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Conversion of Uridine into Isouramil Nucleosides and Related Reactions

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Diazocoupling reaction of uracil derivatives bearing various substituents at position 5 was examined. The uracil derivatives having strong electron-releasing groups at this position were able to be coupled with a number of aryl diazonium salts to give the corresponding 6-arylazouracil derivatives. Catalytic reduction of 5-hydroxy-6-phenylazouridine (IX) prepared from 5-hydroxyuridine gave isouramil nucleoside (X). Stability of X in a pH-range (2.2 —8.2) was examined and it was found that X was comparatively stable at pH *ca.* 3 in aqueous solution. The nucleoside having a new ring system, oxazolo(4,5-*d*)pyrimidine nucleoside (XII) was prepared from X.

We have been interested in 6-substituted pyrimidine nucleosides as potential antimetabolites because orotidine 5′-phosphate plays an important role in the biosyntheses of the nucleotide components of nucleic acids and 6-arylazopyrimidine specifically inhibits biosyntheses of deoxyribonucleic acid.[2]

A number of papers dealing with 6-substituted pyrimidine nucleosides has appeared. Thus, 6-amino- and 6-hydroxyuridines have been prepared by cyclization of blocked D-ribosylurea with ethyl cyanoacetate or diethyl malonate.[3] 6-Methyl pyrimidine nucleosides have been synthesized by the condensation of protected ribosyl halide and pyrimidine derivatives.[4] 6,5′-Anhydro-6-hydroxy-5-iodo-2′-deoxyuridine, a kind of 6-substituted pyrimidine nucleosides was prepared from 2′-deoxycytidine by treatment with iodine-iodic acid and then with base.[5] 6-Hydroxy- and 6-aminouridines have been also prepared by solvolysis and ammonolysis of 6,5′-cyclouridine, respectively, which was obtained from 5-bromouridine by treatment with sodium ethoxide or sodium hydroxide.[6] Ueda and Inoue have prepared 6-cyanouridine from a 5-bromouridine derivative.[7]

The formation of these cyclonucleosides and cyanation of the 5-halogenopyrimidine nucleoside are facilitated by electron-attracting effect of the halogen.

On the other hand, electrophilic substitution on position 6 of uracils or cytosines may be generally difficult, with the exception of the pyrimidines bearing electron-releasing groups such as hydroxyl or amino group on position 5. Thus, Mannich reaction was successively applied to 5-hydroxyuracils[8] and Claisen rearrangement could be also effected with 5-allyloxy- or 5-allylaminouracilis.[9] It has been also reported that 5-hydroxypyrimidines were coupled with aryl diazonium salts to give 6-arylazopyrimidine derivatives.[10]

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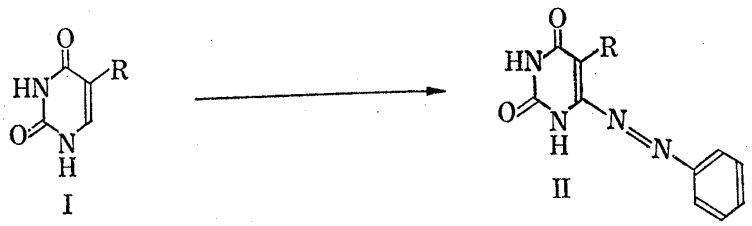
With the above-mentioned information on the synthesis of 6-substituted pyrimidine nucleosides in mind, we tried to work out conversion of uridine into isouramil riboside, a kind of uric acid 3-riboside analogs.

TABLE I. Yields and Properties of Arylazouracil Derivatives (II)

Compound (II) R	Yield (%)	Absorption λ_{max} (nm) pH 1	pH 7	pH 11	Formula	Calcd. (%) (Found) C	H	N
a) NH_2	94	485	480	495	$C_{10}H_9O_2N_5$	51.95 (52.26)	3.90 (3.74)	30.30 (30.71)
b) NHMe	95	500	485	495	$C_{11}H_{11}O_2N_5$	53.87 (54.00)	4.49 (4.52)	28.57 (28.28)
c) NMe_2	81	502	485	495	$C_{12}H_{13}O_2N_5$	55.50 (55.23)	5.02 (5.11)	27.03 (26.84)
d) $NHCH_2$-C$_6$H$_5$ [a]	77		520 [b]		$C_{17}H_{15}O_2N_5$	63.54 (63.28)	4.71 (4.80)	21.79 (21.66)
e) NHCHO	—	—	—	—	—	— (—)	— (—)	— (—)
f) NHCOMe	—	—	—	—	—	— (—)	— (—)	— (—)
g) OMe [c]	—	—	—	530 [d]	—	— (—)	— (—)	— (—)
h) OH	84	—	500 [e]	—	$C_{10}H_8O_3N_4$	51.72 (51.54)	3.47 (3.49)	24.13 (24.16)

a) purified by reprecipitation with $CHCl_3$–hexane b) in $CHCl_3$
c) *p*-Diazobenzenesulfonic acid was used as aryl diazonium salt.
d) in 1N NaOH e) in 10% aqueous dioxane

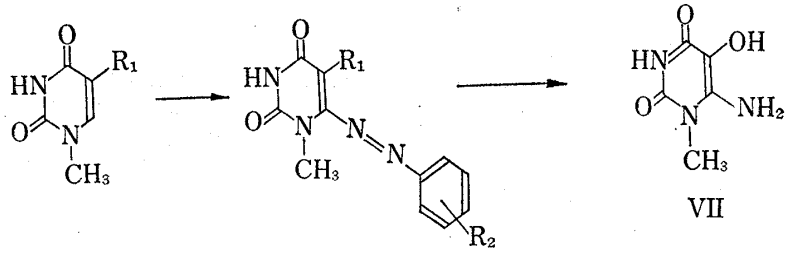


I

II

a : R=−NH_2
b : R=−NHMe
c : R=−NMe_2
d : R=−$NHCH_2$-C$_6$H$_5$

e : R=−NHCHO
f : R=−$HNCOCH_3$
g : R=−OMe



III : R_1=−OH
V : R_1=−NH_2

IVa : R_1=−OH, R_2=−H
IVb : R_1=−OH, R_2=−CO_2Na (*ortho*)
IVc : R_1=−OH, R_2=−SO_3Na (*para*)

VII

VI : R_1=−NH_2, R_2=−H

Chart 1

In this regards, it was felt that diazocoupling, reduction and subsequent ring closure leading to the analog were one of the promissing routes for that goal.

Therefore, as the preliminary experiments, diazocoupling reaction of uracil derivatives having various substitutents at position 5 other than 5-hydroxyl was undertaken. Results obtained are summarized in Table I. As shown, 5-amino-(Ia), 5-methylamino-(Ib), 5-dimethylamino-(Ic) and 5-benzylamino-(Id) uracils were reactive enough to form 6-phenylazo derivatives, whereas 5-formamido-(Ie) and 5-acetamido-(If) uracil were not reactive. 5-Methoxyuracil (Ig) reacted with diazobenzenesulfonic acid only in strong alkaline solution. The product failed to be separated from the solution because of its unstable nature in the alkaline solution. These coupling behavior of these compounds to diazonium salts is reminiscent of the effect of these substituents in diazocoupling reaction on monosubstituted benzenes.

In the next step, extention of the diazocoupling reaction to 1-methyl-5-hydroxy-(III) or 1-methyl-5-aminouracil (V) was tried. The compound (III) was treated with phenyldiazonium salt in acetate buffer at 40—45°. The color of the solution changed to red and subsequently red precipitate deposited from the solution. The precipitate showed λ_{max} 500 nm in the absorption spectrum and the elementary analysis was in good agreement with the theoretical value of 1-methyl-5-hydroxy-6-phenylazouracil (IVa). Compound (III) was also treated with *o*-diazobenzenecarboxylic acid and *p*-diazobenzenesulfonic acid to give 1-methyl-5-hydroxy-6-(*o*-carboxylphenylazo)uracil (IVb) and 1-methyl-5-hydroxy-6-(*p*-sulfonylphenylazo)uracil (IVc), respectively. 1-Methyl-5-aminouracil (V) reacted with phenyldiazonium salt to give 1-methyl-5-amino-6-phenylazouracil (VI) as expected. Compound (IVb) and (IVc) were reduced with aqueous sodium hydrosulfite to give crystalline 1-methyl-isouramil (VII, 1-methyl-5-hydroxy-6-aminouracil), which showed UV λ_{max} 282 nm both in neutral and acidic media, whereas in alkaline solution, showed only end absorption which did not return to the original absorption on acidification. This behavior is common with isouramil derivatives.[10]

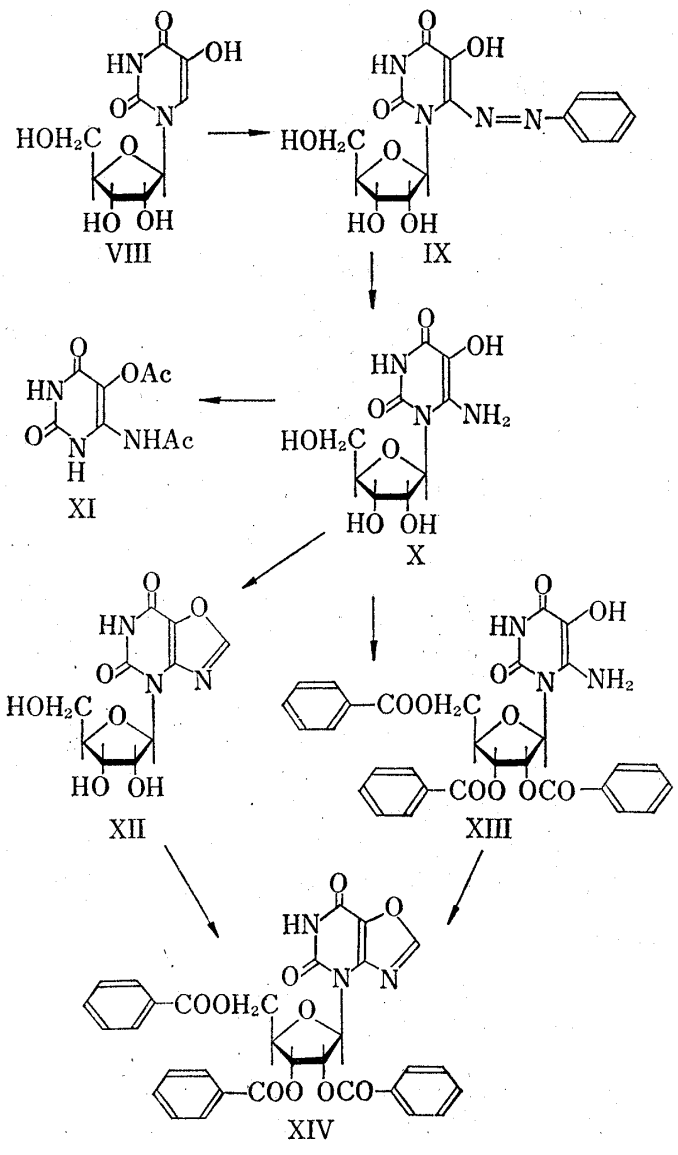


Chart 2

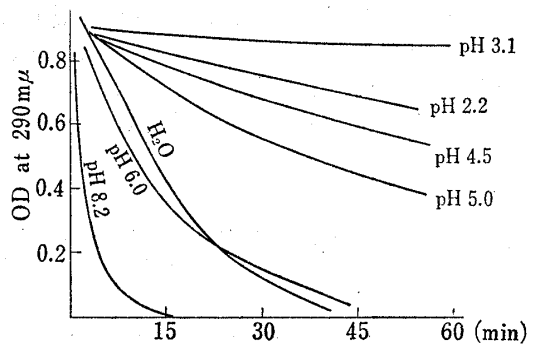


Fig. 1. Optical Density-time Profile of a Buffered Solution of X whose OD at Zero Time was 1, was measured at 290 mμ

Nucleoside, 5-hydroxyuridine (VIII) was treated with phenyldiazonium salt in acetate buffer at 0—5° to give 5-hydroxy-6-phenylazouridine (IX), which decomposed on heating at 202° and showed λ_{max} 510 nm in 5% dioxane solution. When compound (IX) was reduced with aqueous ammonium sulfide or sodium hydrosulfite, the solution showed UV λ_{max} 288—290 nm which may be ultraviolet (UV) spectra of isouramil nucleoside. But, during a work-up, UV spectrum of the solution changed to end absorption, and nucleosidic material could not be isolated. The catalytic reduction of IX was attempted for simple isolation[11]: compound (IX) was reduced with Raney nickel-W 2 in aqueous ethanol at an atmospheric pressure and then resulting precipitate was dissolved in a small volume of hot water. After removal of the catalyst, the solution was quickly cooled to give crystalline isouramil nucleoside(X, 5-hydroxy-6-aminouridine). The nucleoside (X) exhibited UV absorption at λ_{max} 290 nm both in neutral and acidic solution. The optical density of X at 290 nm gradually decreased on standing in water to show finally only end absorption. Rate of the decrease depended on the hydrogen ion concentration of the solution. Oxygen, sodium sulfide and thioglycolic acid were not effective to prevent the decomposition. The rate was minimum at pH about 3 in aqueous solution (see Fig. 1). The structure of a compound having end absorption was not further examined.

In the nuclear magnetic resonance (NMR) spectrum of (X) in D_2O, proton signals of sugar moiety of X appeared at δ 3.90 (5′ H), 4.0—4.5 (2′, 3′ and 4′ H) and 6.3 ppm (1′ H) and no other signals appeared. Heating of X in acetic anhydride containing a trace of pyridine gave diacetylisouramil which resulted from acetolysis of N-glycosyl bond. NMR spectra of this compound in dimethyl sulfoxide-d_6, showed signals at δ 2.18 (N- or O-acetyl), 2.25 (O- or N-acetyl), 10.39 (N_1- or N_3-H), 10.92 (N_1- or N_3-H) and 11.30 ppm (N-H of amide). In the D_2O solution, signals appeared at δ 2.18 and 2.25 ppm. On the basis of these observations, this compound was assigned the 5-acetoxy-6-acetamidouracil (XI) structure.

For cyclization to a new ring system: oxazolo(4,5-*d*)pyrimidine type (XII), compound (X) was heated with formic acid, formamide or diethoxymethylacetate. On heating with formic acid, glycosyl linkage of X was severed, whereas on heating with formamide or diethoxymethylacetate, UV spectra of reaction mixture showed only end absorption. It was well documented that polyphosphate ester[12] was an excellent reagent for cyclization to heterocycles[13]: the polyphosphate ester was therefore tested for the cyclization of X to oxazolopyrimidine. Compound (X) was benzoylated with benzoyl chloride in pyridine. After usual work-up, crude benzoylated product was obtained which was used for cyclization without further purification. Benzoylated isouramil nucleoside (XIII) was heated with formamide and polyphosphate ester at 70° to yield 4-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)4,5,6,7-tetrahydrooxazolo(4,5-*d*)pyrimidine-5,7-dione (KIV). Debenzoylation of XIV failed because the oxazolopyrimidine ring of XIV was not stable enough in both acidic and basic solution. To obtain unblocked nucleoside (XII), compound (X) was cyclized under the same conditions as cylization of XIII to yield XII. UV spectra of XII showed $\lambda_{max}^{H_2O}$ 274, 224 (infl.), λ_{max}^{pH1} 274 and λ_{max}^{pH11} 315 (infl.), 277, 240 nm (infl.). Paper chromatograms of XII gave a single spot on the two solvent systems. Compound (XII) was benzoylated with benzoyl chloride in pyridine to yield XIV which was found to be identical with the compound obtained by cyclization *via* benzoylation of X, by mixed melting point (mp 202°) and elementary analyses.

Experimental

5-Benzylaminouracil (Id)——A suspension of 5-bromouracil (5.0 g) in 25 g of benzylamine was heated at 110—120° for 5 hr. The product was collected by filtration, washed successively with water, ethanol

11) The solution on standing for long time and complex isolation might lead to decomposition of X.

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and ether, and was crystallized from a large proportions of ethanol to give 5.1 g of (Id) (mp 280°, 90% yield). UV λ_{max} nm: 264 (pH 1), 300, 235 (pH 7) and 293 (pH 11). *Anal.* Calcd. for $C_{11}H_{11}O_2N_3$: C, 60.83; H, 5.10; N, 19.35. Found: C, 60.55; H 4.99; N, 19.52.

General Procedure for Diazocoupling Reaction of 5-Substituted Uracils (I)——A solution of aryl amine (10 mmoles) in 30 ml of 1N HCl was treated with a solution of sodium nitrite (10 mmoles) in 15 ml of water at 0—5°. To the diazotized solution was added with stirring sodium acetate (1.5 g) at 0—5°. A solution of I (10 mmoles) in 20 ml of 1N NaOH[14] was added dropwise to the bufferized diazonium solution. The mixture was allowed to stand at 5° and then at room temperature overnight. The resulting precipitate was collected by filtration, washed successively with water, ethanol and dried to give the corresponding 6-arylazouracils (II). The yields and elementary analyses of II were shown on Table I.

1-Methyl-5-hydroxy-6-arylazouracils (IV)——A solution of arylamine (7 mmoles) in 1N HCl (21 ml) was with stirring treated with sodium nitrite (7 mmoles) in water (10 ml) at 0—5°. To the solution was added sodium acetate (1.72 g) and a solution of 1-methyl-5-hydroxyuracil (III) (980 mg) in water (10 ml) containing sodium carbonate (742 mg). After stirring for an hour, the solution was heated at 40—45° for 2 hrs. The product was filtrated, successively washed with water, ethanol and ether until washings were colorless. Yield of IVa was 90% yield. Absorption spectrum: λ_{max} 500 nm (10% aqueous dioxane). *Anal.* Calcd. for $C_{11}H_{10}O_3N_4$: C, 53.66, H, 4.07; N, 22.76. Found: C, 53.54; H, 4.13; N, 22.65. Compound (IVb) was obtained as mono sodium salt in 75% yield. Absorption spectrum: λ_{max} 518 nm (10% aqueous dioxane). *Anal.* Calcd. for $C_{12}H_9O_5N_4Na$: C, 46.15; H, 2.88; N, 17.95. Found: C, 46.32; H, 3.01; N, 18.26. Compound (IVc) was obtained as mono sodium salt in 80% yield. Absorption spectrum: λ_{max} 522 nm (10% aqueous dioxane). *Anal.* Calcd. for $C_{11}H_9O_6N_4SNa$: C, 37.93; H, 2.59; N, 16.06. Found: C, 38.21; H, 2.44; N, 15.81.

1-Methyl-5-amino-6-phenylazouracil (VI)——A solution of aniline (10 mmoles) in 30 ml of 1N HCl was treated with 10 mmoles of sodium nitrite in 15 ml of water at 0—5°. To the solution was added sodium acetate (1.6 g) and then 1-methyl-5-aminouracil (1.41 g) in 20 ml of 1N NaOH at 0—5°. After the mixture was stirred for an hour, it was allowed to stand in ice box overnight to deposit a solid material. The precipitate was collected by filtration and successively washed with water and ethanol to give 1.8 g of VI (74% yield). Compound (VI) was soluble in ether containing ethanol. Absorption spectrum: λ_{max} 482 nm (H_2O). *Anal.* Calcd. for $C_{11}H_{11}O_2N_5$: C, 53.87; H, 4.49; N, 28.57. Found: C, 53.87; H, 4.36; N, 28.40.

1-Methyl-5-hydroxy-6-aminouracil (VII)——Method A: Compound (IVb) (1.0 g) and sodium hydrosulfite (2.0 g) were suspended in 10 ml of water. The mixture was heated at 60—65° for half an hour. The red-colorled phenylazo compound came gradually into solution and precipitate deposited concomitantly with disappearance of the coloration. After the colorless mixture was cooled to a room temperature, the precipitate was collected by filtration, successively washed with water, ethanol and ether, and dried to yield 250 mg of VII (56% yield). UV λ_{max} nm: 282 (pH 1), 282, 233 (infl.) (pH 7). *Anal.* Calcd. for $C_5H_7O_3N_3$: C, 38.22; H, 4.99; N, 26.71. Found: C, 37.89; H, 4.71; N, 26.41.

Method B: Compound (IVc) (1.0 g) was reduced with sodium hydrosulfite (2.0 g) under the same conditions as in reduction of IVb. Yield of VII was 260 mg (52%). This compound was found to be identical with the compound obtained by reduction of IVb on the criteria of spectral properties (UV and IR).

1-(β-D-Ribofuranosyl)-5-hydroxy-6-phenylazouracil (IX)——To a solution of aniline (930 mg) in 1N HCl (30 ml) was added saturated solution of sodium nitrite (690 mg) at 0—5°. To the diazonium solution was added sodium acetate (1.5 g) at 0—5°. A solution of 5-hydroxyuridine (VIII) (2.6 g) in 50 ml of water containing 1.06 g of sodium carbonate was with stirring added dropwise to the bufferized diazonium solution at 0—5°. After the mixture was allowed to stand for an hour at 0—5°, it was warmed at 40—50° for 2 hr. Chocolate-colorled precipitate was collected by filtration and successively washed with cold water, ethanol and ether to yield powdery substance which was continuously extracted with ether by the use of a Soxlet extractor. Solid obtained, was washed with ether and dried to yield 3.2 g of chocolate colored (IX) (87%, mp 202° (decomp.)). Absorption spectrum: λ_{max} 510 nm (5% aqueous dioxane). *Anal.* Calcd. for $C_{15}H_{16}O_7N_4$: C, 49.45; H, 4.40; N, 15.39. Found: C, 49.26; H, 4.60; N, 15.29.

5-Hydroxy-6-aminouridine (X)——A suspension of IX (3.64 g) in 30 ml of 20% aqueous ethanol was reduced with Ranyl nickel W 2 (1 g) under atomospheric pressure at room temperature. The chocolate colored mixture changed into the mixture containing white precipitate. After absorption of a theoretical volume of hydrogen, precipitate was filtrated off. The filtrate was evaporated under reduce pressure. The combined residue and precipitate were dissolved in hot water. After the catalyst was filtrated off, crystals deposited from the clear solution on quick cooling. The crystals were recrystallized from water to yield 1.8 g of X (65%, mp 184° (decomp.)). UV λ_{max} nm: 290, 232 (infl.) (H_2O), 290 (pH 2). The molar absorptivity of X at 290 nm, gave 19900 after 10 minutes from dissolution in absolute methanol. *Anal.* Calcd. for $C_9H_{13}O_7N_3$: C, 39.27; H, 4.73; N, 15.27. Found: C, 39.31; H, 4.86; N, 15.10.

5-Acetoxy-6-acetamiduracil (XI)——A solution of X (1.0 g) in acetic anhydride containing 2 drops of pyridine was heated for 75 min at 100°. After cooling, precipitate was filtrated and crystallized from

14) 5-Benzylaminouracil was dissolved in 0.6N NaOH containing 40% ethanol.

water to yield 500 mg of XI (61%, mp >260°). UV λ_{max} nm: 286 (pH 1 and 7), 344 (pH 11). NMR (DMSO-d_6) ppm: 2.18 (3H), 2.25 (3H), 10.38 (1H), 10.92 (1H), 11.30 (1H). *Anal.* Calcd. for $C_8H_9O_5N_3$: C, 42.29; H, 3.96; N, 18.50. Found: C, 42.29; H, 4.19; N, 19.00.

4-(β-D-Ribofuranosyl)-4,5,6,7-tetrahydrooxazolo(4,5-*d*)pyrimidine-5,7-dione (XII)——Compound (X) (275 mg) and formamide (225 mg) were added to polyphosphate ester[15] (2.7 g). The mixture was heated at 70° for 4 hr. After cooling, the mixture was added to ethanol (500 ml) with stirring. The coloidal precipitate was collected by centrifugation and washed with ethanol at several times. The precipitate was dissolved in water and insoluble material was filterated off. The clear solution was adjusted to pH 4.5 with aqueous ammonia and passed through a column of anion exchange resin (Dowex 1×8 formate form). The column was washed with water. The eluent was evaporated to dryness under reduced pressure and the residue was crystallized from aqueous methanol to yield 114 mg of hydroscopic powder (XII) (mp 115—122°). UV λ_{max} nm: 274, 224 (infl.) (H_2O), 274 (pH 1), 315 (infl.), 277, 240 (infl.) (pH 11). On the paper chromatogram, the powder showed a single spot: *Rf*: 0.28 (solvent A) and 0.49 (solvent B)[16] as determined by UV lamp.

4-(2′,3′,5′-Tri-O-benzoyl-β-D-ribofuranosyl)-4,5,6,7-tetrahydrooxazolo(4,5-*d*)pyrimidine-5,7-dione (XIV)——Method A: A solution of benzoyl chloride (110 mg) in benzene (0.7 ml) was added to a solution of XII (70 mg) in pyridine (3 ml) at 0—5°. After the solution was allowed to stand for 7 days at room temperature, ice-water (30 ml) was added to the solution. The resulting gummy precipitate was solidified by trituration, collected by filtration and crystallized from $CHCl_3$ to yield 128 mg of XIV (mp 202°). *Anal.* Calcd. for $C_{31}H_{23}O_{10}N_3$: C, 62.31; H, 3.88; N, 7.03. Found: C, 62.49; H, 4.02; N, 6.99.

Method B: Benzoyl chloride (1.2 ml) was dropwise added to a solution of X (832 mg) in pyridine (25 ml) at 0—5°. After the solution was allowed to stand for 4 days at room temperature, the solution was concentrated to 10 ml under reduced pressure and then poured into 500 ml of ice-water. The precipitate was collected by filtration and dried to yield 1.5 g of powdery substance containing (XIII). The powder was used to subsequent reaction without further purification. After a mixture of the powder (1.0 g), formamide (168 mg) and polyphosphate ester (10 g) was heated at 70° for 3 hr., there was dropwise added to one liter of ice-water with stirring. The precipitate was collected by filtration and washed with water. Compound (XIV) was extracted with $CHCl_3$ (30 ml ×10) and the $CHCl_3$ solution was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was crystallized from $CHCl_3$ to yield 624 mg of (XIV) (mp 202°). *Anal.* Calcd. for $C_{31}H_{23}O_{10}N_3$: C, 62.31; H, 3.88; N, 7.03. Found: C, 62.35; H, 3.97; N, 7.11.

Acknowledgement The present authors are grateful to staffs of analytical laboratory of this Faculty for elementary analyses and NMR spectral determination.

15) Polyphosphate ester used was "material D" by reference 12.
16) Solvent systems used were solvent A: *n*-propanol–water (7: 3), solvent B: *n*-propanol–5% ammonium chloride (4: 6).