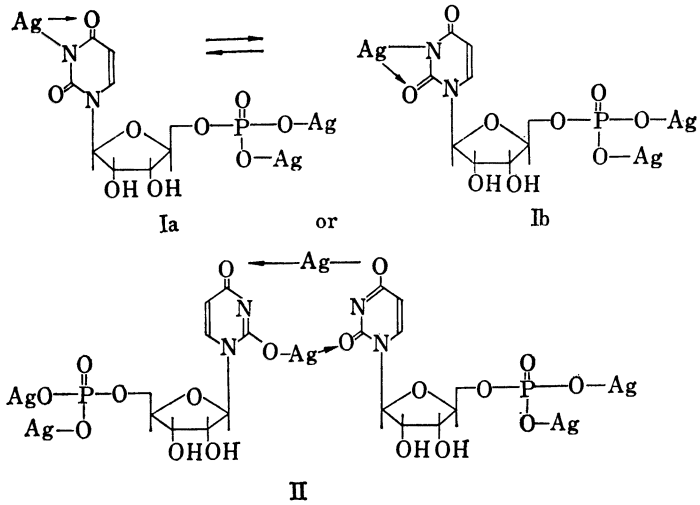

Chart 1. Proposed Structure of Ag-5′-UMP Complex

In the cases of zinc and cadmium, only a little spectral changes were noticed in the region of 1700—1600 cm^{-1}, and that these metals seemed to be predominantly bound to the phosphate group in 5′-UMP.

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Formation and Characterization of 5′-Deoxy-5′,6-epimino-5,6-dihydro-2′,3′-O-isopropylideneuridine

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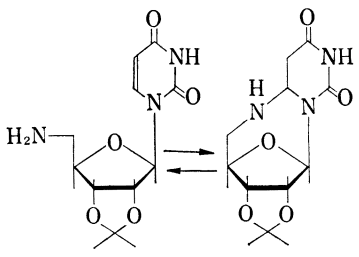
The susceptibility of uracil and cytosine to nucleophilic attack at the 5,6-double bond constitutes an important chemistry of their ring systems. It has recently been shown that the addition of an external nucleophile is involved in the mechanism of cleavage of uracil by hydroxylamine,[2] in deuterium or tritium exchange process of uracil,[3] in the mode of action of thymidine synthetase,[4] in the synthesis of triazolopyrimidines from 5-nitrouracil,[5]

1) Location: *a*) *Wako-shi, Saitama*; *b*) *Honkomagome, Bunkyo-ku, Tokyo.*
2) J.H. Phillips and D.M. Brown, *Progr. Nucleic Acid Res. Mol. Biol.*, **7**, 349 (1967).
3) *a*) S.R. Heller, *Biochem. Biophys. Res. Commun.*, **32**, 998 (1968); *b*) R.J. Cushley and S.R. Lipsky, and J.J. Fox, *Tetrahedron Letters*, **1968**, 5393; *c*) W.J. Wechter, *Collection Czech. Chem. Commun.*, **35**, 2003 (1070); *d*) K. Kai, Y. Wataya, and H. Hayatsu, *J. Am. Chem. Soc.*, **93**, 2089 (1971).
4) D.V. Santi and C.F. Brewer, *J. Am. Chem. Soc.*, **90**, 6236 (1968).
5) *a*) H.U. Blank and J.J. Fox, *J. Am. Chem. Soc.*, **90**, 7175 (1968); *b*) H.U. Blank, I. Wempen, and J.J. Fox, *J. Org. Chem.*, **35**, 1131 (1970).

in the synthesis of 5-mercaptouracil derivatives from 5-bromouracils,[6] in the selective deamination[7] or transamination[8] of cytosine, and facile decarboxylation of 5-carboxyuracils.[9] A number of chemical transformation of uracil or cytosine nucleosides have also shown to proceed through the intramolecular addition of a sugar substituent such as hydroxyl[10] and thiol[11] groups. We now wish to describe that the sugar amino group also adds across the 5,6-double bond of uracil in anhydrous condition to form an epimine and the behavior of this compound in neutral, acid, and alkaline media.

In the synthetic study[12] of 5′-N-aminoacyl-5′-amino-5′-deoxyuridine by the coupling of 5′-amino-5′-deoxy-2′,3′-O-isopropylideneuridine (**1**) with activated N-carbobenzoxyamino acids in anhydrous dioxane containing catalytic amount of triethylamine, we often observed in a reaction mixture a weakly positive ninhydrin-spot which showed no ultraviolet (UV) absorption and had a higher *Rf* value than that of the starting compound **1**,[13] on silica gel thin-layer chromatography (TLC). The same spot was also detected when aminonucleoside **1** alone was heated in anhydrous dioxane containing catalytic amount of triethylamine.[14] After refluxing for 11 hrs, the reaction mixture was separated on silica gel chromatography with benzene–acetone (1: 1). From the first fraction, crystals of the reaction product (**2**) were obtained in 36% yield, and 40% of the unchanged aminonucleoside **1** was recovered from the second fraction. To eliminate the possibility of the formation of **2**, during the catalytic hydrogenation of 5′-azido-5′-deoxy-2′,3′-O-isopropylideneuridine, the hydrogenated



1 2

Chart 1

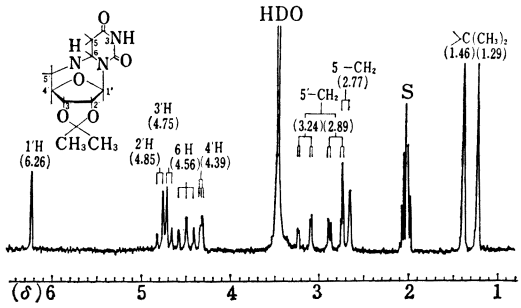


Fig. 1. 100 MHz NMR Spectrum of 5′-Deoxy-5′,6-epimino-5,6-dihydro-2′,3′-O-isopropylideneuridine (**2**) in Acetone-d_6 (plus D_2O); Internal Standard, TMS

6) L. Szabo, T.I. Kalman, and T.J. Bardos, *J. Org. Chem.*, **35**, 1434 (1970).
7) *a*) R. Shapiro, R.E. Servis, and M. Welcher, *J. Am. Chem. Soc.*, **92**, 422 (1970); *b*) H. Hayatsu, Y. Wataya, and K. Kai, *J. Am. Chem. Soc.*, **92**, 724 (1970); *c*) H. Hayatsu, Y. Wataya, K. Kai, and S. Iida, *Biochem.*, **14**, 2858 (1970); *d*) W.J. Wechter and R.C. Kelly, *Collection Czech. Chem. Commun.*, **35**, 1991 (1970).
8) R. Shapiro and J.M. Weisgras, *Biochem. Biophys. Res. Commun.*, **40**, 839 (1970).
9) K. Isono, S. Suzuki, M. Tanaka, T. Nanbata, and K. Shibuya, *Tetrahedron Letters*, **1970**, 425.
10) *a*) J.J. Fox, N.C. Miller, and R.J. Cushley, *Tetrahedron Letters*, **1966**, 4927; *b*) B.A. Otter and J.J. Fox, *J. Am. Chem. Soc.*, **89**, 3663 (1967); *c*) B.A. Otter, E.A. Falco, and J.J. Fox, *J. Org. Chem.*, **33**, 3593 (1968); *d*) *Idem*, *Tetrahedron Letters*, **1968**, 2967; *e*) *Idem*, *J. Org. Chem.*, **34**, 1390 (1969); *f*) D. Lipkin, C. Cori, and M. Sano, *Tetrahedron Letters*, **1968**, 5993; *g*) R.E. Notari, M.L. Chin, and A. Cardoni, *Tetrahedron Letters*, **1969**, 3499.
11) *a*) B. Bannister and F. Kagan, *J. Am. Chem. Soc.*, **82**, 3363 (1960); *b*) R.W. Chambers and V. Kurkov, *ibid.*, **85**, 2160 (1963); *c*) E.J. Reist, A. Benitez, and L. Goodman, *J. Org. Chem.*, **29**, 554 (1964).
12) K. Isono, T. Azuma, and S. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **19**, 505 (1971).
13) The *Rf* value of this compound (**2**) was 0.61 with benzene–acetone (1: 1). That of **1** was 0.14 in the same run.
14) Heating and the presence of triethylamine are not always necessary. Considerable amount of **2** was detected on TLC after standing few hrs at room temperature. Refluxing condition was employed to have the maximum yield of **2**.

solution was checked on TLC at the end of hydrogenation, giving no spot of **2**. However, after evaporation of the solvent, the residual white foam of **1** showed the presence of small amount of **2** on TLC. Transformation to **2** is seemed to occur to some extent during the concentration process. From this reason, it is difficult to isolate pure **1** free from **2**.

The compound **2** has the formula, $C_{12}H_{17}O_5N_3$ isomeric to the starting aminonucleoside **1**, as confirmed by a molecular ion peak in a high resolution mass spectrum. It had no absorption in UV region and was weakly positive to a ninhydrin test. The NMR spectrum of **2** in acetone-d_6 (plus a drop of D_2O) with assignment is shown in Fig. 1. An A_2X system of 5,6-protons (H-5,5,d, δ 2.77; H-6, 4.56; $J_{5,6}$=8.6 Hz) and an ABX system of 4′,5′-protons (H-4′, q, 4.39; H-5′,5′, 2.89, 3.24; $J_{5',5'}$=14.2, $J_{4',5'}$=2.2, 1.4) were confirmed by a double resonance technique. In acetone-d_6 without addition of D_2O, multiplicity of H-5′ and H-6 increased because of the additional coupling with a proton on epimine-N, whereas virtually no change was observed in the signals of H-5 and H-4′ supporting the above assignment. No appreciable coupling between 1′ and 2′ protons and 3′ and 4′ protons in 2′,3′-O-isopropylideneribocyclonucleoside were well demonstrated.[10e,15] Thus the anomeric proton appeared as a sharp singlet at δ 6.26 and the 2′,3′-protons constituted an AB system at δ 4.85 and 4.75 ($J_{2',3'}$=6.0 Hz). The configuration at C-6 is unknown. However, in the reaction mixture, we always observed a minor spot having a little lower *Rf* value[16] than **2**. This compound was too small to be isolated but is assumed to be a C-6 epimer of **2**.

It is evident that the 5′-amino group attacked as an nucleophile the C-6 of an uracil ring to form a 5,6-dihydro-5′,6-epimino compound. This bond formation would be promoted by the presence of an isopropylidene group. The striking differences were observed in the reactivity between 5′-deoxy-5′-thiouridine and its 2′,3′-O-isopropylidene ketal.[10e] It is believed that the isopropylidene ring forces the furanose ring into a conformation that favors the proximity of the 5′-substituent group to the uracil double bond.[10d,e,11c] This is also the case for this compound, *i.e.*, no formation of the corresponding epimino compound was observed with 5′-amino-5′-deoxyuridine in a similar condition. This may also be due to the conformational factor.

Epimine **2** has essentially no UV absorption in acid and neutral condition. However, the time course of the absorption is interesting. As illustrated in Fig. 2, the gradual increase of the UV absorption in an acid medium indicates the elimination of 5′,6-epimine as the result of the protonation on epimine-N and the subsequent generation of an uracil ring. Thus the absorbancy reached the highest after 7—13 days at room temperature, which was comparable to that of uridine (ε, 10100 at 262 mμ in 0.1N HCl[17]). The generation of **1** in an acid medium was also detected on cellulose TLC (BuOH–AcOH–H_2O, 4:1:2). On treatment with 50% acetic acid overnight at room temperature, a spot corresponding to **1**

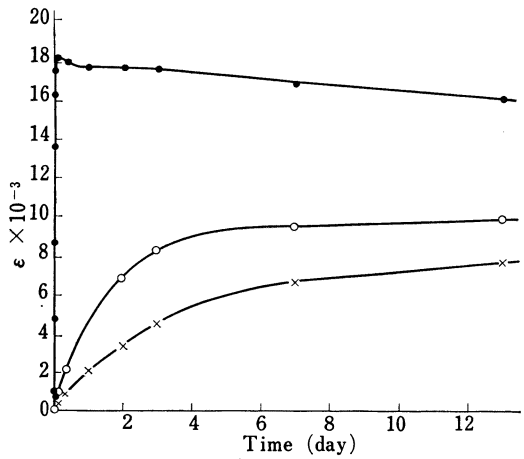


Fig. 2. Time Course of UV Absorption (258 mμ) of **2** in 0.1N HCl (—○—), in Phosphate Duffer (M/10, pH 7), (—×—), and in 0.1N NaOH (—●—)

15) R.L. Tolman, R.K. Robins, and L.B. Townsend, *J. Am. Chem. Soc.*, **91**, 2102 (1969).
16) The *Rf* value was 0.54 with benzene–acetone (1:1) on silica gel TLC, when that of **2** was 0.61.
17) "CRC Handbook of Biochemistry," The Chemical Rubber Co., Cleavland, Ohio, 1968, p. G36.

appeared, whereas no more spot corresponding to **2** was observed. After heating at 60° for 1 hr, one more spot appeared corresponding to 5′-amino-5′-deoxyuridine,[18] thus indicating the elimination of the epimine preceeded to the hydrolysis of the isopropylidene group. The conversion of **2** to **1** on silicic acid was also observed. When a silica gel thin-layer spotted with **2**, was left to stand overnight before development, considerable amount of **1** was formed as judged by the appearance of UV-absorbing ninhydrin-positive spot having the identical *Rf* value to **1**. In a neutral medium (M/10 phosphate buffer, pH 7), the increase in absorbancy was much slower. After 2 weeks at room temperature, the absorption reached 78% of that of uridine (ε, 10100 at 262 mμ, pH 7[17]) and still increasing very slowly. It is to be assumed that **1** is predominant in the slow **1**$\rightleftarrows$**2** equilibrium in aqueous buffer solution.

The change in UV absorption in 0.1N NaOH was striking. The rapid increase in UV absorbancy at 258 mμ reached the maximum in 3 hrs at room temperature. This absorption (ε, 18200 at 258 mμ) is clearly not that of uridine (ε, 7100 at 262 mμ, pH 13[17]). It was fairly stable in alkaline condition, although some slight decrease was observed on standing at room temperature (Fig. 2). On acidification, this absorption was irreversibly lost. All attempts to characterize this absorption were unsuccessful, however, it may be attracting to consider that N-1 of a dihydrouracil ring instead of epimine-N was eliminated in the alkaline condition resulting in the opening of a dihydrouracil ring in contrast to uracil ring generation in acid solution. Simultaneous well-documented facile cleavage at the 3,4 positions[19] may lead to the formation of the α,β-unsaturated carboxylate with an imine on the β-position, which may directly or indirectly be responsible for the UV absorption. The behavior of this epimino compound is in sharp contrast to the corresponding episulfide,[11] which remains unchanged in acid but generates 2′,3′-O-isopropylidene-5′-thiouridine in alkaline condition.

Experimental

5′-Deoxy-5′,6-epimino-5,6-dihydro-2′,3′-O-isopropylideneuridine (2)——A solution of 850 mg of 5′-azido-5′-deoxy-2′,3′-O-isopropylideneuridine[18] in 30 ml of EtOH was hydrogenated over 30 mg of palladium black under 3.5 kg/cm^2 pressure at room temperature for 3 hr. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to dryness, yielding white foam of **1**, which was dried over P_2O_5 *in vacuo* at 60°. The whole residue was dissolved in 20 ml of anhydrous dioxane and 10 drops of Et_3N were added. The resulting solution was refluxed for 11 hrs and the solution was concentrated to dryness. The residue was subjected to silica gel chromatography with benzene–acetone (1: 1). From the first ninhydrin-positive fraction, 303 mg of crystalline **2** was obtained, which was recrystallized from EtOH, mp 228—230° (decomp.), $[\alpha]_D^{22}$ −93.2° (c=0.980, H_2O). *Anal.* Calcd. for $C_{12}H_{17}O_5N_3$: C, 50.88; H, 6.05; N, 14.83. Found: C, 51.18; H, 5.92; N, 15.04. Mass Spectrum *m/e*: 283.118 (M^+). Calcd. for $C_{12}H_{17}O_5N_3$: 283.118. From the second ninhydrin-positive and UV-absorbing fraction, 342 mg of yellowish foam of unreacted **1** was recovered on evaporation of the solvent.

Acknowledgement We thank Dr. S. Suzuki of this institute for reading the manuscript, and Dr. H. Kuzuhara and Mr. H. Ohrui of this institute for their discussion.

18) J.P. Horwitz, A.J. Thomson, J.A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).
19) R.D. Batt, J.K. Martin, J. McT. Ploeser, and J. Murray, *J. Am. Chem. Soc.*, **76**, 3663 (1954).