CHEMISTRY OF GLYCOSYL PHOSPHATES AND THEIR DERIVATIVES

COMMUNICATION 3. SYNTHESIS OF 2-DEXOSY-α-D-ARABINOHEXOPYRANOSYL PHOSPHATE

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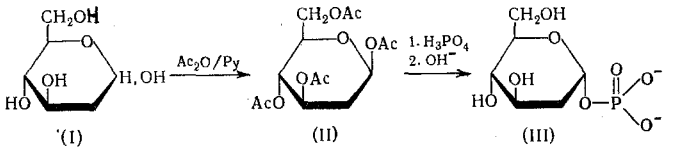
In a previous communication [1] we described the preparation of some desoxyglycosyl phosphates, containing the desoxy unit at C_6, C_4 and C_3 of the monosaccharide moiety, by the phosphorylation of the corresponding β-acetates. The transition to the 2-desoxyhexose derivative is associated with an increase in the lability of the compound due to the presence of the desoxy unit at C_2. Nevertheless, the data on the biochemical synthesis of the phosphorus-containing esters of 2-desoxyglucose [2] made it possible to expect the successful preparation of 2-desoxy-α-D-glucopyranosyl phosphate by a chemical route.

An important difficulty in the synthesis of 2-desoxy-D-glycopyranosyl phosphate is associated with the absence of stereochemical control of the phosphorylation. The data [3] that are available on the mechanism of this reaction make it possible to assume that substitution at C_1 in the acetates of monosaccharides proceeds with a participation of the acetyl group at C_2 and the primary reaction product is usually the β-anomer, which is then anomerized to the α-glycosyl phosphate. In the case of the 2-desoxy derivatives it could be expected that the initial product will be either a mixture of the α- and β-anomers, when the reaction proceeds by the S_N1 mechanism, or only the α-glycosyl phosphate (when reaction is by the S_N2 mechanism). The latter compound should be the predominant reaction product after anomerization of the primary products.

The synthesis of 2-desoxy-α-D-arabino-hexopyranosyl phosphate (III) was accomplished by the following scheme:



(I) (II) (III)

The acetylation of 2-desoxy-D-glucose (I) with Ac_2O in pyridine [4] led to a mixture of the α- and β-acetates (1:2), from which the pure β-anomer of 1,3,4,6-tetra-O-acetyl-2-desoxy-D-arabino-hexopyranose (II) could be isolated in 61% yield. The subsequent phosphorylation of the β-acetate (II) was accomplished by treatment with anhydrous H_3PO_4 [5,6]. Taking into account the high lability of the formed phosphate (III), it was necessary to find the optimum reaction time, for which the phosphorylation at 50°C was run for 15, 30, 60 and 120 min. The acid-labile phosphorus [7] in the reaction mixture was determined after deacetylation and removal of inorganic phosphate. It was found that the highest yield of organic phosphate is observed when the reaction is run for 30 min.

As is known [5,6], the main condition for the successful progress of the phosphorylation reaction is the absence of water in the reaction mixture. We observed that the time of drying the mixture of reactants importantly affects the yield of the end glycosyl phosphate. The highest yield of the sugar phosphate was obtained when the mixture was dried for 45 min, whereas dehydration for either 5 or 90 min led to a decrease in the yield of the glycopyranosyl phosphate, in which connection marked tarring of the reaction mixture occurred in the latter case.

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TABLE 1. Results of Ion-Exchange Chromatography

Fraction	Isolated amount		$[\alpha]_D^{20}$ (C; H_2O)	$[M]^{20}$				
	mg	% *		589 nm	578 nm	546 nm	436 nm	365 nm
134—142	53,9	20,5	+45,9°(0,22)	202	210	260	428	601
143—145	19,1	7,8	+45,7°(0,28)	201	208	245	286	542
146—155	7,2	2,1	+47,0°(0,37)	207	214	256	412	573

*Based on the starting pyranose (II).

As a result, the preliminary experiments made it possible to select the optimum conditions for the phosphorylation of the β-anomer (II). The deacetylation and simultaneous removal of inorganic phosphate from the reaction mixture was accomplished by treatment with LiOH. After removal of the excess Li^+ ions the obtained lithium salt of the desoxysugar phosphate was converted to the dicyclohexylammonium salt (DCHA).

In order to separate any possible impurity of the β-anomer of the 2-desoxy-D-arabino-hexopyranosyl phosphate we attempted to use ion-exchange chromatography on Dowex-AG-1 × 8 (HCO_3 form) [8].

For the separation we used a large excess (500 ml) of the anionite, with elution using a linear gradient of triethylammonium bicarbonate. The fraction of organic phosphates elutes from the column as one peak, and here both the $[\alpha]_D$ and optical rotation dispersion values (Table 1) remain constant within the limits of experimental error, both on the ascending and descending branches of the elution curve.

Taking into account the somewhat higher mobility of the β-anomers of glycosyl phosphates when chromatographed on paper [9, 10], we also attempted to separate the postulated mixture of α- and β-anomers by partition chromatography on cellulose in the system: 7:1.:2 isopropanol–ammonia–water. All of the organic phosphate is eluted from the column as one peak, and here the $[\alpha]_D$ and optical rotation dispersion values remain constant under the given chromatographing conditions (Table 2).

These results make it possible to assume that either a separation of the α- and β-anomers of the 2-desoxyglucose phosphate fails to occur under ion-exchange and partition chromatography conditions, or else that in general the β-anomer of 2-desoxy-D-arabino-hexopyranosyl phosphate is absent in the reaction mixture. The absence of the β-anomer was shown by recrystallizing the DCHA salts by the generally accepted method for separating the α- and β-anomers of glycosyl phosphates [11]. Four recrystallizations of phosphate (III) as the DCHA salt failed to lead to a noticeable change in the optical rotation and, as a result, corroborated the presence of only the α-desoxyglycosyl phosphate.

As evidence in support of the α-configuration of the phosphate group at C_1 is the high $[\alpha]_D$ value ($[\alpha]_D^{20}$ + 72.5°) for the ammonium salt of 2-desoxy-α-D-arabino-hexopyranosyl phosphate. Conclusive proof of the α-configuration for the glycoside center follows from the NMR spectrum, in which the signal of the anomeric proton at C_1 is similar in the chemical shift (δ 5.64 ppm) and in the spin–spin splitting constants ($J_{1,2}$ 3.0, $J_{1,P}$ 7.5 Hz) to the C_1-H signal of α-D-glucopyranosyl phosphate (δ = 5.53 ppm, quadruplet, $J_{1,2}$ 3.5, $J_{1,P}$ 7.5 Hz [12]). A broadening in the lines of the observed quadruplet is due to the additional splitting of the signal of the proton at C_1 with the proton in the equatorial position at C_2 ($J_{1,2e} \leq 1$ Hz). The absence of signals in the 4.95-5.00 ppm region, characteristic for the C_1-H of β-D-glucopyranosyl phosphates [13], testifies to the α-configuration of the obtained phosphate.

TABLE 2. Results of Partition Chromatography on Cellulose

Fraction*	Isolated amount		$[\alpha]_D^{20}$ (C; H_2O)	$[M]^{20}$				
	mg	%†		589 nm	578 nm	546 nm	436 nm	365 nm
10—12	16,5	9,89	+73,0°(0,5)	203	211	240	296	594
13—14	14,9	8,90	+72,1°(0,87)	201	207	236	393	594
15—18	15,9	9,49	+72,5°(0,6)	202	206	234	390	586
19—21	5,7	3,40	+72,4°(0,28)	202	209	238	394	592

*2-Desoxy-D-arabinohexose (R_f ~ 0.81 in system A) was detected in fractions 3-7, while inorganic phosphate (R_f ~ 0.07 in system A) was detected in fractions 25-40.
†Based on the starting β-acetate (II).

The analytical data for the DCHA salt of (III) correspond to the assigned structure. A coinciding of the electrophoretic mobility with the mobility of α-D-glucopyranosyl phosphate corroborates the fact that the obtained desoxysugar phosphate is the glycosyl phosphate. This is also evidenced by the data on the acid hydrolysis and the reaction with phosphoglucomutase. A quantitative decomposition of the 2-desoxyglucosyl phosphate to inorganic phosphate and 2-desoxyglucose occurs under the conditions for the specific cleavage of the glycoside linkage (pH 1.0, 100°, 15 min). A transfer of the phosphate from C_1 to C_6 is observed when reaction is with phosphoglucomutase [14], which is characteristic only for the α-anomers of glycosyl phosphates. The higher R_f values when compared with α-D-glucopyranosyl phosphate corroborate the presence of the desoxy unit in the molecule of the obtained phosphate (III).

As a result, from the above enumerated facts it follows that the sole phosphorylation product of the β-acetate (II) is 2-desoxy-α-D-arabino-hexopyranosyl phosphate (III). The absence of even traces of the β-anomer makes it possible to asume that substitution at C_1 proceeds by the bimolecular mechanism.

EXPERIMENTAL METHOD

The paper chromatography was run in the system: 7:1:2 isopropanol–ammonia–water (system A), while the electrophoresis on paper was run in 0.05 M triethylammonium bicarbonate (TEAB) buffer solution with pH 7.5 (system B) [1]. The phosphorus-containing compounds were detected on the chromatograms and electrophoregrams employing the Hanes–Isherwood reagent [15], while the 2-desoxyglucose derivatives were detected using a solution of perchloric acid in acetone [16]. The 2-desoxysugar phosphate solutions were evaporated at 20-25° and pH 7.5-8.0. The acid-labile and total phosphorus were determined as described in [7].

The NMR spectrum was taken on a Varian XL-100 instrument in D_2O. The $[\alpha]_D$ values and the optical rotation dispersion data were obtained on a Perkin–Elmer 141 instrument. The melting points were determined on a Kofler block. For the partition chromatography we used cellulose that was labeled Whatman column chromedia, while for the ion-exchange we used Dowex-AG-1 × 8 (100-200 mesh).

1,3,4,6-Tetra-O-acetyl-2-desoxy-β-D-arabino-hexopyranose (II). To a solution of 10 g of 2-desoxy-D-glucose (I) in 40 ml of absolute pyridine was added 15 ml of Ac_2O; the obtained suspension was stirred at ~12° until solution was complete and then the solution was allowed to stand at 20° for 16 h. The reaction mixture was poured into 400 ml of ice water, stirred for 1 h, and extracted with $CHCl_3$ (3 × 100 ml). The chloroform extract was washed in succession with chilled 20% HCl solution (3 × 200 ml), water (2 × 200 ml) and saturated $NaHCO_3$ solution; then the solution was dried over Na_2SO_4, the filtrate was evaporated, and the sirup was dissolved in 50 ml of isopropanol. After 15 h at 20° the top layer of crystals (prisms) was carefully decanted along with the mother liquor; the main precipitates was filtered, washed with a small volume of isopropanol, and dried. We obtained 12.32 g (61%) of pyranose (II), mp 85-91°. After three recrystallizations from isopropanol, mp 91-92°; $[\alpha]_D^{20}$ + 2.7° (C 1.0; $CHCl_3$); based on the data given in [4]: mp 92.2-93.2°; $[\alpha]_D^{25}$–2.8° (C 3.54; $CHCl_3$).

2-Desoxy-α-D-arabino-hexopyranosyl Phosphate (III). After being carefully dried in a vaccum-desiccator, 441 mg of H_3PO_4 and 190 mg of pyranose (VII) were dissolved in 2-3 ml of absolute THF, the solution were combined, and the THF was removed by distillation, avoiding the entrance of moisture. The obtained residue was dried in vacuo at 20° for 45 min, and then it was heated at 50° for 30 min. The reaction mixture was dissolved in 4 ml of THF, 15 ml of 1 N LiOH solution was added (0°), and the stirred mixture was allowed to stand at 20° for 18 h, and then at 4° for 2 h. The obtained precipitate was removed by centrifuging at 5000 rpm and then washed with 1 N LiOH solution. To the supernatant liquor (pH 11.5) was added Dowex-50 resin (H^+ form) to pH 8.5-9.0; the ion-exchange resin was filtered, while the filtrate was passed through a column (1 × 10 cm) filled with Dowex-50 resin (CHA form). Based on the chromatography (system A) and paper electrophoresis (system B) data, the elute contains 2-desoxyglucose (R_f ~0.81), the desoxysugar phosphate (R_f ~0.25 in system A, R_{G-1-P} ~1.02 in system B), and a trace amount of inorganic phosphate as impurity (R_f ~0.09 in system A, R_{G-1-P} ~1.2 in system B). The yield of the DCHA salt of the 2-desoxyglucose phosphate was 250 μM (41.5%). The elute was evaporated at ~20° to a sirup, which was then separated either by ion-exchange chromatography or by partition chromatography on cellulose.

Ion-exchange chromatography. The obtained sirup was dissolved in 200 ml of 0.05 M TEAB solution and then transferred to a column (3.3 × 63 cm) containing Dowex-AG-1 × 8 resin (HCO_3 form), that had been previously equilibrated by the passage of ~4 liters of 0.05 M TEAB solution. The column was washed with 100 ml of 0.05 M TEAB solution, and then the compounds were eluted employing a linear gradient of TEAB solutions ranging from 0.05 M (2 liters) to 0.3 M (2 liters), collecting the fractions as 15-ml aliquots (1 ml/min); then the acid-labile phosphorus was determined in aliquots of the fractions. The fractions that contained a homogeneous substance, when based on the chromatography and paper electrophoresis data, were combined, evaporated at 20°, and steam-distilled several times in order to remove the TEAB. Three main fractions were obtained in this manner: 134-142, 143-145 and 146-155, which contained the triethylammonium salt of (III) (R_f ~0.25 in system A, R_{G-1-P} ~1.02 in system B); the reagents for phosphorus and for 2-desoxyglucose were used for detection. The $[\alpha]_D$ values, the optical rotation dispersion data, and the isolated amounts are given in Table 1. The total yield of the 2-desoxy-D-arabino-hexopyranosyl phosphate was 30.4%.

Partition chromatography on cellulose. The obtained mixture of the DCHA salt of the desoxysugar phosphate, H_3PO_4 and 2-desoxyglucose was dissolved in 3 ml of system A, transferred to a column (2.2 × 28 cm) containing cellulose (20 g), and eluted with system A. The acid-labile phosphorus was determined in aliquots of the fractions (6.5 ml/h); the separation was also checked by chromatography and by paper electrophoresis. The fractions, containing the desoxysugar phosphate, were combined, evaporated, and the optical rotation values and amount of acid-labile phosphorus were measured. The chromatography results are given in Table 2. The total yield of the diammonium salt of (III) was 31.6%.

Dicyclohexylammonium Salt (DCHA) of 2-Desoxy-α-D-arabino-hexopyranosyl Phosphate. A solution of 90 mg of the diammonium salt of the desoxysugar phosphate in 10 ml of water was passed through a column (1 × 5 cm) containing Dowex-50 resin (CHA form) [13] and then eluted with ~25 ml of water. The eluate was evaporated at 20° to ~1 ml, acetone was added to the appearance of cloudiness, and the whole was allowed to stand at ~20° for 18 h. The obtained crystals of the DCHA salt of (III) were filtered, washed with acetone, then with ether, and dried to constant weight; the yield was 87.8 mg (61.8%), and mp 133-138°; $[\alpha]_D^{20}$ +44.8° (C 0.5; H_2O). An additional 32.5 mg of the DCHA salt was obtained from the mother liquor. The total yield of the desoxysugar phosphate was 120 mg (84.7%). After four recrystallizations, mp 136-141°; $[\alpha]_D^{20}$ +45.0° (C 0.5; H_2O). Found, %: C 44.92; H 8.80; N 5.66; P 7.03%. $C_{18}H_{39}N_2O_8P \cdot 2H_2O$. Calculated, %: C 45.20; H 8.81; N 5.86; P 6.49%. The acid-labile phosphorus [7]: total phosphorus [7]: desoxysugar [17] ratio was equal to 1.00 : 1.01 : 1.02.

Acid Hydrolysis of 2-Desoxy-α-D-arabino-hexopyranosyl Phosphate. A solution of 1 μM of compound (III) in 1 ml of 0.1 N HCl solution was heated at 100° for 15 min, evaporated to dryness, and steam-distilled to remove traces of HCl. Analysis of the obtained mixture by paper chromatography in system A disclosed the presence of 2-desoxy-D-arabino-hexose (R_f ~ 0.81) and inorganic phosphate (R_f ~ 0.07).

Reaction of 2-Desoxy-α-D-arabino-hexopyranosyl Phosphate with Phosphoglucomutase. A mixture of 1.6 μM of compound (III) in 0.1 ml of water and 12 mg of phosphoglucomutase in 0.4 ml of 0.05 M tris–HCl buffer solution (pH 8) was incubated at 37° for 3 h. At the end of reaction the mixture was separated by paper electrophoresis in system B, eluting the zone that contained the desoxysugar phosphate. The ratio of acid-labile to total phosphorus in the eluate was equal to 1.0 : 3.9. In an analogous experiment with the α-D-glucopyranosyl phosphate this ratio was 1.0 : 4.8. Similar ratios were retained on longer incubation (5 h), which indicates the equilibrium character of this reaction.

CONCLUSIONS

1. Conditions were found for the phosphorylation of 1,3,4,6-tetra-O-acetyl-2-desoxy-β-D-arabino-hexopyranose.

2. The chemical synthesis of 2-desoxy-α-D-arabino-hexopyranosyl phosphate on a preparative scale was accomplished.

3. The absence of the β-anomer in the reaction products was observed.

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