$$\begin{array}{c}
 \text{H}_2\text{N}-\overset{\text{O}}{\parallel}\text{P}-\text{O}-\text{CH}_2-\text{C}_5\text{H}_4\text{OR}' \\
 | \\
 \text{OH}
 \end{array}
 + (\text{C}_2\text{H}_5)_3\text{N}^+\text{H} - \text{O}-\overset{\text{O}}{\parallel}\text{P}-\text{O}-\overset{\text{O}}{\parallel}\text{P}-\text{O}-\text{H} + (\text{C}_2\text{H}_5)_3\text{N}^+\text{H}
 \xrightarrow{\hspace{1cm}}$$

$$\begin{array}{c}
 \text{HO}-\overset{\text{O}}{\parallel}\text{P}-\text{O}-\overset{\text{O}}{\parallel}\text{P}-\text{O}-\overset{\text{O}}{\parallel}\text{P}-\text{O}-\text{CH}_2-\text{C}_5\text{H}_4\text{OR}' \\
 | \quad | \quad | \\
 \text{OH} \quad \text{OH} \quad \text{OH}
 \end{array}$$

R=adenine, uracil, or cytosine
 R'=OH or H

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Synthesis of Uridine Diphosphate-Glucuronic Acid

A solution of 123 mg. (0.128 m.mole) of the dicyclohexylguanidinium salt of UMP-NH₂⁵⁹

*1 Abbreviations used : UDPGA, uridine diphosphate-glucuronic acid; UDPG, uridine diphosphate-glucose; UMP, uridine 5'-phosphate; UMP-NH₂, uridine 5'-phosphoramidate; UDP, uridine 5'-diphosphate; DUPP, P¹,P²-diuridine 5'-pyrophosphate; DPN, diphosphopyridine nucleotide; GA-1-P, glucuronic acid 1-phosphate

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3) J. Solms, W. Z. Hassid : J. Biol. Chem., **228**, 357(1957).

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(I) in pyridine or *o*-chlorophenol was added to a solution of the triethylammonium salt of α -GA-1-P(II) (prepared from 393 mg. (1.01 m.moles) of the tripotassium salt of α -GA-1-P^{6,7)} in the same solvent and the mixture was left to stand at 37° for 10 days. The solvent was distilled off from the reaction mixture and an aqueous solution of the residue was subjected to chromatography on a column of 15 cc. of Dowex-1 $\times$ 8 (chloride form). The column was treated first with 0.003*N* HCl+0.01*M* NaCl to elute UMP and UMP-NH₂, then with 0.003*N* HCl+0.04*M* NaCl to wash out a fraction supposed to be a mixture of DUPP and UDP, and finally with 0.003*N* HCl+0.1*M* NaCl to elute a fraction appearing to contain UDPGA (III). The final fraction was adsorbed on charcoal and eluted with 50% ethanol containing 0.5% of ammonia, and the sole presence of UDPGA in the concentrated eluate was confirmed by the following tests: a) Paper electrophoresis (0.1*M* acetate buffer, pH 3.9, 11 V/cm., 500 V applied, 2.5 hr.) gave only one ultra-violet-absorbing spot at $R_{UMP-NH_2} = 2.0$; b) the analytical values of the UDPGA fraction (Table I); c) an aliquot was heated with 0.1*N* HCl at 100° for 10 min. and paper electrophoresis as well as paper partition chromatography of the hydrolysate showed the presence of UDP besides UMP, which fact seems to explain the sequence of the components; and d) the p*K* value indicated the presence of one free carboxyl group (p*K*_a=3.2).

TABLE I. Analysis of UDPGA Fraction

	Ratio (referred to uridine)	
	Found	Calcd. for UDPGA
Uridine	1	1
Total phosphate	1.992	2
Acid-labile phosphate	0.945	1
Inorganic phosphate	0	0
Glucuronic acid	0.893	1
Reducing value	0	0
Reducing value after hydrolysis*	0.885	1

* Hydrolysis with 1*N* HCl for 10 min. at 100°.

Chart 1.

*² Ratio of the migration distance of the sample divided by that of UMP-NH₂.

6) C. A. Marsh: J. Chem. Soc., 1952, 1578.

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Next, (I) was allowed to react with the triethylammonium salt of β -GA-1-P⁸⁾ (IV) under the same conditions as in the case of the α -compound, giving β -UDPGA (V). Test of α - and β -UDPGA for their ability to form *o*-aminophenol glucuronide, using the UDPGA-transferase present in microsomes of the liver of a guinea pig^{4,9),*3} showed that only the α -compound is active.

Therefore, barium acetate and ethanol were added to the concentrated α -UDPGA eluate mentioned above and the resulting barium salt was reprecipitated from hydrous ethanol to give a colorless powder (yield, 37.7 mg.), which gave only one spot corresponding to UDPGA in paper electrophoresis (*Anal.* Calcd. for $C_{15}H_{19}O_{18}N_2Ba_{1/2}$: C, 22.90; H, 2.42; N, 3.56; P, 7.89. Found: C, 22.73; H, 2.71; N, 3.39; P, 7.61).

As another synthetic method for α -UDPGA(III), oxygen was introduced into an aqueous solution of the sodium salt of α -UDPG(VI) at 40° for 10 hr., in the presence of a platinum catalyst, and paper electrophoresis of the reaction mixture showed the formation of UDPGA, which also had the ability to form *o*-aminophenol glucuronide.

That natural UDPGA takes α -structure is evident from the fact that the ability to form β -glucuronide was found in α -UDPGA prepared by the two methods but not in β -UDPGA. In addition, it was made clear that oxidation of α -UDPG to UDPGA by UDPG-dehydrogenase is not accompanied with Walden inversion, but the inversion takes place when α -UDPGA participates in the glucuronide formation by the action of transferase.

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May 25, 1960.

*3 The enzymatic assay was conducted with the aid of Mr. K. Hatanaka of the Biochemical Department, School of Medicine, Kyoto University.

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