UDC 547.854.4'456'118.5'457 : 577.15

81. Tohru Ueda: Studies on Coenzyme Analogs. V.*1 Syntheses of Analogs of Uridine Diphosphate Glucose.

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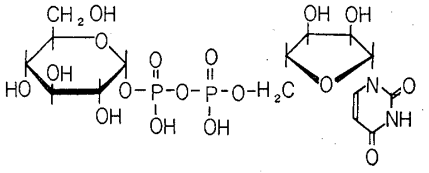
Uridine diphosphate glucose(UDPG*3) is now known as one of the most important sugar nucleotides and has the structure (I) in Fig. 1.[1] It acts as glucose carrier for enzymatic



UDPG(I)

—→ Sucrose, glycogen, etc., α-type condensation.

—→ Cellulose, lactose, etc., β-type condensation.

UDP-α-glucuronic acid —→ β-Glucuronides

Fig. 1.

syntheses of sucrose and glycogen in which α-glycosidic linkages are retained. On the other hand, in the case of syntheses of cellulose and lactose, β-form is produced in spite of α-configuration of the coenzyme. Furthermore, in the case of glucuronide formation, UDPG is converted to UDP-glucuronic acid by the aid of UDPG-dehydrogenase and gives β-glucuronides with inversion of α-glucuronidic linkage of the coenzyme.[2] To investigate the stereospecificity of these glucosyl transferring reaction, the syntheses of UDPG-analogs are required. For this purpose, UDP-β-glucose (IV), in which the glycosidic center of natural one was inverted to β, was synthesized.

The syntheses of UDPG-analogs, in which the uracil moiety of the coenzyme is substituted by several uracil derivatives known as the anticancer agents (i.e. 5-bromo, 5-mercapto, 6-aza, etc.), is very interesting. Among these, the synthesis of 5-bromouridine diphosphate glucose (VI) is reported in this paper.

It was described in the preceding paper*1 that the best synthetic procedure for pyrophosphate is a phosphoramidate method. As stated in the preceding paper, however, attempted synthesis of UDPG (V) by the reaction of UMP with β-D-glucose 1-phosphoramidate failed and, therefore, uridine 5'-phosphoramidate (UMP-amidate) (IIIa) and β-D-glucose 1-phosphate were reacted by a slightly modified Khorana's method for UDPG synthesis.[3]

β-D-Glucose 1-phosphate[4] was converted to its monobrucinium salt, which was obtained as a solid mass, easily soluble in pyridine and suitable for the reaction. This brucinium salt and UMP-amidate were reacted in pyridine for 6 days at room temperature. UDP-β-glucose (IV) was obtained as its lithium salt by ion exchange chromatography from the reaction mixture, the yield being 50% as calculated from the pattern

*1 Part IV : This Bulletin, 8, 459(1960).

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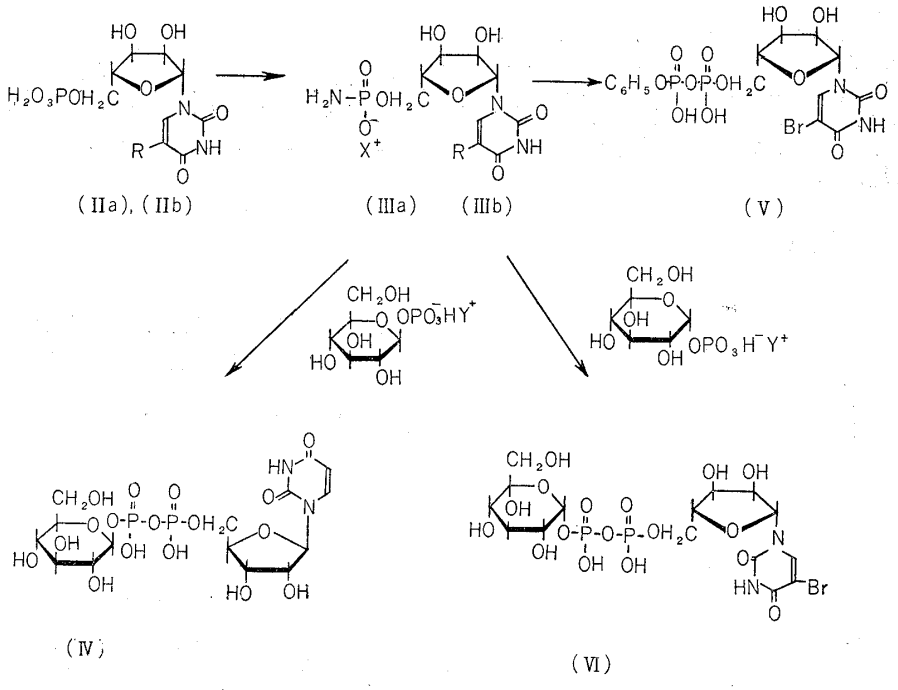
*3 Following abbreviations are used in this paper : UDPG, uridine diphosphate glucose; UMP, uridine 5'-phosphate; UDP, uridine 5'-diphosphate.

1) L. F. Leloir : "Phosphorus Metabolism," **1**, 67(1951). Johns Hopkins Press, Baltimore, U. S. A.
2) Recent general discussions are reviewed by W. Z. Hassid, *et al.* : Proc. Natl. Acad. Sci., **45**, 905 (1959).
3) R. W. Chambers, H. G. Khorana : J. Am. Chem. Soc., **80**, 3752(1958).
4) M. L. Wolfrom, *et al.* : *Ibid.*, **64**, 23(1942).

of the chromatogram. It was 70~73% pure on a weight basis as estimated by ultraviolet absorption and phosphorus analysis. When dibrucinium salt of β-D-glucose 1-phosphate[4] was used instead of monobrucinium salt, the yield decreased to 38%. The chemical behavior of UDP-β-glucose was tested by acid hydrolysis according to the method adopted by natural UDPG.[5] Glucose, UDP, and a small amount of UMP (IIa) were detected in the hydrolyzate at 100° for 20 minutes in 0.01*N* hydrochloric acid. UDP-β-glucose is similar to natural UDPG in chemical and chromatographic properties except for its specific rotation. Biological activity of UDP-β-glucose will be reported elsewhere.

5-Bromouridine 5'-phosphate (IIb), described in a previous paper,[6] was converted to its phosphoramidate (IIIb) by reaction with dicyclohexyl carbodiimide and ammonia in formamide and *tert*-butanol at 80° for 7 hours. Bromine in 5-position was not affected by any substitution reaction under this condition. When this amidate was reacted with phenyl phosphate in pyridine, 5-bromouridine phenyl diphosphate (5-Bromo-UDP-phenol) (V) was obtained as detected by paper and ion exchange chromatography, which proved the reactivity of this amidate for phosphorolysis. Monobrucinium salt of α-D-glucose 1-phosphate was prepared and reacted with 5-Bromo-UMP-amidate (IIIb) in pyridine in analogous reaction conditions in the synthesis of UDP-β-glucose. 5-Bromo-UDP-α-glucose (VI) was detected by paper chromatography. Ion exchange chromatography also showed the presence of (VI) but the yield was low and unreacted amidate, 5-Bromo-UMP, and probably P^1,P^2-bis(5-bromouridin-5'-yl) pyrophosphate due to the hydrolysis of amidate, were found together, and 5-Bromo-UDP-α-glucose (VI) could not be isolated in pure form. β-D-Glucose 1-phosphate, instead of α-anomer, was also reacted with 5-Bromo-UMP-amidate and similarly gave 5-Bromo-UDP-β-glucose.



(IIa), (IIb) (IIIa) (IIIb) (V)

(IV) (VI)

a : series, R=H
b : series, R=Br

X^+ : dicyclohexylguanidinium
Y^+ : brucinium

5) A. C. Paladini, L. F. Leloir : Biochem. J., **51**, 426(1952).
6) Part III. T. Ueda : This Bulletin, 8, 455(1960).

Experimental

Rf Values in Paper Chromatography

Solvent system	I	II	III
UMP	0.44	0.16	0.29
UDP	0.18		0.16
UMP-amidate (IIIa)	0.40	0.40	
UDP-β-glucose (IV)	0.32	0.30	
5-Bromo-UMP (IIb)	0.44	0.16	
5-Bromo-UMP-amidate (IIIb)	0.40		
5-Bromo-UDP-α-glucose (VI)	0.33	0.30	

Uridine Diphosphate β-Glucose (IV)—Dibrucinium β-D-glucose 1-phosphate[4] was dissolved in water and passed through a column of ion exchange resin (Amberlite IR-120). To the filtrate equal amount of the dibrucinium salt was added and evaporated to dryness under a reduced pressure. The resulting solid was used as monobrucinium β-glucose 1-phosphate. Dicyclohexylguanidinium salt of UMP-amidate*4(IIIa) (180 mg.) and monobrucinium β-D-glucose 1-phosphate (880 mg., 4 moles) were dissolved in pyridine (18 cc.) and the solution was allowed to stand for 6 days at 23° in a sealed tube. After pyridine was evaporated, water was added to make the whole volume 50 cc., adjustd to pH 8.0, and applied to the top of a column (2×8 cm.) of Amberlite IRA-400 (Cl^- form). After washing with water (500 cc.), the column was eluted with 0.003*N* HCl containing LiCl. UMP-amidate (IIIa) and UMP (IIa) (876 O.D. unit) eluted with 0.01*N* LiCl. UDP-β-glucose (IV) (1066 O.D. unit, 50%) eluted with 0.06*N* LiCl. This fraction was adjusted to pH 7.0 with LiOH and evaporated under a reduced pressure, below room temperature. MeOH (20 cc.) and a large excess of Me_2CO (200 cc.) were added to the residue and precipitated white solid was collected by centrifugation. The solid thus obtained was dissolved in a small volume of water and reprecipitated in the same manner, washed with EtOH and Et_2O, and dried (40 mg., 20%).

This material was found to be homogeneous by paper chromatography, and 70% and 73% pure on a weight basis as estimated by UV calculation and phosphorus analysis, respectively. The ratio of uridine to phosphorus is 1 : 2.10 (theoretical, 1 : 2.0). $[\alpha]_{589}^{16}$ +7.8° (c=0.57, H_2O) (natural UDPG, $[\alpha]_D$ +43.6° by Khorana[7]). UDP-β-glucose was completely hydrolyzed in 20 min. at 100° in 0.01*N* HCl and the products were glucose, UDP, and UMP.

Dibrucinium β-D-glucose 1-phosphate (100 mg., 5 moles) and UMP-amidate (11.6 mg.) were dissolved in pyridine (1.5 cc.) and allowed to stand for 4 days at 20° in a sealed tube. The solution was added to water, concentrated to a small volume, and adjusted to pH 8.0. This solution was submitted to ion exchange chromatography as described above. The formation of (IV) decreased to 38% in yield (Fig. 2).

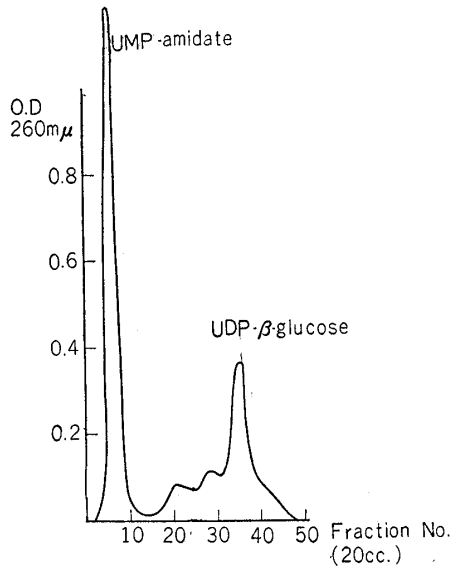


Fig. 2. Reaction of UMP-amidate and Dibrucinium Glucose 1-Phosphate

Formation of UDP-β-glucose=38%

Concave gradient elution method: 0.003*N* HCl; 0.01*N* LiCl; 0.1*N* LiCl

*4 UMP-amidate was synthesized by Khorana's method[3] but could not be obtained in crystal form. This is 70% pure as estimated by ion exchange chromatography. Reaction with phenyl phosphate gave UDP-phenol in 78% yield.

7) J. G. Moffatt, H. G. Khorana : J. Am. Chem. Soc., **80**, 3756(1958).

5-Bromouridine 5′-Phosphoramidate (IIIb) and 5-Bromo-UDP-phenol (V)—5-Bromouridine 5′-phosphate (IIb) (3 m. moles) was dissolved in 2*N* NH_3 (7.5 cc.) and formamide (5 cc.). Dicyclohexyl carbodiimide (3 g.) was dissolved in *tert*-BuOH and this solution was added to the above solution. The two-phase mixture was kept at 80° for 7 hr. in a stoppered flask. The solution, after cool, was filtered and precipitated dicyclohexylurea was washed with water. *tert*-BuOH was removed under a reduced pressure and the aqueous formamide solution was extracted 3 times with Et_2O. Water was removed under a reduced pressure and dry acetone was added to precipitate the amidate. The solid mass was collected by filtration, washed with Me_2CO, and dried (450 mg.). UV: λ_{max} 276 mμ. This gave positive test for Br_2 and negative to $FeCl_3$ reagent.*5 This was 70% pure as estimated by ion exchange chromatography.

A solution of the amidate (IIIb) and phenyl phosphate dissolved in pyridine was allowed to stand for 3 days at room temperature in a sealed tube and the solution was submitted to ion exchange chromatography. 5-Bromo-UDP-phenol (V) formed in 67% yield, calculated from the chromatogram (Fig. 3).

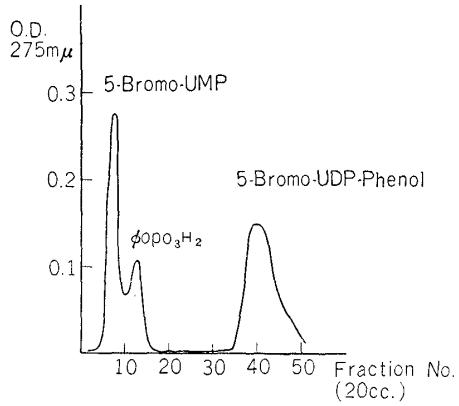


Fig. 3. Reaction of 5-Bromouridine 5′-Phosphoramidate and Phenylphosphate

Formation of 5-Bromo-UDP-phenol = 67%

Concave gradient elution method: 0.003*N* HCl; 0.01*N* LiCl; 0.5*N* LiCl

5-Bromouridine Diphosphate Glucose (5-Bromo-UDP-α-glucose) (VI) and its β-Anomer—Barium α-D-glucose 1-phosphate[8] was converted to its monobrucinium salt as described above and dried over P_2O_5. A solution of 5-Bromo-UMP amidate (IIIb) and this salt dissolved in pyridine was allowed to stand for 4 days at room temperature in a sealed tube. Paper chromatography showed the presence of (VI) but the amidate remained unreacted. Ion exchange chromatography was carried out with this aliquot and formation of (VI) was confirmed, but appreciable amount of the amidate or 5-Bromo-UMP (IIb) and a by-product, probably P^1,P^2-bis(5-bromouridin-5′-yl) pyrophosphate, was also detected (Fig. 4). β-D-Glucose 1-phosphate, in place of α-anomer, was similarly reacted with the amidate and 5-Bromo-UDP-β-glucose was detected on paper chromatogram.

Paper chromatography was carried out in three solvent systems: (I) EtOH-*N* AcOH (5 : 2), (II) EtOH-0.5*N* $AcONH_4$ (5 : 2). (III) BuOH-AcOH-H_2O (5 : 2 : 3). Detection: Base by ultraviolet absorption; P by $HClO_4$-molybdate spray.[9]

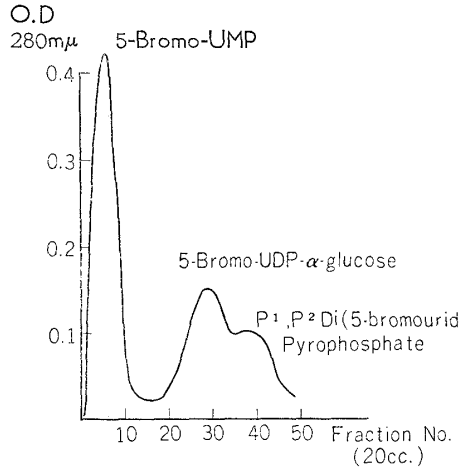


Fig. 4. Reaction of 5-Bromouridine 5′-Phosphoramidate and Monobrucinium α-Glucose 1-Phosphate

Concave gradient elution method: 0.003*N* HCl; 0.003*N* HCl; 0.2*N* LiCl

*5 5-Hydroxyuracil derivatives give blue or violet color with this reagent. See Part III of this series.[6]

8) T. Posternak, *et al.*: J. Am. Chem. Soc., **72**, 4824(1950).
9) C. S. Hanes, F. A. Isherwood: Nature, **164**, 1107(1949).

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Summary

Syntheses of UDP-β-glucose and 5-Bromo-UDPG was attempted to investigate the stereospecificity of coenzyme UDPG. UMP-amidate and monobrucinium β-D-glucose 1-phosphate were reacted in pyridine, and UDP-β-glucose was obtained as its lithium salt by the aid of ion exchange chromatography. The structure of (Ⅶ) thus obtained was ascertained by acid hydrolysis.

5-Bromo-UMP was converted to the phosphoramidate and reacted with α-D-glucose 1-phosphate. 5-Bromo-UDPG was detected on paper chromatogram and ion exchange chromatogram, but the yield was low and 5-Bromo-UMP and another by-product were detected.

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82. Ken'ichi Takeda and Taichiro Komeno : Bile Acids and Steroids. XII.*2 Thiosteroids (1). The Fission of Epoxide in the C-Ring of Steroids by Thiocyanic Acid.

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There have been a few attempts[1] to introduce a sulfur atom into steroids, but no evidence that a sulfur atom has been introduced into the C-ring. These methods mostly consisted of substitution reactions, in which halides or tosylates are converted to thiocyanates or thiols by treatment with thiocyanato or mercapto ion, respectively. In the study of alkene episulfide, van Tamelen[2] found that cyclohexene and cyclopentene epoxides could be converted into the corresponding thiocyanatohydrins on being treated with thiocyanic acid in ether. Thus, this method was applied to steroidal epoxides and succeeded in introducing thiocyanato groups into the C-ring. The present paper is concerned with the fission of steroidal epoxides by thiocyanic acid.

When methyl 3α-acetoxy-11α,12α-epoxycholanate[3] (Ⅱ) was treated with thiocyanic acid in ether at room temperature for 60~70 hours, the compound (Ⅲ), m.p. 169~171°, which showed peaks at 3390 cm^{-1} (OH) and 2151 cm^{-1} (SCN) in its infrared spectrum, was

*1 Imafuku, Amagasaki, Hyogo-ken (武田健一, 米野太一郎).

*2 Part XI. K. Takeda, K. Igarashi : J. Biochem. (Japan), **46**, 1313(1959).

1) (3β-SH) H. R. Rosenberg, S. G. Turnball : U. S. Pat., 2,375,873, 2,375,874 (C. A., **39**, 5049(1954)); L. King, R. M. Dodson, L. A. Subluskey : J. Am. Chem. Soc., **70**, 1176(1948); S. Bernstein, K. J. Sax : J. Org. Chem., **16**, 685(1951). (3-Thione) L. Jirousek : Chem. Listy, **47**, 726(1953). (7-SCN and 7-SH) S. Frederikson, Sr. Liiberg : Chem. Ber., **88**, 684(1955). (21-SH) C. Djerassi, A. L. Nussbaum : J. Am. Chem. Soc., **75**, 3700(1953). (1α- and 7α-SH) R. M. Dodson, R. C. Tweit: *Ibid.*, **81**, 1224(1959).

2) E. E. van Tamelen : J. Am. Chem. Soc., **73**, 3444(1951).

3) J. Press, T. Reichstein : Helv. Chim. Acta, **25**, 878(1942); G. H. Ott, T. Reichstein : *Ibid.*, **26**, 1799(1943).