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137. **Morio Ikehara**: Studies on Coenzyme Analogs. VII. The Synthesis of 2-Oxo-3-β-D-ribofuranosyl-2,3-dihydropyrimidine 5'-Di- and 5'-Triphosphates.

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In the preceding paper[1] the synthesis of 2-oxo-3-β-D-ribofuranosyl-2,3-dihydropyrimidine (6-deoxyuridine)(I) and its 5'-monophosphate (6-deoxy-UMP[*2])(II) was reported. To examine the effect of 6-substituent on the pyrimidine ring for specificity in the reaction of some enzyme systems, 5'-di- (III) and 5'-triphosphates (IV) were now synthesized.

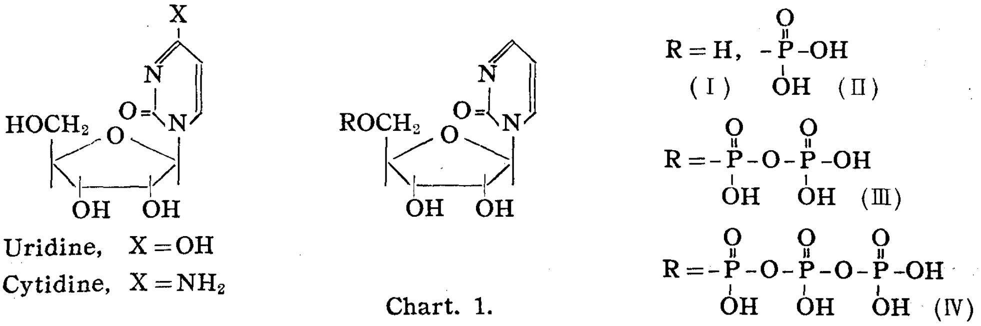

Chart. 1.

Natural ribonucleoside 5'-diphosphates were proved to be direct precursors of polynucleotides in the bacterial polynucleotide phosphorylase system.[2] Several false nucleoside diphosphates, such as 6-azauridine diphosphate[3] or ribosylthymine diphosphate,[4] were also examined for activity on this enzyme.

Nucleoside 5'-triphosphates, represented by ATP, are known to be the important cofactors in many biochemical reactions. Among these, the ATPase activity of muscle fiber[5] must be a suitable system for examining the effect of the change of structure of triphosphate.[6~8]

The synthesis was carried out according to Khorana's general method[9] for the synthesis of ribo- and deoxyribonucleoside 5'-di- and 5'-tri-phosphates. Barium salt of 6-deoxyuridine 5'-monophosphate[1](I) was converted to its pyridinium salt by passage through Amberlite IR-120 ion-exchange resin and reacted with 85% orthophosphoric acid in the presence of excess DCC. Tightly stoppered reaction vessel was set aside at room temperature (ca. 15~20°) for 48 hours. Reaction mixture was purified on the column of Amberlite IRA-400 by stepwise elution technique with dilute hydrochloric acid and lithium chloride (Fig. 1). Two major peaks were evaporated and tested by paper chromatography in several solvent systems (see Experimental). Both were converted to their barium salt in order to obtain non-hygroscopic material.

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[*2] Following abbreviations are used: UMP, uridine 5'-phosphate; ATP, adenosine 5'-triphosphate; DCC, dicyclohexylcarbodiimide.

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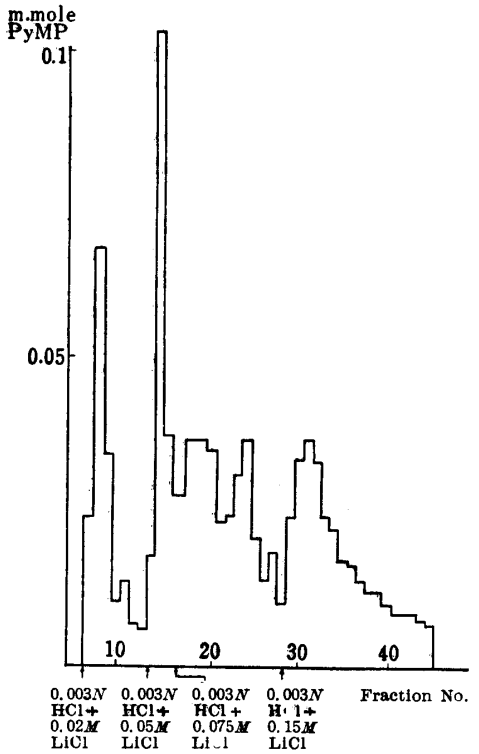

Fig. 1. Ion-exchange Chromatogram of Phosphorylation of 6-Deoxy-UMP

From the elementary analysis data of barium salt of the diphosphate, molecular formula of diphosphate–$Ba_{1.5}$ and the ratio of base to total P and labile P were given as 0.92:2.05:1.00, which shows the structure of 6-deoxyuridine diphosphate correctly. Yield was 15.0% on the weight basis.

Triphosphate material was analyzed to have the structure of triphosphate–$Ba_2 \cdot 2H_2O$ and had a ratio of base:total P:labile P of 1.15:3.08:2.00. The migratory behavior and ion-exchange pattern together with the above data gave proof of this material as 6-deoxyuridine triphosphate.

Acid hydrolysis of both phosphates was carried out with 1*N* hydrochloric acid at 100° for 7 minutes or with 2*N* hydrochloric acid at 100° for 30 minutes. In each case monophosphate, inorganic phosphate, and pyrimidine were detected on paper chromatogram. This fact corresponded to the instability of starting nucleoside previously investigated.[1]

The biological activity of these di- and tri-phosphate thus obtained is now under investigation and will form a separate communication.

Experimental

Reaction of 6-Deoxy-UMP with Orthophosphoric Acid—A solution of 100 mg. of 6-deoxy-UMP Ba salt dissolved in 10 cc. of water was passed repeatedly through a column (1×5 cm.) of Amberlite IR-120 (H^+-form, 100~200 mesh). The effluent was added with 1 cc. of pyridine and evaporated *in vacuo* below 25° to a syrup. The residue was dissolved in 2 cc. of pyridine, 116 mg. of 85% H_3PO_4 was added, and followed by the addition of 1.03 g. of DCC and 0.5 cc. of $(Bu)_3N$ (ratio of monophosphate : DCC was 1:50). The whole was kept in a stoppered flask in a desiccator at 20° for 48 hr. An aliquot of reaction mixture was extracted several times and tested for the presence of monophosphate by paper chromatography. Monophosphate was almost one-half of the initial quantity at 24th hr. and gradually decreased to 1/4 by visual investigation of UV absorbing spot on the paper chromatogram. After 48 hr., the precipitate that appeared was removed by filtration and the filtrate was diluted with water (5 cc.). This was evaporated under a reduced pressure below 25° to afford a syrup, which did not have the odor of pyridine (pH was adjusted to 8 by the addition of LiOH). The solution was diluted to 25 cc. with water and submitted to ion-exchange chromatography.

Ion-exchange Chromatography—The above solution (total optical density, 15000) was applied on the top of a column (0.8×7 cm.) of Amberlite IRA-400 (Cl′ form, 100~200 mesh) at a flow-rate of 1 cc./min. After washing with water (500 cc.), the column was eluted with 0.003*N* HCl + 0.02*M* LiCl (800 cc.), 0.003*N* HCl + 0.05*M* LiCl (400 cc.), 0.003*N* HCl + 0.075*M* (1200 cc.), 0.003*N* HCl + 0.15*M* LiCl (1500 cc.), and 0.003*N* HCl + 0.5*M* LiCl (900 cc.) used as the eluting solution. Results are shown in Fig. 1, in which fraction Nos. 14~22 (A) contained diphosphate and the fraction Nos. 30~39 (B) contained triphosphate from the examination of paper chromatogram. Mixed fractions in between were discarded.

Fraction (A) and (B) were neutralized with LiOH and evaporated separately in a rotary evaporator under a reduced pressure, below 25°, dried over P_2O_5 for 8 hr. at 2 mm. Hg, and the resulting white powder was washed with dehyd. MeOH to remove LiCl. In order to avoid the hygroscopic nature of this material, (A) and (B) were each dissolved in a small amount of water and added with equivalent amount of $(AcO)_2Ba$ at pH 8. Resulting white precipitate was removed by centrifugation and the supernatant was added with 2 volumes of EtOH. The precipitate that appeared was collected by centrifugation, washed with EtOH, EtOH-Et_2O, and dehyd. Et_2O, and dried over P_2O_5 for 5 hr. *Anal.* Calcd. for $C_9H_{10}O_{11}N_2Ba_{1.5}P_2$ (A): C, 18.59; H, 1.53; N, 4.82; P, 10.69. Found: C, 18.50; H, 1.25; N, 4.03; P, 10.60. Ratio of bese:total P:labile P=0.92:2.05:1.00. Purity, 85.5% (on the basis of ε_{298} 2365 of 6-deoxyuridine). Yield, 22.3 mg. (15.3%). *Anal.* Calcd. for $C_9H_{11}O_{14}N_2Ba_2P_3 \cdot 2H_2O$ (B): C, 14.20; H, 1.96; N, 3.66; P, 12.13. Found: C, 14.46; H, 1.92; N, 3.15; P, 12.29. Ratio of base: total P:labile P=1.15:3.08:2.00. Purity, 74.2% (on the basis of ε_{298} 2365 of 6-deoxyuridine).

Treatment of fraction Nos. 6~13 analogously as above gave only 5.3% of recovered monophosphate. This may be due to contamination with inorganic phosphates eluted along with the earlier effluent.

Paper Chromatography—Applied on Toyo Roshi No. 51A. Results are listed on Table I. In each experiment inorganic orthophosphate was used as reference by cochromatography technique. Migratory behavior of diphosphate and triphosphate are just between corresponding uridine and adenosine 5′-di- and 5′-tri-phosphates.

TABLE I.

		Rf Value					
		Base	Monophosphate	Diphosphate	Triphosphate	Ribose-P	Pi
Solvent	I		0.25	0.15	0.06		0.55
	II		0.70	0.55	0.47		0.76
1*N* HCl, 100°, 7 min.	(I)		0.23				0.54
2*N* HCl, 100°, 30 min.	(I)		0.23				(+)
	(III)	0.48				0.29	

I : iso-PrOH-1%:$(NH_4)_2SO_4$=2:1 ↓
II : iso-PrOH (75 cc.)-H_2O (25 cc.)-CCl_3COOH (5 g.)-NH_4OH (0.25 cc.) ↓
III : BuOH:H_2O=86:14 ↑

Acid Hydrolysis (cf. Table I)—Hydrolysis with 1*N* HCl at 100° for 7 min. (7 min.-P) gave the spot of monophosphate and inorganic phosphate. While hydrolysis with 2*N* HCl at 100° for 30 min. gave the spot of 2-hydroxypyrimidine, monophosphate, and inorganic phosphate. Ribose phosphate was also detected. Detection was carried out as follows: Base by visual investigation of UV absorbing area, sugar by IO_4'-benzidine spray, and P by Hanes-Isherwood method.

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Summary

2-Oxo-3-β-D-ribofuranosyl-2,3-dihydropyrimidine 5′-diphosphate and 5′-triphosphate were synthesized by the reaction of 2-oxo-3-β-D-ribofuranosylpyrimidine 5′-phosphate with orthophosphoric acid in the presence of DCC. The structure of both compounds was chemically elucidated.

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