Synthesis of 2-deoxy-α-D-glucopyranosyl and 2-deoxy-α-D-galactopyranosyl phosphates

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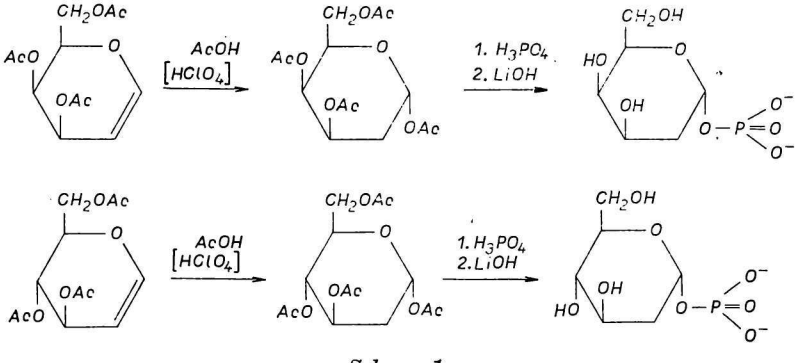
2-Deoxy-α-D-glucopyranosyl and 2-deoxy-α-D-galactopyranosyl phosphates have been prepared by phosphorylation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose with crystalline H_3PO_4. Acetylation of 2-deoxysugars introduced by Bonner has been applied in the synthesis of crystalline 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose.

2-Deoxyglycosyl phosphates, analogues of natural glycosyl phosphates, are important intermediates in the syntheses of nucleoside diphosphate 2-deoxysugars. The absence of the hydroxyl group in the molecule of glycosyl phosphate causes its acid lability which apparently account for the fact that only small amounts of 2-deoxyglycosyl phosphates have been so far prepared, *e.g.* 2-deoxy-α- and 2-deoxy-β-D-ribofuranosyl phosphate [1], 2-amino-2-deoxy-α-D-glucopyranosyl phosphate [3], and 2-deoxy-α-D-glucopyranosyl phosphate [2].

The present paper describes the preparation of glycosyl phosphates of 2-deoxy-D-galactopyranose and 2-deoxy-D-glucopyranose. Phosphorylation was effected by the action of cryst. H_3PO_4 on the α anomers of per-*O*-acetylated derivatives of 2-deoxy-D-galactopyranose and 2-deoxy-D-glucopyranose (Scheme 1).

The α anomers were chosen with the intention to find out their behaviour in the phosphorylation reaction. It is known that the phosphorylation of the α anomers of per-*O*-acetylated derivatives of D-glucopyranose [4] and D-galactopyranose [5] with cryst. H_3PO_4 affords the corresponding α-glycosyl phosphates in much lower yields than that of the β anomers.



Scheme 1

Experimental

Chromatography of phosphorylated sugars was done on Whatman No. 1 paper in isopropyl alcohol—ammonia—water 7 : 1 : 2 (Solvent *A*) and electrophoresis in 0.05 M triethylamine-bicarbonate buffer, pH 7.5 (Solvent *B*) on the same paper. The reagent of *Hanes* and *Isherwood* [10] was used for detection in both cases. Acetylated saccharides were chromatographed on thin layers of Silica gel in benzene—ethyl acetate 7 : 3 (Solvent *C*). Optical rotation was measured with a Perkin—Elmer 141 Polarimeter. Melting points were determined on a Kofler stage. N.m.r. spectra of 2-deoxyglycosyl phosphates (in D_2O) and acetyl derivatives of 2-deoxyglucopyranose and 2-deoxygalactopyranose (in $CDCl_3$) were measured with a Tesla BS 483B/80 MHz equipment using TMS as an internal standard.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose was prepared as described by *Bonner* [8], m.p. 109—110°C, $[\alpha]_D^{20}$ 106.5° (*c* 1, $CHCl_3$). Ref. [8] m.p. 109—110°C, $[\alpha]_D^{20}$ 107° ($CHCl_3$).

1,3,4,6-Tetra-O-acetyl-2-deoxy-α-D-galactopyranose

To cooled acetic anhydride (0°C; 184 ml), firstly 70% $HClO_4$ (21.5 g) and then a solution of tri-*O*-acetyl-D-galactal (27.5 g) in glacial acetic acid (109 ml) were gradually added with stirring so that the temperature of the reaction mixture did not exceed 0°C. When the dropping was finished the mixture was stirred for additional 10 minutes, then anhydrous sodium acetate (12.3 g) in glacial acetic acid (176 ml) was added dropwise at a rate to keep the temperature between −5 and 0°C. The reaction mixture was poured into ice-cold water (1800 ml), stirred for 90 minutes, and extracted with chloroform (3 × 300 ml). The chloroform extracts were combined, washed with water (5 × 250 ml), and dried with anhydrous Na_2SO_4. The filtrate was evaporated *in vacuo* to sirup, which was dissolved in ether (500 ml), decolourized with charcoal, and evaporated again. Resulting product was dissolved in ether (50 ml) and left to crystallize. The crystals (6 g) were filtered off, washed with ether, and dried. Recrystallization from ethanol gave chromatographically homogeneous compound; m.p. 105—106°C, $[\alpha]_D^{20}$ 135° (*c* 1, methanol), $[\alpha]_D^{20}$ 122.6° (*c* 1, $CHCl_3$).

For $C_{14}H_{20}O_9$ (332.19) calculated: 50.60% C, 6.06% H; found: 50.59% C, 6.19% H. N.m.r.data: signal of anomeric proton at δ 6.28, $J_{1,2}$ 1.5 Hz, $J_{1,2'}$ 3.0 Hz.

2-Deoxy-α-D-galactopyranosyl phosphate

A solution of cryst. H_3PO_4 (882 ml; 9.6 mmoles, dried in a desiccator over $MgClO_4$) in anhydrous tetrahydrofuran (5 ml) was mixed with a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose (380 ml; 1.2 mmole) in the same solvent (5 ml). Tetrahydrofuran was removed by distillation at *ca.* 1 torr and room temperature. The residue was kept further at this pressure for 45 minutes at room temperature and thereafter for 30 minutes at 50°C. The residue was then dissolved in tetrahydrofuran (6 ml) and after addition of 1 N-LiOH (0°C; 30 ml) the mixture was stirred for 16 hours at room temperature. The precipitate formed was filtered off and the solution was neutralized with Dowex 50 H^+ (pH 7) and then poured on a Dowex 50 (cyclohexylammonium form) column (2.5 × 16.5 cm). Paper chromatographic examination (Solvent *A*) showed that the column eluate contained besides 2-deoxygalactopyranosyl phosphate (R_F 0.16, R_F of galactosyl phosphate 0.1) small amounts of inorganic phosphate. The latter was separated as follows:

1. *Separation on a Cellulose column*

The eluate was evaporated and dissolved in Solvent *A* (3 ml) and chromatographed on a Cellulose column (3 × 50 cm) in the above solvent system at a rate 5 ml/hour. Separation of components was followed by paper chromatography. Fractions containing pure 2-deoxy-α-D-galactopyranosyl diammonium phosphate were combined and evaporated *in vacuo* below 40°C to sirupy residue (130.6 mg; 39.1%), $[\alpha]_D^{20}$ 70.1 (*c* 0.5, water).

2. *2-Deoxy-α-D-galactopyranosyl cyclohexylammonium phosphate dihydrate*

The phosphorylation of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose was done as in the case of preparation of 2-deoxy-α-D-galactopyranosyl diammonium phosphate. The eluate of the reaction mixture from a Dowex 50 (cyclohexylammonium form) column was evaporated. Sirup was dissolved in water (1 ml) and acetone was added to a slight turbidity. Crystals separated after 16-hour standing at 0°C were filtered off and dried. Yielded product: 183 mg (34.5%), $[\alpha]_D^{20}$ 41.1° (*c* 0.5, water), m.p. 127—132°C. Traces of inorganic phosphate were removed by twofold recrystallization from aqueous acetone. Thus obtained 2-deoxy-α-D-galactopyranosyl cyclohexylammonium phosphate dihydrate was chromatographically homogeneous and had $[\alpha]_D^{20}$ 43° (*c* 0.5, water) and m.p. 130—134°C.

Cyclohexylammonium salt of 2-deoxy-α-D-galactopyranosyl phosphate was also prepared from the diammonium salt (see Procedure *1*). Optical rotation of thus obtained 2-deoxy-α-D-galactopyranosyl cyclohexylammonium phosphate dihydrate was $[\alpha]_D^{20}$ 42.8° (*c* 0.5, water).

For $C_{18}H_{39}N_2O_8P \cdot 2H_2O$ (478.15) calculated: 45.2% C, 8.81% H, 5.86% N, 6.49% P; found: 45.44% C, 8.74% H, 5.76% N, 7.03% P.

N.m.r. data: signal of anomeric proton at δ 5.55, $J_{H-1,P}$ 8.0 Hz, $J_{1,2}$ 1.5 Hz, $J_{1,2'}$ 2.2 Hz.

2-Deoxy-α-D-glucopyranosyl phosphate

Cryst. H_3PO_4 (882 mg; 9.6 mmoles) dried in a desiccator over $MgClO_4$ and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose (380 mg; 1.2 mmole) were dissolved separately in 5-ml portions of anhydrous tetrahydrofuran. After mixing the solutions, the solvent was distilled off and the residue was kept at a pressure of 1 torr at first for 45 minutes at room temperature and then for 30 minutes at 50°C. The residue was further processed similarly as in the preparation of 2-deoxy-α-D-galactopyranosyl phosphate. The eluate was concentrated and chromatographed on a Whatman Cellulose column (3 × 50 cm) in Solvent *A* at a rate 8 ml/hour. Separation was followed by paper chromatography in Solvent *A* (R_F of 2-deoxy-α-D-glucopyranosyl phosphate 0.23, R_F of standard glucopyranosyl phosphate 0.13). Fractions containing 2-deoxy-α-D-glucopyranosyl diammonium phosphate were pooled, concentrated *in vacuo* at a temperature below 40°C to give chromatographically homogeneous sirup (111 mg; 33%) having $[\alpha]_D^{20}$ 72.4° (*c* 0.5, water).

2-Deoxy-α-D-glucopyranosyl diammonium phosphate was passed through a column of Dowex 50 (cyclohexylammonium form). The eluate was concentrated *in vacuo* and the residue was crystallized from aqueous acetone for 16 hours at 0°C to give 2-deoxy-α-D-glucopyranosyl cyclohexylammonium phosphate dihydrate, m.p. 135—141°C, $[\alpha]_D^{20}$ 45.4° (*c* 0.5, water).

For $C_{18}H_{39}N_2O_8P \cdot 2H_2O$ (478.15) calculated: 45.2% C, 8.81% H, 5.86% N, 6.49% P; found: 45.05% C, 8.72% H, 5.69% N, 6.90% P.

N.m.r. data: signal of anomeric proton at δ 5.50, $J_{H-1,P}$ 8.0 Hz, $J_{1,2}$ 1.5 Hz, $J_{1,2'}$ 2.2 Hz.

Discussion

Phosphorylation of per-*O*-acetyl derivatives of saccharides with cryst. H_3PO_4 is greatly influenced by the arrangement at the anomeric centre. The reaction is more effective with 1,2-*trans* derivatives than with 1,2-*cis* derivatives [4, 5]. Thermodynamic conditions also play an important role in the phosphorylation [6]. It is supposed that the phosphorylation of per-*O*-acetylated sugars with cryst. H_3PO_4 proceeds as an S_N2 reaction, rate of which is considerably increased by the cooperative effect of the acetyl group at C-2 (formation of the *ortho* ester) [7].

In the case of 2-deoxysugars the formation of the *ortho* ester cation is excluded so that the phosphorylation should depend on the configuration of the starting acetate. Phosphorylation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose with cryst. H_3PO_4 afforded 2-deoxy-α-D-glucopyranosyl phosphate [2]; the β anomer was not found in the reaction mixture. This fact suggested that the reaction was an S_N2 one since in the case of the S_N1 mechanism both α and β anomers should be formed. The phosphorylation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose with cryst. H_3PO_4 described in this paper also gave 2-deoxy-α-D-glucopyranosyl phosphate. Similar phosphorylation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose led to 2-deoxy-α-D-galactopyranosyl phosphate. β Anomers were not evidenced in the reaction mixtures. From the above said it may be assumed that the main role in the phosphorylation of 2-deoxysugars plays the relatively higher stability of the anomer possessing the axial arrangement at C-1 of the glycosyl phosphate to be formed (α-glucosyl and α-galactosyl phosphate). The extreme acid lability of 2-deoxyglycosyl phosphates in comparison with glycosyl phosphates (due to the absence of the hydroxyl group at C-2) thus causes that the eventually formed β anomer of a glycosyl phosphate is hydrolyzed to inorganic phosphate and free deoxysugar.

The yields of 2-deoxy-α-D-glucopyranosyl phosphate seem to be independent of the configuration of the starting peracetate; phosphorylation of the α-acetate gave 33% yield, that of the β-acetate about 35% yield [2]. 2-Deoxy-α-D-galactopyranosyl phosphate was obtained in 39% yield. Chromatographically homogeneous 2-deoxy-D-gluco- and 2-deoxy-D-galactopyranosyl phosphates showed higher R_F values than D-gluco- and D-galactopyranosyl phosphates. They gave inorganic phosphate and the corresponding 2-deoxysugar upon hydrolysis. Electrophoretic mobility of 2-deoxyglycosyl phosphates was identical with that of the corresponding standard glycosyl phosphates. The α configuration of the prepared 2-deoxyglycosyl phosphates was confirmed by n.m.r. spectroscopy.

The starting α anomer of per-*O*-acetyl-D-galactopyranose was prepared from 3,4,6-tri-*O*-acetyl-D-galactal by addition of $HClO_4$ followed by reesterification of thus obtained perchloric adduct into peracetylated compound. This procedure has been described for the first time by *Bonner* [8] for the preparation of the α anomer of peracetylated 2-deoxy-D-glucopyranose. We extended this method for the synthesis of crystalline 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose. This way of the preparation of the α anomer of 2-deoxy-D-galactose from tri-*O*-acetyl-D-galactal is more convenient than the classical procedure, *i.e.* the acid catalyzed acetylation of 2-deoxy-D-galactose with acetic anhydride [9]. The synthesis of the α anomer of peracetylated 2-deoxy-D-galactose was shortened by two steps (deacetylation of tri-*O*-acetyl-D-galactal and its hydration) and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-galactopyranose was obtained in crystalline form contrary to the sirupy product of the older procedure [9].

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