starch product began to be precipitated. After 2 min, the precipitate was collected, successively washed thoroughly with water and acetone, and dried. The yield and i.r. spectrum of the product were similar to those of the product obtained with **1** in the methyl sulfoxide system.

This investigation is being continued along a broad front to determine the generality of the reaction. Exploratory experiments have shown that starch and other polysaccharides react with various cyclic *trans*-carbonates of glycosides. The only apparent restriction for the sugar derivative is that it should possess the *trans*-fused structure. When the cyclic group is *cis*-fused, as with methyl 4,6-*O*-benzylidene-α-D-mannopyranoside 2,3-carbonate, no reaction with starch occurs under the reaction conditions described.

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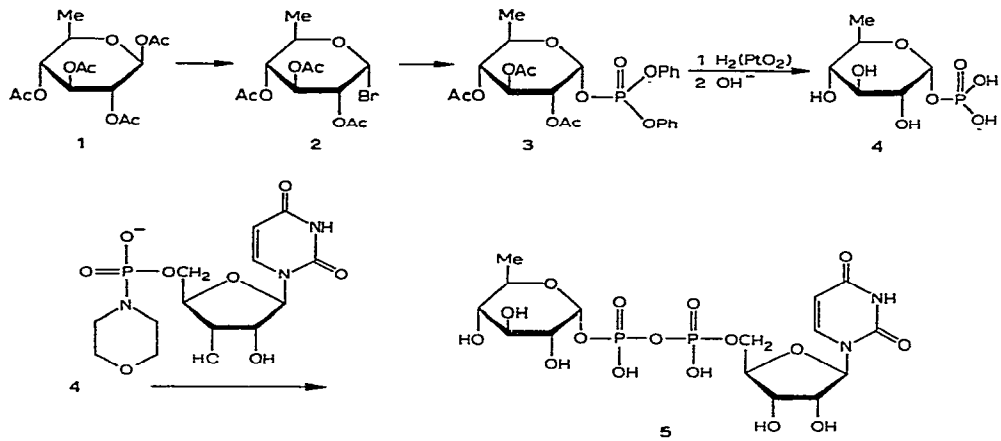
The synthesis of uridine 5-(6-deoxy-α-D-glucopyranosyl dihydrogen pyrophosphate) and its interaction with UDP-glucose dehydrogenase

Syntheses of analogues of uridine 5-(α-D-glucopyranosyl dihydrogen pyrophosphate) (UDPG) having a modified heterocyclic nucleus have been reported[1]. The behaviour of these analogues in chemical and enzymic reactions[2] is in good agreement with the hypothesis[3] that relates intramolecular interactions of glycosyl esters of nucleotides and their biological specificity.

We now report the synthesis and some biochemical properties of a UDPG analogue having the monosaccharide moiety modified at C-6, *viz.*, uridine 5-(6-deoxy-α-D-glucopyranosyl dihydrogen pyrophosphate) (UDP-quinovose, UDPQ). It is of interest to know whether UDPQ can participate in UDPG enzymic reactions, in order to obtain information on the significance of the hydroxyl group at C-6 of the monosaccharide residue in the biological activity of UDPG. Also, UDPQ is a potential inhibi-

tor of enzymic reactions at C-6 of UDPG, for example, the conversion of UDPG into UDP-glucuronic acid, catalyzed by UDPG-dehydrogenase (EC 1,1,1,22)[4]. UDPQ may also be a precursor in the biosynthesis of plant glycosides, containing 6-deoxy-D-glucose.

The synthesis of UDPQ (5) was performed as follows:



Treatment of tetra-*O*-acetyl-6-deoxy-β-D-glucopyranose[5] (1) with a 40% solution of hydrogen bromide in glacial acetic acid gave tri-*O*-acetyl-6-deoxy-α-D-glucopyranosyl bromide (2, 89% yield), m.p. 125–127°, $[\alpha]_D^{20}$ +242° (*c* 0.43, chloroform) (lit.[6,7], m.p. 135–137°, $[\alpha]_D^{20}$ +226°; and m.p. 150–152°, $[\alpha]_D^{20}$ +247°). Treatment of a boiling solution of 2 in benzene with four equivalents of silver diphenyl phosphate for 4 h gave 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-glucopyranosyl diphenyl phosphate (3), isolated by t.l.c. on silica gel (chloroform–methanol, 20:1; R_F 0.60–0.65). Hydrogenation of 3 over platinum and deacetylation with ammonia gave 6-deoxy-α-D-glucopyranosyl phosphate (4) which was purified on Dowex-1 (HCO_3^-) and isolated as the triethylammonium salt. The product was homogeneous by paper chromatography and paper electrophoresis. The ratio (1:0.95) of deoxy sugar[8] to acid-labile phosphate[9], and an electrophoretic mobility equal to that of α-D-glucopyranosyl phosphate (Table I)

TABLE I

PAPER CHROMATOGRAPHIC AND ELECTROPHORETIC DATA[a]

Substance	*Paper chromatography*		*Paper electrophoresis*	
	A	*B*	*C*	*D*
α-D-Glucopyranosyl phosphate	—	1.14	1.25	—
6-Deoxy-α-D-glucopyranosyl phosphate	—	1.15	1.25	—
UDPG	1.09	—	0.80	1.50
UDPQ	1.12	—	0.80	1.50

[a]Mobilities relative to that of uridine 5-phosphate. [b]Solvent systems: *A*, ethanol–M ammonium acetate (5:2) (pH 7.5); *B*, isopropyl alcohol–ammonia–water (7:1:2); *C*, 0.05M triethylammonium hydrogen carbonate (pH 7.5); *D*, 0.05M triethylammonium acetate (pH 4.0).

prove **4** to be a sugar monophosphate. The α-D-configuration of the glycosidic bond in **4** is indicated by the large, positive, optical rotation ($[\alpha]_D^{20}$ +88.6° in water), and by the n.m.r. signal for H-1 (τ 4.70, quartet, $J_{1,2}$ 3Hz and $J_{1,P}$ 7Hz), which closely corresponds to the H-1 signal for α-D-glucopyranosyl phosphate.

For the synthesis of **5**, the triethylammonium salt of **4** was treated with triethylammonium uridine 5′-phosphomorpholidate[10] (3 equivalents) in anhydrous methyl sulfoxide for 3 h at 60°. UDPQ (**5**) was isolated by ion-exchange chromatography on Dowex-1X2 (Cl^-) and desalted by gel filtration[11] on Sephadex G-25. The resulting sodium salt of **5** was homogeneous by paper chromatography and paper electrophoresis, and its mobilities were closely similar to those of UDPG (Table I). The proportions of reducing sugar[12] after acid hydrolysis, to acid-labile phosphate, to total phosphate were 1:1.07:2.02 and correspond to the structure of UDPQ.

The interaction of UDPQ with UDPG-dehydrogenase was investigated with a purified[4,13] enzyme preparation. No reduction of NAD (nicotinamide adenine dinucleotide) under the influence of UDPG-dehydrogenase was obtained in the presence of UDPQ. When UDPQ was added to a mixture of UDPG, NAD, and UDPG-dehydrogenase, a characteristic change in the rate of NAD-reduction was seen. Initially, the rate was very low, but, after several minutes, it increased and became comparable with the rate of reaction in the absence of UDPQ (Fig. 1).

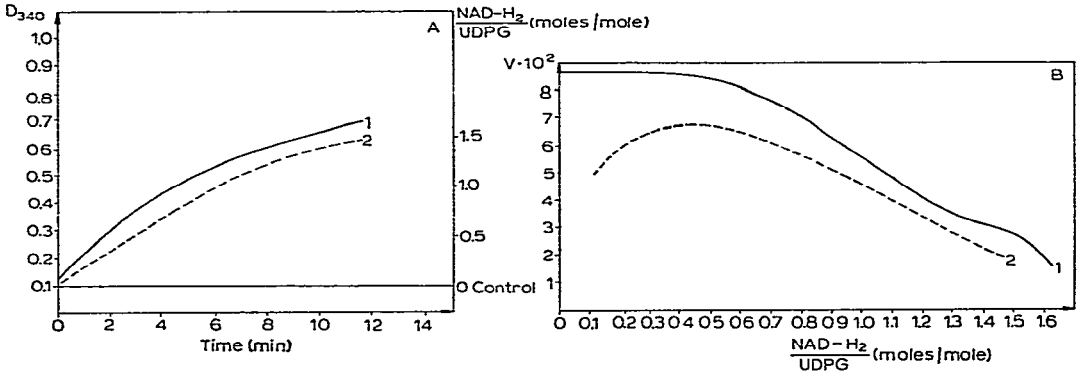


Fig. 1. Kinetics of the interaction of UDPG with UDPG-dehydrogenase in the presence of UDPQ. A. Change of absorbance at 340 nm (D_{340}) *versus* time. B. The rate of NAD-reduction (expressed as the change of D_{340} per min) *versus* the quantity of reduced NAD (calculated from D_{340}, assuming $\varepsilon_{340}=6.22\times10^3$). The curves in Fig. 1B were obtained by graphical differentiation of the curves in Fig. 1A.
Curve 1 (————). The incubation mixture contains enzyme (41 units[4]), glycine buffer (510 μmoles) pH 8.75, NAD (3 μmoles), and UDPG (0.17 μmoles) in a total volume of 3 ml. Curve 2 (— — — —). The incubation mixture contains UDPQ (0.56 μmoles) in addition to the above constituents. The reaction was begun by addition of the enzyme, and the scanning with a Hitachi ESP-2 recording spectrophotometer was begun within 20 sec. All solutions (except that of the enzyme) and the cell compartment of the spectrophotometer were kept at 30°.

Simonart *et al.*[14] have shown that the interaction of UDPG with NAD catalyzed by UDPG-dehydrogenase is a two-step reaction having a large difference in the

velocities of the stages:

$$\text{UDPG} + \text{NAD} \rightarrow \text{X-UDPG} + \text{NADH} + \text{H}^+ \quad (1)$$

$$\text{X-UDPG} + \text{NAD} \rightarrow \text{UDP-glucuronic acid} + \text{NADH} + \text{H}^+ \quad (2)$$

The initial rate of NAD-reduction is close to the rate of stage (1), but the rate of the final section of the kinetic curve is approximately that of the rate of stage (2). The two-step character of the kinetic curve in the reduction of UDPG may be clearly seen in Fig. 1 B.

The effect of UDPQ on the UDPG-dehydrogenase reaction may be interpreted as strong inhibition of stage (1) with weak or no inhibition of stage (2). Differential inhibition of stages (1) and (2) by the same inhibitor may be connected with catalysis of stages (1) and (2) of the UDPG-dehydrogenase reaction by different active sites of the enzyme or by different enzymes. A more-detailed study of enzyme reaction kinetics in the presence of UDPQ is now under investigation in this laboratory.

These results show the importance of UPDQ for the study of the mechanism of enzymic reactions of glycosyl esters of nucleotides.

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