Glycols with hydroxyls on either terminal ends or in 1,2-positions showed a break point in the foregoing physical constants and were considered to form a micelle-like structure.

Glycols with one hydroxyl at a terminal end and the other in a median position also forms a micelle-like structure but those having both hydroxyls in median positions and none at the terminal position do not form such a structure.

The concentration of glycols at which they form a micelle-like structure decreases with increasing number of carbon atoms and the concentration of dodecanediol becomes approximately equal to the critical micelle concentration of nonionic surfactant.

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124. Tohru Ueda : Studies on Coenzyme Analogs. XII.*1 Synthesis of 5-Dimethylaminouridine- and 3-Methyluridine 5′-Phosphate.

(*Faculty of Pharmaceutical Sciences, School of Medicine, Hokkaido University**2)

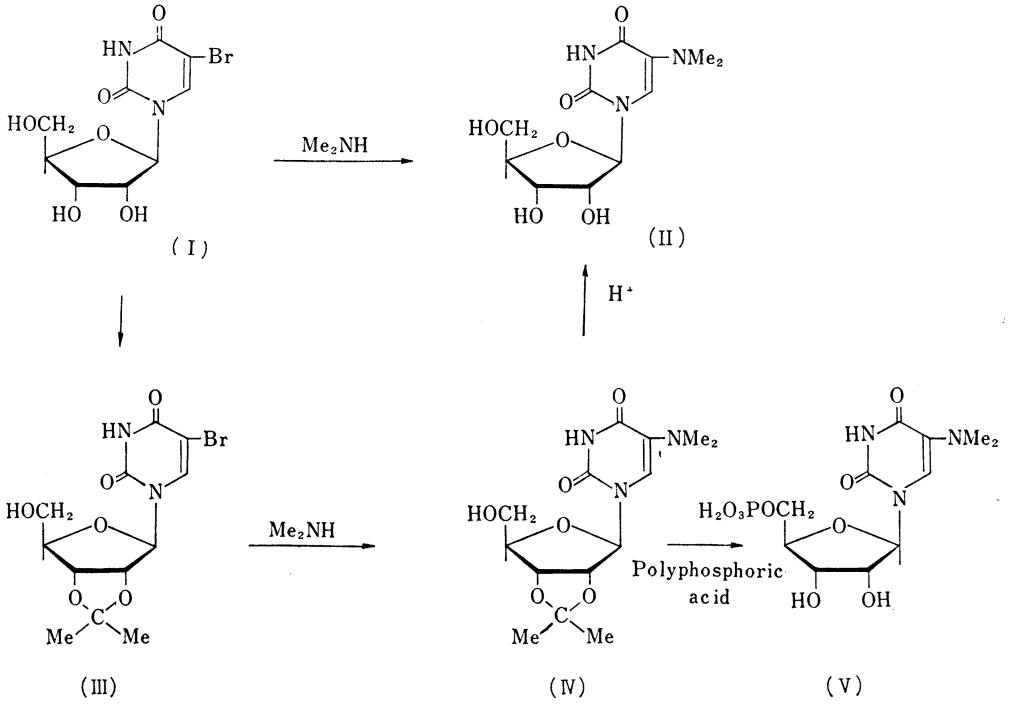
In order to elucidate the hitherto unclarified substrate specificity of snake venom 5′-nucleotidase[1] in the level of submolecular structure, the syntheses of several nucleoside 5′-monophosphates were required. Among these compounds, 5-bromo-, 5-hydroxy-, and 5-morpholino-uridine 5′-phosphate, in which the pyrimidine moiety was modified at C_5, were already prepared by the present author.[2] In the present paper the synthesis of 5′-phosphate of 3-methyluridine and 5-dimethylaminouridine is described.

As to the biological activity of modified pyrimidine nucleosides, such as 5-bromo-, 5-chloro-, 5-amino-, 5-hydroxy-, 3-methyl-, and 3-methyl-5-bromo-derivatives of uridine and cytidine,[3] some works have been done and inhibitory effect of these nucleosides was observed against the incorporation of natural pyrimidine (base or nucleotides) into ribonucleic acid of pyrimidine requiring *Neurospora strain-1298* or *E. coli K-12*.[4] Accordingly, from the stand point of pharmaceutical interests for cancerostatic agents, it is worthwhile to synthesize these nucleotides stated above.

Various 5-substituted aminouracils were already synthesized by Philips[5] from 5-bromouracil by the reaction with amines at elevated temperature. However, reports dealing with this kind of replacement reaction in the nucleoside level were scanty and the synthesis of 5-aminouridine was reported solely by Visser, *et al.*[3a] and Fox, *et al.*

*1 Part XI : This Bulletin, **9**, 767 (1962).
*2 Kita 12-jo, Nishi 5-chome, Sapporo, Hokkaido (上田 亨).

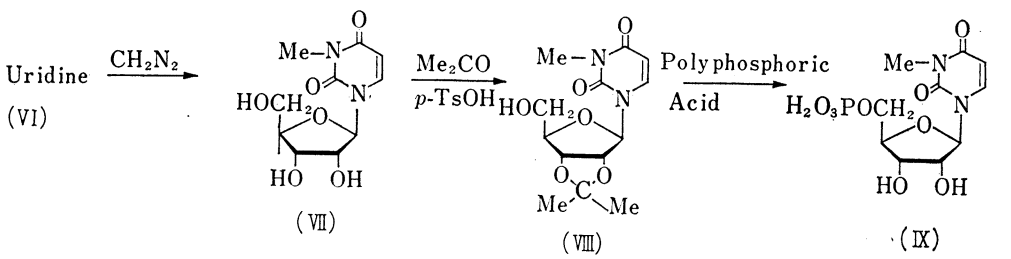
1) Y. Mizuno, *et al.* : This Bulletin, **9**, 388 (1961).
2) T. Ueda : *Ibid.*, 8, 455 (1960).
3) *a*) M. Roberts, D. W. Visser : J. Am. Chem. Soc., **74**, 668 (1952); *b*) H. T. Miles : Biochim. et Biophys. Acta, **22**, 247 (1956); *c*) T. K. Fukuhara, D. W. Visser : J. Biol. Chem., **190**, 95 (1951); *d*) T. K. Fukuhara, D. W. Visser; J, Am. Chem. Soc., **77**, 2393 (1955); *e*) R. Belz, D. W. Visser: *Ibid.*, **77**, 736 (1955).
4) *a*) M. Roberts, D. W. Visser : J. Biol. Chem., **194**, 695 (1952); *b*) G. Barron, R. Beltz, D. W. Visser : J. Am. Chem. Soc., **75**, 2017 (1953).
5) A. P. Philips : J. Am. Chem. Soc., **73**, 1061 (1951).



by the reduction of 5-nitrouridine.[6] As stated in the preceding report,[2] 5-morpholinouridine was obtained rather easily by the reaction of 5-bromouridine and morpholine.

The reaction of various amines with 5-bromouridine (I) was now investigated. Methylamine, cyclohexylamine, benzylamine and diethylamine gave scanty results as described in the experimental part. Dimethylamino derivative (II) was obtained easily by the heating of 5-bromouridine with anhydrous dimethylamine at 70° for 20 hours in a sealed tube and was isolated as crystals, m.p. 188~189°. Diethylamine failed to react even at 120° for 15 hours. Cyclohexylamine decomposed prior to the condensation. Though in the case of methylamine and benzylamine, 5-bromouridine seemed to undergo changes which were able to be observed by ultraviolet absorption spectra, the product could not be isolated in the crystalline state.

The condensation of dimethylamine with 5-bromo-2′,3′-isopropylideneuridine (III)[2] proceeded as smoothly as mentioned above and afforded a crystalline substance, m.p. 138~139°. This was confirmed as 5-dimethylamino derivative (IV) on the basis of the optical behavior, analytical data and hydrolytic evidences. The phoshorylation of this



6) I. Wempen, I.L. Doerr, L. Kaplan, J.J. Fox : J. Am. Chem. Soc., 82, 1624 (1960).

isopropylidene derivative (IV) was achieved by usual method[7] and 5-dimethylaminouridine 5'-phosphate was obtained as the barium salt in 43% yield.

The synthesis of 3-methyluridine 5'-phosphate (IX) was attempted next. The starting material, 3-methyluridine (VII), was already synthesized by Levene and Tipson,[8] Visser, *et al.*[4b] and Miles[3b] and reported as having the inhibitory effect on the growth of *Neurospora strain-1298*.[4a] This inhibitory effect may come from the blocking of tautomerism at the N_3-C_4 bond on uracil nucleus. With respect to this fact, the phosphate (IX) is expected to have the interesting activity to venom 5'-nucleotidase.

3-Methyluridine (VII) was obtained by the methylation of uridine (VI) with diazomethane by the slightly modified method of Miles, having the m.p. of 111°. The melting point of Leven's sample, m.p. 108~110°, is different from that of Visser and Miles' sample, m.p. 120° or 122°, and the both substances were proved to be nonconvertible each other.[3b] The present author's substance was confirmed as 3-methyluridine on the basis of the ultraviolet absorption spectrum. (VII) was then converted to 3-methyl-2',3'-isopropylideneuridine (VIII), m.p. 116~118°, by the condensation with acetone in the presence of *p*-toluenesulfonic acid[9,2] and then phosphorylated with polyphosphoric acid to give 3-methyluridine 5'-phosphate (IX) in 41% yield.

Experiments on the hydrolytic cleavage by venom 5'-nucleotidase of these two 5'-phosphates were done and the result obtained have been reported in a separate communication.[1]

Experimental

5-Dimethylaminouridine (II)——5-Bromouridine (1.9 g.) and anhyd. dimethylamine (10 cc.) were heated at 70° for 18 hr. in a sealed tube. After evaporation of amine, the residue was dissolved in EtOH and Et_2O was added dropwise until precipitation occurred. The separated precipitate was recrystallized from EtOH-H_2O. Yield, 1.0 g., m.p. 188~189°. UV : $\lambda_{max}^{H_2O}$ 290 mμ; λ_{max}^{1NHCl} 265 mμ. *Anal.* Calcd. for $C_{11}H_{17}O_6N_3$: C, 45.99; H, 5.92; N, 14.63. Found : C, 45.75; H, 5.99; N, 14.47.

5-Dimethylamino-2',3'-isopropylideneuridine (IV)——5-Bromo-2',3'-isopropylideneuridine (III) (2.0 g.) and anhyd. dimethylamine (10 cc.) were heated for 20 hr. at 70° in a sealed tube, and treated as above. Dimethylamine hydrobromide was precipitated first by addition of Et_2O, which filtered off, then precipitated material was recrystallized from EtOH-hexane, to yield white crystals (1.4 g.), m. p. 138~139°. UV λ_{max}^{EtOH} mμ : 292, 230. $\lambda_{max}^{0.1NHCl}$ 263 mμ. *Anal.* Calcd. for $C_{14}H_{21}N_3O_6$: C, 51.37; H, 6.73; N, 12.84. Found : C, 50.89; H, 6.65; N, 12.42.

Rf value of paperchromatography in the solvent system of iso-PrOH-conc. NH_4OH-H_2O (7:1:3) was 0.51 for 5-dimethylaminouridine (II) and 0.85 for (IV). When (IV) was heated in 0.1*N* HCl at 100° for 5 min. it showed the Rf value of 0.51 (equal to (II)) and became positive for periodate-benzidine spray test.

5-Dimethylaminouridine 5'-Phosphate (V)——Mixture of (IV) (1.0 g.) and polyphosphoric acid (P_2O_5, 4 g. and 85% H_3PO_4, 5.2 g.) were heated for 2 hr. at 60° and treated similarly as the synthesis of 3-methyluridine 5'-phosphate. Yield, 650 mg. as Ba salt of (V) (43%). UV $\lambda_{max}^{H_2O}$ mμ : 292, 230. $\lambda_{max}^{pH\,1}$ 263.5 mμ. This was 84% pure as estimated by ion exchange chromatography and impurity (probably diphosphate) was 7.8%.

Reaction of 5-Bromouridine (I) and other Amines——Cyclohexylamine (5 cc.) and (I) (1 g.) were heated for 1~1.5 hr. at 70~75°, then the solution turned to dark brown and ultraviolet absorption of λ_{max}^{EtOH} 314 mμ was noted. But the reaction product was H_2O-insoluble resin. 5-Cyclohexylaminouracil, prepared by the method of Philips,[5] has its ultraviolet absorption maximum at 295 mμ in H_2O. Diethylamine (10 cc.) and (I) (1.0 g.) were heated at 120° for 15 hr. in a sealed tube, but ultraviolet absorption was unchanged and recovered 900 mg. of (I). (II) also did not react with diethylamine at this reaction condition. Methylamine and benzylamine were reacted with (II), but their reaction products were not isolated as pure substances and further experiment was not attempted.

3-Methyluridine (VII)——Et_2O solution of CH_2N_2 [prepared from 17 g. of *p*-toluenesulfonyl (N-

7) R.H. Hall, H.G. Khorana : J. Am. Chem. Soc., **77**, 1871 (1955); A.M. Michelson : J. Chem. Soc., **1958**, 1957.
8) P.A. Levene, R.S. Tipson : J. Biol. Chem., **104**, 385 (1934).
9) A. Hampton, D.I. Magrath : J. Am. Chem. Soc., **79**, 3252 (1957).

methyl, N-nitrosoamide)] was added to MeOH solution of uridine (VI) (2 g.) and resulting solution was left to stand overnight. After evaporation of the solvent, the residue was dissolved in small volume of EtOH and Et_2O was added to slight turbidity. The crystals which separated after few days were recrystallized from Et_2O-EtOH to yield 1.2 g. of substance, m.p. 108~111°. UV : $\lambda_{max}^{H_2O}$ 260 mμ, $\lambda_{max}^{0.1N\,NaOH}$ 262 mμ. *Anal.* Calcd. for $C_{10}H_{14}O_6N_2$: C, 46.49; H, 5.47; N, 10.55. Found : C, 46.09; H, 5.41; N, 10.50.

3-Methyl-2′,3′-isopropylideneuridine (VIII)——A solution of 650 mg. of (VII) dissolved in Me_2CO solution of *p*-toluenesulfonic acid (5 g. in 50 cc.) was stirred for 20 min. Powdered $NaHCO_3$ (15 g.) was added to this solution and stirred for 2 hr. and the precieitate was collected by filtration and washed with hot Me_2CO. Filtrate and washings were combined and evaporated in a reduced pressure. The residue was solidified on standing. Yield, 650 mg., m.p. 116~118° (not recrystallized). *Anal.* Calcd. for $C_{13}H_{18}O_6N_2 \cdot H_2O$: N, 8.36. Found : N, 8.64.

3-Methyluridine 5′-Phosphate (IX)——650 mg. of (VIII) and polyphosphoric acid (mixture of 3.0 g. of P_2O_5 and 3.9 g. of 85% H_3PO_4) were heated for 2 hr. at 60°, then H_2O (30 cc.) was added and heated for 25 min. on a boiling water bath. The solution was neutralized to pH 7.0 with $Ba(OH)_2$ solution, precipitated $Ba_3(PO_4)_2$ was removed by filtration and washed with hot H_2O, and combined filirate and washings were concentrated to a small volume *in vacuo*. Two volumes of EtOH were added to this solution and the precipitated Ba salt was collected by centrifugation. This was reprecipitated by H_2O-EtOH and dried. Yield, 400 mg. (41%). *Anal.* Calcd. for $C_{10}H_{13}O_9N_2BaP \cdot H_2O$: C, 23.56; H, 3.34; N, 5.50; P, 6.09. Found : C, 23.36; H, 3.43; N, 5.12; P, 6.56.

Purity of Ba salt (as anhydrous) as estimated by ultraviolet absorption and ion exchange chromatography was 92%.

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Summary

The reaction of 5-bromo-2′,3′-isopropylideneuridine and dimethylamine gave 5-dimethylamino derivative and this was phosphorylated to 5-dimethylaminouridine 5′-phosphate by polyphosphoric acid. 3-Methyluridine 5′-phosphate was also synthesized from 3-methyl-2′,3′-isopropylideneuridine by polyphosphoric acid.

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