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79. **Tohru Ueda**: Studies on Coenzyme Analogs. III.[*1] Syntheses of 5-Substituted Uridine 5'-Phosphates.

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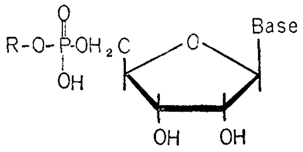
It was established by Ochoa, *et al.*1) that nucleoside 5'-diphosphate (Ia) is a direct precursor for the biosynthesis of ribonucleic acid. In the course of studies on anticancer agents, it seemed of great interest to synthesize the 5'-phosphate (Ib) and 5'-diphosphate (Ia) of nucleoside analogs and to investigate inhibitory activity of these compounds for the polynucleotide phosphorylase action. Furthermore, 5'-phosphates of nucleoside analogs are of much value for the investigation of substrate specificity of 5'-nucleotidases.



Base: Purines or pyrimidines

(I) R=H

(Ia) R=PO_3H_2

In this paper, the sytheses of 5'-phosphates of uridine analogs, i.e. 5-bromo-, 5-hydroxy-, and 5-morpholino-uridine 5'-phosphate, are reported.

5-Bromouridine 5'-phosphate (5-Bromo-UMP[*3]) (Ⅳa) was synthesized by Michelson2) in 1958 by way of bromination of UMP with N-bromosuccinimide. In the present work, (Ⅳa) was obtained by phosphorylation of 5-bromouridine3) (Ⅱ). The reaction of 5-bromouridine with acetone in the presence of *p*-toluenesulfonic acid4) gave 5-bromo-2',3'-isoproylideneuridine (Ⅲ) in a good yield. Phosphorylation of (Ⅲ) was carried out with both phosphoryl chloride-pyridine and phosphorus pentoxide-phosphoric acid mixture. Treatment of (Ⅲ) with the former reagent at -10° for 2 hours gave barium salt of 5-bromouridine 5'-phosphate (Ⅳa), but it was revealed that this salt contained appreciable amount of 5'-di- and 5'-tri-phosphates of 5-bromouridine analyzed by paper and ion exchange chromatography (Fig. 1). (Ⅳa) was isolated from the mixture by ion exchange chromatography as its lithium salt.

This observation agrees with Khorana's report5) on the formation of GDP and GTP during phosphorylation of 2',3'-isopropylideneguanosine by phosphoryl chloride-pyridine method, and this proved to be a convenient method for the direct pyro- and tri-phosphorylation of nucleosides. Studies along this line are now being undertaken.

Alternately, (Ⅲ) and phosphorus pentoxide-phosphoric acid mixture were heated at 60° for 2 hours as in the case of UMP-synthesis2,6) and barium salt of 5-Bromo-UMP (Ⅳa) was obtained in a fairly good yield. Bromine at 5-position was not affected during the course of this reaction. Two specimens of 5-Bromo-UMP synthesized as above were quite identical.

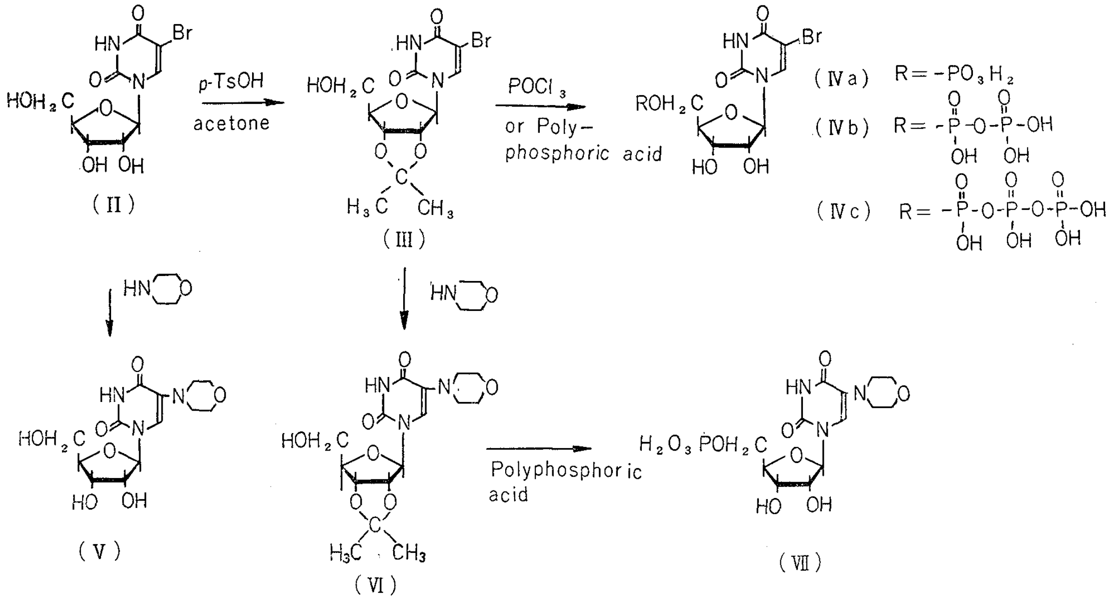
The direct bromination of UMP was attempted in order to obtain 5-Bromo-UMP.

[*1] Part Ⅱ. M. Ikehara: This Bulletin, 8, 367(1960).

[*2] Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (上田 亨).

[*3] Following abbreviations are used: UMP, uridine 5'-phosphate; GDP, guanosine 5'-diphosphate; GTP, guanosine 5'-triphosphate.

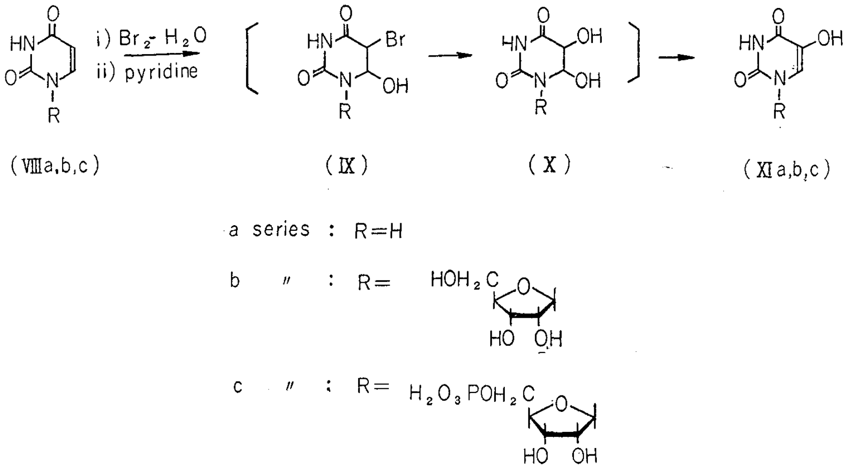
1) M. Grunberg-Manago, P.J. Ortiz, S. Ochoa: Science, **122**, 907(1955).
2) M. Michelson: J. Chem. Soc., **1958**, 1957.
3) T.K. Fukuhara, D.W. Visser: J. Biol. Chem., **190**, 95(1951).
4) A. Hampton, D.I. Magrath: J. Am. Chem. Soc., **79**, 3252(1957).
5) H.G. Khorana, *et al.*: *Ibid.*, **79**, 3747(1957).
6) R.M. Hall, H.G. Khorana: *Ibid.*, **77**, 1871(1955).



When bromine water was added to the aqueous solution of UMP, rapid decolorization and disappearance of ultraviolet absorption at 260 mμ occurred. On heating the solution with addition of excess pyridine or other aliphatic amines, ultraviolet absorption maximum at 278 mμ appeared. The product isolated was not the desired 5-Bromo-UMP but 5-hydroxyuridine 5′-phosphate (5-Hydroxy-UMP) (XIc). This substance showed positive test to ferric chloride reaction and ultraviolet absorption in dilute alkaline medium characteristic of 5-hydroxyuracil derivatives.

Recently, after the present experiment was completed, Wang[7] reported the formation of 5-hydroxyuracil (XIa) from uracil (VIIIa) by treatment with bromine water and successive heating in sodium hydrogencarbonate solution. 5-Hydroxy-UMP (XIc) must also have been formed similarly through the intermediates (IX) and (X) by treatment with bromine water and pyridine. Expectedly, 5-hydroxyuridine[8] (IXb) was obtained from uridine (VIIIb) by analogous procedure in a fairly good yield.

Number of 5-substituted aminouracils were synthesized from 5-bromouracil and amines by Philips,[9] but as to the synthesis of 5-amino derivatives of uridine, only the



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[7] S. Y. Wang : *Ibid.*, **81**, 3786(1959).

[8] M. Roberts, D. W. Visser : *Ibid.*, **74**, 668(1952).

[9] A. P. Philips : *Ibid.*, **73**, 1061(1951).

synthesis of 5-aminouridine from 5-bromouridine was reported in the literature[8] and its 5′-phosphate is not known.

5-Morpholinouridine (V) was obtained by refluxing (II) with morpholine. Considering this finding, (III) was treated with morpholine in analogous manner and the resulting vitreous 5-morpholino-2′,3′-isopropylideneuridine (VI) was treated with polyphosphoric acid in a usual way. 5-Morpholinouridine (5-Morpholino-UMP) (VII) was obtained as barium salt in a low yield. This relatively low yield may be due to the vitreous nature of isopropylidene derivative. Syntheses of a number of other 5-substituted aminouridines and their phosphates will now be undertaken.

The phosphates described in this paper were tested as a substrate for venom 5′-nucleotidase. The result will be reported elsewhere.

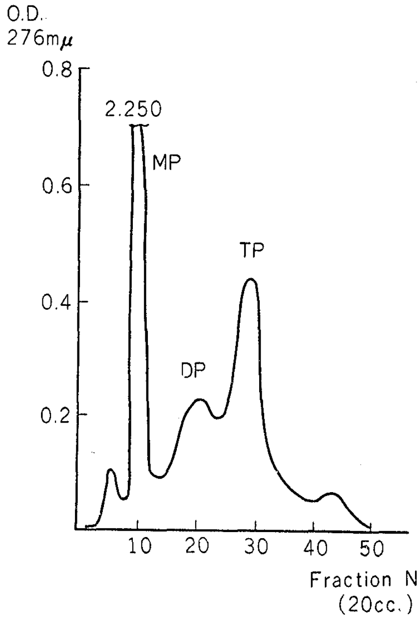

Fig. 1. Ion Exchange Chromatogram of 5-Bromouridine 5′-Phosphates

0.003 *N* HCl } concave gradient
0.003 HCl+0.5 *N* LiCl } elution method.

Column : Amberlite IRA-400 (100~200Mesh) resin 0.8 × 7 cm.

MP : 5-Bromo-UMP 43%
DP : 5-Bromo-UDP 21%
TP : 5-Bromo-UTP 35%

Experimental

5-Bromo-2′,3′-isopropylideneuridine (III)—Finely powdered 5-bromouridine (II) (3 g.) was added to 300 cc. of Me_2CO containing 30 g. of *p*-toluenesulfonic acid and the mixture was stirred vigorously for 30 min. at room temperature. Then 30 g. of powdered $NaHCO_3$ was added to the clear solution and stirred for another 1 hr. The precipitate was collected, washed twice with hot Me_2CO, and the combined filtrate and washings was evaporated under a reduced pressure. The residue was crystallized from Me_2CO-hexane or hot water to white needles (2.5 g.). *Anal.* Calcd. for $C_{12}H_{15}O_6N_2Br$: C, 39.8; H, 4.14; N, 7.74. Found : C, 39.83; H, 4.01; N, 7.94. UV : $\lambda_{max}^{H_2O}$ 276 mμ.

5-Bromouridine 5′-Phosphate (5-Bromo-UMP) (IVa)—a) Phosphorylation with $POCl_3$ and pyridine : A solution of $POCl_3$ (0.5 cc.) dissolved in 10 cc. of pyridine was added dropwise into pyridine solution (30 cc.) of (III) (1.35 g.) under stirring at −10° and stirring was continued for 2 hr. Then 50% pyridine-water (15 cc.) was added, stirred for additional 30 min., the solution was poured into ice water (40 cc.), and adjusted to pH 8.7 with $Ba(OH)_2$ solution. After the solution was evaporated *in vacuo*, water was added to the residue and acidified by addition of H_2SO_4 to its 0.1*N* concentration. The mixture was warmed at 60° for 15 min., neutralized with $Ba(OH)_2$, precipitate was filtered off, and the filtrate was concentrated to a small volume under reduced pressure. Two volumes of EtOH was added to the solution, centrifuged, and 660 mg. of Ba salt was obtained. This salt was found to contain three products, i.e. 5-Bromo-UMP (IVa), -UDP (IVb), and -UTP (IVc), by ion exchange chromatography (Fig. 1) and paper chromatography (Rf : 0.44, 0.18, and 0.08, respectively, in solvent system of EtOH-*N* AcOH (5 : 2)). The salt was dissolved in water and applied to the top of a column (2×8 cm.) of Amberlite IRA-400 (Cl^-). The column was eluted with 0.003*N* HCl containing 0.01*N* LiCl. 5-Bromo-UMP fraction was collected, adjusted to pH 7.0 with LiOH, and evaporated under a reduced pressure. Large excess of Me_2CO was added to the residue, the precipitate was collected by centrifugation, washed with EtOH and Et_2O, and dried (150 mg.). The identity of this product with 5-

Bromo-UMP synthesized by polyphosphoric acid (v.i.) was proved by paper chromatography, and UV and IR spectra.

b) Phosphorylation with polyphoshoric acid : (III) (3 g.) was added to the mixture of P_2O_5 (10 g.) and 85% H_3PO_4 (13 g.), and heated for 2 hr. at 60° with occasional stirring. To the mixture was added 100 cc. of water and heated for 30 min. on a boiling water bath. The solution was neutralized to pH 7.0 with $Ba(OH)_2$ solution, precipitated barium phosphate was collected, and washed with hot water. Filtrates and washings were combined, concentrated *in vacuo* to 40 cc., and 2 volumes of EtOH was added. The precipitated Ba salt was collected by centrifugation, washed with EtOH and Et_2O, and dried (2.5 g.). *Anal.* Calcd. for $C_9H_{10}O_9N_2BaBrP\cdot5H_2O$: C, 17.19; H, 3.18; N, 4.46; P, 4.93. Found C, 17.49; H, 2.80; N, 4.23; P, 4.74. UV mμ : $\lambda_{max}^{H_2O}$ 276, $\lambda_{min}^{H_2O}$ 245, $\lambda_{max}^{pH\ 9}$ 276, $\lambda_{min}^{pH\ 9}$ 250.

5-Hydroxyuridine 5′-Phosphate (5-Hydroxy-UMP) (XIc)—Ba salt of UMP (VIIIc) (500 mg.) was dissolved in water and Br_2 water was added until the solution colored yellow. Air was bubbled through the solution until the solution became colorless. Pyridine (5 cc.) was added and the solution was heated for 15 hr. on a boiling water bath. The solution colored yellow. $Ba(OH)_2$ solution was added to pH 7.5 and precipitated barium phosphate was filtered off. The filtrate was concentrated to a small volume and 2 volumes of EtOH was added. Precipitated Ba salt of (XIc) was collected by centrifugation, washed with EtOH and Et_2O, and dried (200 mg.). UV mμ : λ_{max}^{pH9} 300, 236, λ_{min}^{pH9} 268, λ_{max}^{pH6} 278, λ_{min}^{pH6} 245. This salt was 60% pure on a weight basis as estimated by ion exchange chromatography. A diluted solution of $FeCl_3$ in (VIc)[8] gives a purple color.

5-Hydroxyuridine (XIb)—Uridine (VIIIb) (1 g.) was treated with Br_2 water and pyridine (5 cc.) for 10 hr. as described above and the solution was evaporated to dryness under a reduced pressure. Hot EtOH was added to the residue. When cool, 5-hydroxyuridine (XIb) separated as fine needles which recrystallized from EtOH-H_2O (430 mg.). m.p. 230~232°. UV mμ : $\lambda_{max}^{H_2O}$ 278, λ_{max}^{pH9} 304, 237, λ_{min}^{pH9} 269, 230. *Anal.* Calcd. for $C_9H_{12}O_7N_2$: N, 10.77. Found : N, 10.54. A dilute solution of $FeCl_3$ gives a blue color with (XIb).[8]

5-Morpholinouridine (V)—A mixture of 5-bromouridine (II) (2 g.) and morpholine (10 cc.) was refluxed gently for 90 min. and allowed to stand overnight. Separated morpholine hydrobromide was filtered off and the filtrate was evaporated under a reduced pressure. The resulting gummy substance was dissolved in hot EtOH, hexane was added until faint turbidity appeared, and allowed to stand overnight. Fine crystals separated (900 mg.). m.p. 229~231°. UV mμ : $\lambda_{max}^{H_2O}$ 290, $\lambda_{max}^{0.01N\,HCl}$ 265. *Anal.* Calcd. for $C_{13}H_{19}O_7N_3$: N, 12.76. Found : N, 12.73.

5-Morpholinouridine 5′-Phosphate (5-Morpholino-UMP) (VII)—(III) (800 mg.) in morpholine was gently refluxed for 90 min., cooled, precipitated morpholine hydrobromide was filtered off, and the filtrate was evaporated to dryness under a reduced pressure. This vitreous residue (VI) (550 mg.) was mixed with P_2O_5 (1.5 g.) and 85% H_3PO_4 (2 g.), and kept for 2 hr. at 60°. To this mixture 50 cc. of water was added and heated for 30 min. on a boiling water bath. $Ba(OH)_2$ solution was added to pH 7.0, the precipitate was collected, washed with hot water, and the combined filtrate and washings was evaporated to a small volume under a reduced pressure. Two volumes of EtOH was added, the precipitated Ba salt of 5-morpholino-UMP (VII) was collected by centrifugation, washed with EtOH and Et_2O, and dried (220 mg.). UV mμ : $\lambda_{max}^{H_2O}$ 290, $\lambda_{min}^{H_2O}$ 260, $\lambda_{max}^{0.01N\,HCl}$ 265. *Anal.* Calcd. for $C_{13}H_{18}O_{10}N_3$-$BaP\cdot5H_2O$: P, 4.89. Found : P 4.69. This was ca. 70% pure on a weight basis as estimated by ion exchange chromatography.

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Summary

The syntheses of 5-substituted uridines and their 5′-phosphates were described. 5-Bromo-2′,3′-isopropylideneuridine was phosphorylated with phosphoryl chloride and from the mixture of 5-Bromo-UMP, -UDP, and -UTP obtained, 5-Bromo-UMP was isolated by ion exchange chromatography. Phosphorylation of 5-bromo-2′,3′-isopropylideneuridine with polyphosphoric acid afforded 5-Bromo-UMP in a good yield. 5-Hydroxyuridine and its 5′-phosphate were synthesized by the addition of bromine to uridine or UMP and successive treatment with pyridine. 5-Morpholinouridine and its isopropylidene derivative were obtained from corresponding 5-bromo derivatives and morpholine, and 5-Morpholino-UMP was obtained by phosphorylation of 5-morpholino-2′,3′-isopropylideneuridine with polyphosphoric acid.

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