resonances; except for C-6, these were all upfield of the corresponding resonances for β-D-glucopyranosyl phosphate (see Table II). The ^{31}P-n.m.r. spectrum of the diester shows a striking shift, compared with that of the sugar phosphate (**5**). At pD 9.4, the phosphorus resonance lies about 6 p.p.m. upfield of the corresponding resonance for D-glucopyranosyl phosphate. The resonance is split into the expected triplet, *J* 8.5 Hz. The i.r. spectrum had a strong band at 1250 cm^{-1} (P=O). Catalytic deacetylation of **2** with sodium methoxide gave **3**, which showed a single spot in electrophoresis and t.l.c. (see Table I). The hydrolytic behavior of **2** and **3** on treatment with HCl is summarized in Scheme 1.

When the phosphorylation mixture was treated with M LiOH, instead of ammonium hydroxide, no phosphoric diester was formed. β-D-Glucopyranosyl phosphate (**5**) was the sole product detected after the usual isolation-procedure.

The phosphorylation reaction was ordinarily conducted for 10 min at 25°. If the reaction mixture was allowed to stand for 2 h, a minor, faster-moving spot

appeared upon electrophoresis (R_p 1.4, *vs.* 1.34). As reacetylation with acetic anhydride caused the disappearance of this new spot, we presume that it represents the formation of 3,4,6-tri-*O*-acetyl-D-glucopyranosyl phosphate (**4**), as had been reported[1].

Formation of the diester. — The formation of the diester **2** in the neutralization is dependent upon the presence of phosphoric anhydride in the reaction mixture, as shown by the following experiments. When the reaction was conducted with an excess of the ortho ester (to act as drying agent) but no P_2O_5, the initial reaction gave a mixture of the mono- and di-ester. Diester formation at this stage is the expected product of reaction of the initially formed glycosyl phosphate with an excess of the ortho ester. However, on neutralization with ammonia, no additional diester is formed.

In a second experiment, the reaction mixture containing ortho ester and phosphoric acid in the ratio of 1 : 1 in THF was dried by treating very briefly with P_2O_5, and then filtering before much of the anhydride had had time to dissolve. Under these conditions, little diester formation was observed following neutralization with ammonia, and the monoester was the major product. We attempted to show the formation of the diester by treatment of solutions of peracetylated D-glucopyranosyl phosphate (prepared by the MacDonald method[10]) with P_2O_5. These attempts failed, apparently due to the presence of an excess of D-glucose pentaacetate, as no diester was formed when D-glucose pentaacetate was present in the ortho ester reaction-mixture.

Survey of suitable solvents. — We have examined the suitability of a number of solvents (see Experimental section) as, although oxolane is an excellent medium for the reaction with phosphoric acid itself, we are interested in extending this general synthesis to "sugar nucleotides" and their analogs[11], and nucleotides are essentially insoluble in THF. The tests showed that a variety of ethers, as well as a number of *N*,*N*-dimethylamides, are suitable solvents. Among the unsuccessful solvents, it should be noted that several fail because either the ortho ester or the phosphoric acid is insufficiently soluble therein. Phosphoric anhydride reacts with formamide and *N*-methylformamide. Reactions in Me_2SO, and sulfolane, also failed to yield any phosphorylated product. The case of hexamethylphosphoric triamide (HMPT) is interesting; the phosphorylation proceeds in this solvent, but so slowly that acceptable yields require heating for 10 days at 105°, in contrast to the usual reaction conditions (10 min at 25°). On the other hand, mixtures of HMPT and THF were quite satisfactory, although the reaction proceeded somewhat more slowly (30 min at 25°) than with THF alone.

Success in the addition of phosphoric acid and its derivatives to a sugar ortho ester depends on (*a*) the maintenance of strictly anhydrous conditions, (*b*) protonation of the orthoester, and (*c*) use of a solvent system compatible with all of the components. Anhydrous conditions are necessary, because of the rapid reaction of water with the protonated ortho ester[12]. We have been unsuccessful in attempts to maintain sufficiently dry conditions with molecular sieves under various conditions. Phosphoric anhydride is extremely effective in this regard; its drawbacks are (*a*) its

reactivity with certain functional groups, (*b*) formation of phosphoric acid on reaction with water, and (*c*) the diester formation described here. An alternative way in which to dry the solvent is to use an excess of the ortho ester, and this is satisfactory if some formation of diester can be tolerated.

Whereas the formation of phosphoric diesters in the initial reaction-mixture can be readily rationalized when an excess of the ortho ester is present, the mechanism of formation of the di- from the mono-ester in the presence of phosphoric anhydride upon neutralization with aqueous ammonia is not yet clear. It is, however, probably related to the interesting chemistry of condensed phosphates described by Pollman and Schramm[13].

EXPERIMENTAL

General methods. — Evaporations were conducted under diminished pressure below 45°. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. T.l.c. was performed on an aluminum support coated with silica gel 60 containing a fluorescent indicator, using either solvent *A* (3:1, v/v, ether–light petroleum) or solvent *B* (60:35:6, v/v, chloroform–methanol–water). Spots were detected by using a sulfuric acid–ethanol spray and heating. Paper chromatograms were developed in 4:1:1 1-butanol–ethanol–water, and processed by the silver nitrate, dip procedure described by Hough and Jones[14]. Paper electrophoresis was conducted on Schleicher and Schuell No. 589 (orange ribbon) paper strips in 0.15M NH_4HCO_3 (pH 7.9). The relative mobilities, $R_{picrate}$, are given by cm(phosphate)/cm(picrate). Phosphate spots were revealed by the spray reagent of Bandurski and Axelrod[15]. D-Glucose orthoester was prepared as described[16]. Anhydrous ethers were prepared by distillation, and storage over molecular sieves 4A. Hexamethylphosphoric triamide was purified by distillation from calcium hydride, and storage over molecular sieves 4A. The microanalyses were performed by Galbraith Analytical Laboratories, Knoxville, Tenn. ^{1}H-N.m.r. spectra were recorded with a Varian T-60 instrument at 60 MHz; ^{1}H-decoupled, ^{13}C-n.m.r. spectra with a Bruker-90 instrument at 22.63 MHz; and ^{31}P-n.m.r. spectra with a Bruker HFX-10 instrument at 36.4 MHz. Solutions in D_2O (concentration, 50–100 mg/mL) were used, with DSS as the internal standard (^{13}C and ^{1}H), and 85% H_3PO_4 as the external standard for ^{31}P-n.m.r. spectra.

Bis(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl) phosphate (**2**). — To a solution of **1** (1 g, 2.48 mmol) in dry oxolane (20 mL) containing dry P_2O_5 (0.2 g, 1.4 mmol) was added anhydrous, crystalline orthophosphoric acid (1.22 g, 12.4 mmol) at room temperature. After the mixture had been shaken occasionally for 10 min at room temperature, t.l.c. (solvent *A*) showed disappearance of the starting material, with concurrent appearance of a spot having zero mobility. This reaction mixture showed a single, phosphate-containing spot in electrophoresis (R_P 1.34). The mixture was made neutral with an excess of concentrated ammonium hydroxide (10 mL, 15M). The excess of phosphoric acid precipitated as triammonium phosphate, which was filtered off with the aid of Celite, and the filtrate was evaporated under an air current

to a syrup which showed a single, slow-moving spot in electrophoresis (R_P 0.38). The syrupy ammonium salt was dissolved in water (5 mL), an excess of AG-50W X-8 (K^+) cation-exchange resin was added, the suspension was stirred for 24 h at room temperature and filtered with Celite, and the solid washed with water (10 mL). The filtrate was evaporated, and the residue dried under vacuum. T.l.c. (solvent *B*) indicated a few, slow-moving, minor spots along with the major spot. Hence, the potassium salt (0.22 g) was dissolved in pyridine (1 mL), acetic anhydride (1 mL) was added, and the mixture was stirred for 24 h at room temperature, after which, t.l.c. (solvent *B*) showed a single spot, R_P 0.38. The reaction mixture was processed by removing the pyridine and the excess of acetic anhydride under diminished pressure, with addition of six 5-mL portions of toluene, to yield a hygroscopic syrup (**2**), 0.223 g (22.3%), $[\alpha]_D^{25}$ +5.5° (*c* 1, water).

Anal. Calc. for $C_{28}H_{38}KO_{22}P$: C, 42.21; H, 4.77; K, 4.89; P, 3.89. Found: C, 41.96; H, 5.34; K, 4.15; P, 3.36.

β-D-Glucopyranosyl [di(cyclohexylammonium) phosphate] (**6**). — To a solution of **1** (0.5 g, 1.24 mmol) in dry oxolane (10 mL) containing P_2O_5 (0.15 g) was added crystalline, anhydrous orthophosphoric acid (0.61 g, 6.2 mmol) at room temperature. After the mixture had been shaken occasionally for 10 min, t.l.c. (solvent *A*) showed complete disappearance of the starting material, and appearance of a spot having zero mobility. The mixture was transferred to another flask, to which cold M lithium hydroxide was added, with stirring, to a pH of 11. This pH was maintained for 22 h by periodic addition of lithium hydroxide solution. The precipitated trilithium phosphate was filtered off, and the pH of the filtrate was adjusted to 9.0 with Dowex-50 (H^+) resin. The resin was removed by filtration, and the solution concentrated *in vacuo* below 40° to 10 mL. In electrophoresis, this solution showed a single spot (R_P 1.36), and no trace of the diester **3** was detected. Barium acetate (600 mg) was added to the solution, followed by ethanol (10 mL). After 2 h at 0°, the precipitate was collected by centrifugation. The crude barium salt was dissolved in water (20 mL), and converted into the dicyclohexylammonium salt by addition of dicyclohexylammonium sulfate (620 mg). The barium sulfate was removed by centrifugation, and the supernatant liquid was dried *in vacuo* for 6 h at 50°, when a crystalline derivative (**6**) appeared. (This treatment removed the excess of cyclohexylammonium acetate.) Compound **6** was recrystallized from ethanol–water, yield 0.2 g (35.3%), $[\alpha]_D^{25}$ +5.4° (*c* 1, water) (lit.[1] $[\alpha]_D$ +5.4°). The ^{1}H-n.m.r. spectrum was identical with that of authentic β-D-glucopyranosyl phosphate (**5**).

Effect of solvent and drying agent. — Compound **1** (~0.1 g) was added to 2 mL of each solvent, and the mixture was dried by using P_2O_5. Crystalline phosphoric acid (0.2 g) was then added, and the reaction mixture was checked for 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phosphate (**4**) by t.l.c. (solvent *A*) and by paper electrophoresis (pH 7.9) after 30 min. The following solvents were satisfactory when dried with P_2O_5: oxolane, 2-methyloxolane, 2,5-dimethyloxolane, 1,4-dioxane, 1,2-dimethoxyethane, bis(2-methoxyethyl) ether, *N*,*N*-dimethylformamide, *N*,*N*-dimethylacetamide, 1,1,3,3-tetramethylurea, and 1-methyl-2-pyrrolidinone. The following solvents

were not suitable, because of insolubility of one of the components: diethyl ether, diisopropyl ether, triethyl orthoformate, tetrahydropyran, oxepane, tetrahydrothiophene, and tetrahydrothiopyran.

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