## Communications to the Editor

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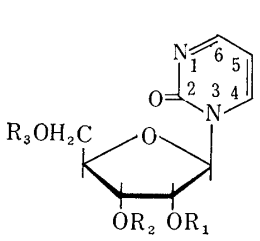
### Syntheses of Unnatural Pyrimidine Nucleosides

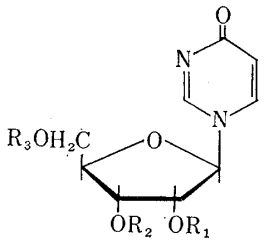
On the structural requirements for substrate of bovine pancreatic ribonuclease I-A recent efforts by several researchers have revealed several useful informations. Failure of the enzyme to catalyze the hydrolysis of methyl $\alpha$-D-ribofuranoside cyclic 2,3-phosphate, methyl $\beta$-D-ribofuranoside cyclic 2,3-phosphate,[1] 5′-(ribose-3-phosphoryl)cytidine, and 5′-(ribitol-3-phosphoryl)cytidine,[2] suggests that some functional group found in the pyrimidine residue of esters of 3′-phosphoryl-uridine or -cytidine might be necessary for the substrate. Further observations of Witzel[3] that an ester of 4,5-dihydrouridine 3′-phosphate was found to keep its ability of serving as a substrate while a hydrolysis product of the latter, the ester of ureidopropionic acid ribofuranoside 3′-phosphate, had lost the ability led him to propose the necessity of the presence of a following functional group in the pyrimidine residue in an ester of a 3′-pyrimidine nucleotide.

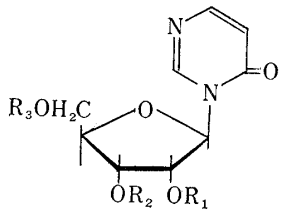
X = O or S
Y = OH or $NH_2$

On the basis of these results, Witzel[3] proposed a mode of action for ribonuclease on an ester of 3′-uridylic and 3′-cytidylic acids, which involves, as the primary important mechanism, the formation of a hydrogen bond between the carbonyl oxygen at C-2 of the pyrimidine ring with the sterically well-situated 2′-hydroxyl of its sugar residue.

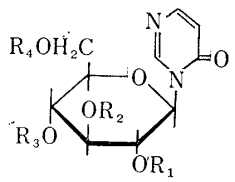
In order to have additional confirmations for the above proposal, attempt was made to synthesize two unnatural pyrimidine cyclic nucleotides, cyclic 2′,3′-phosphates of 3-$\beta$-D-







(I) $R_1, R_2 = >P(=O)OH$ $R_3 = H$ (II) $R_1, R_2 = >P(=O)OH$ $R_3 = H$ (III) $R_1 = R_2 = R_3 = H$
(IV) $R_1 = R_2 = R_3 = H$ (V) $R_1 = R_2 = R_3 = H$ (VIII) $R_1 = R_2 = R_3 = COC_6H_5$
(IX) $R_1 = R_2 = R_3 = COC_6H_5$



(VI) $R_1 = R_2 = R_3 = R_4 = H$
(VII) $R_1 = R_2 = R_3 = R_4 = COCH_3$

1) T. Ukita, M. Irie : This Bulletin, **6**, 445 (1958).
2) H. Witzel : Ann., **635**, 191 (1960).
3) *Idem* : IV Intnatl. Kongr. Biochem. Zusammenfassungen, 33 (1958), Wien.

ribofuranosyl-2-oxo-2,3-dihydropyrimidine (I) and 3-β-D-ribofuranosyl-6-oxo-3,6-dihydropyrimidine (II), each of which lacks one carbonyl group at C-6 or C-2 position in the pyrimidine residue of the cyclic uridylic acid and, to test their ability to serve as a substrate for ribonuclease. The present communication deals with the syntheses of the ribonucleoside (IV), which corresponds to cyclic ribonucleotide (I), of ribonucleoside (III) which was obtained as the reaction product in attempting the synthesis of the ribonucleoside (V), and of glucopyranosyl-4-oxo-3,4-dihydropyrimidine (VI).

The glycosidation of the bases was performed fundamentally according to Fox's mercury-salt procedure[4] to condense a mercury salt of the base with a 1-halogenated protected sugar. This procedure afforded the desired condensation product between the protected ribofuranosyl chloride and the mercury salt of 2-hydroxypyrimidine. In the case of 4-hydroxypyrimidine, however, both protected glucopyranosyl bromide and ribofuranosyl chloride underwent condensation to give not the desired $N_3$-glycosides of 6-oxo-3,6-dihydropyrimidine, but those of 4-oxo-3,4-dihydropyrimidine (VI) and (III).

**3-β-D-Glucopyranosyl-4-oxo-3,4-dihydropyrimidine (VI)**——A suspension of 2 g. of the mercury salt of 4-hydroxypyrimidine (Hg : pyrimidine=1:2) added to 80 cc. of dehyd. toluene was dried by azeotropic distillation of approximately 1/4 volume of the solvent with vigorous stirring, 4.1 g. of tetra-O-acetylglucopyranosyl bromide was added and the mixture was refluxed in an oil bath heated at 150~160° for 1 hr. with stirring. The warm mixture was filtered and to the cooled filtrate petr. ether was added. The precipitate produced was collected, dissolved in $CHCl_3$, and the solution was washed successively with 30% KI solution and water. The dried $CHCl_3$ layer was evaporated to furnish the product, 2′,3′,4′,6′-tetra-O-acetyl-3-β-D-glucopyranosyl-4-oxo-3,4-dihydropyrimidine (VII), which was recrystallized from MeOH, m.p. 209~210°; yield, 31.6%; $[\alpha]_D^{13.7}$ +80.56°(c=1.44, $CHCl_3$). *Anal.* Calcd. for $C_{18}H_{22}O_{10}N_2$ : C, 50.70; H, 5.20; N, 6.57. Found : C, 50.39; H, 4.97; N, 6.42. Rf 0.84 (iso-PrOH-$NH_3$-$H_2O$=7:1:2, ascending), UV $\lambda_{max}^{EtOH}$ mμ(ε) : 218 (5,260), 275 (3,650); IR $\lambda_{max}^{KBr}$ $cm^{-1}$: 1750, 1690, 1600, 1540, 1240.

100 mg. of (VII) dissolved in a mixed solvent of 3 cc. of MeOH and 0.4 cc. of EtOH containing 22% of HCl was set aside at room temperature, with protection from moisture. The colorless prisms that appeared were collected by filtration and washed successively with EtOH and $Et_2O$ to obtain 65 mg. of the hydrochloride of 3-β-D-glucopyranosyl-4-oxo-3,4-dihydropyrimidine (VI). m.p. 162~163° (decomp.), $[\alpha]_D^{13.5}$ +60.58°(c=1.37, $H_2O$). *Anal.* Calcd. for $C_{10}H_{14}O_6N_2 \cdot HCl$ : C, 40.75; H, 5.13; N, 9.51. Found : C, 40.94; H, 5.04; N, 9.12. IR $\lambda_{max}^{KBr}$ $cm^{-1}$ : 3320, ~2660, 1730, 1680, 1560.

The absorption maxima of this compound in UV region at both pH −0.4 and 6.0 were compared with those of 3-N-methyl-4-oxo-3,4-dihydropyrimidine[5] (X), 1-N-methyl-4-oxo-1,4-dihydropyrimidine[5] (XI), and 4-methoxypyrimidine[5] (XII), and as is given in Table I, the maxima of this product were very similar to those of (X) and the product was proved to be 3-glucopyranosyl-4-oxo-3,4-dihydropyrimidine.

Table I.

| Compound | pH | Absorption Maxima mμ(ε) |
|---|---|---|
| (VI) | −0.4 | 223 (7,390), 264 (2,900) |
| | 6.0 | 218 (6,430), 274 (4,100) |
| (X) | −0.4 | 226 (9,080), 258 (2,940) |
| | 5.0 | 221 (6,810), 269 (3,900) |
| (XI) | −0.4 | 229 (10,200), 252 (2.640) |
| | 6.0 | 240 (14,640) |
| (XII) | 0 | 227~228 (7,740), 238* (6,800) |
| | 6.95 | 247~248 (3,350) |

* shoulder

**3-β-D-Ribofuranosyl-4-oxo-3,4-dihydropyrimidine (III)**——10.1 g. of 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose was chlorinated by the usual procedure[4] and was added with stirring to a suspension of 3.9 g. of the above mercury salt of 4-hydroxypyrimidine in 150 cc. of dehyd. xylene in an oil bath heated at 150~160° for 1 hr. The reaction mixture was treated similarly as above and the product, 2′,3′,5′-

4) J. J. Fox, N. Yung, J. Davoll, G. B. Brown : J. Am. Chem. Soc., **78**, 2117 (1956).
5) D. J. Brown, E. Hoerger, S. F. Mason : J. Chem. Soc., **1955**, 211.

tri-O-benzoyl-3-β-D-ribofuranosyl-4-oxo-3,4-dihydropyrimidine (Ⅷ), was recrystallized from a mixture of AcOEt and petr. ether to obtain 7.4 g. (yield, 67%) of colorless prisms, m.p. 157~157.5°, $[\alpha]_D^{13.7}$ +15.39° (c=1.04, $CHCl_3$): Rf 0.95 (BuOH-$H_2O$=86:14, ascending), Rf 0.92 (iso-PrOH-$NH_3$-$H_2O$=7:1:2, ascending). *Anal.* Calcd. for $C_{30}H_{24}O_8N_2$: C, 66.66; H, 4.48; N, 5.18. Found: C, 66.29; H, 4.68; N, 5.04. IR $\lambda_{max}^{KBr}$ $cm^{-1}$: 1730, 1690, 1600, 1540, 1290. UV $\lambda_{max}^{EtOH}$ mμ(ε): 230 (46,500), 257 (7,420).

A solution of 1.0 g. of the benzoate (Ⅷ) dissolved in 50 cc. of EtOH previously saturated with $NH_3$ at 0°, sealed in a tube, was heated in a boiling water bath for 10 hr. The pale yellow solution thus obtained was concentrated to a yellow syrup which was steam-distilled to remove ethyl benzoate and the aqueous residue was shaken with $CHCl_3$ to remove benzamide. The aqueous layer was decolorized with charcoal and concentrated in a reduced pressure. The residue on addition of EtOH formed crystals which were recrystallized from EtOH giving plates, m.p. 170~171°, yield, 83%; Rf 0.28 (BuOH-$H_2O$=86:14, ascending), 0.60 (iso-PrOH-$NH_3$-$H_2O$=7:1:2); $[\alpha]_D^{13.5}$ +91.89° (c=1.48, $H_2O$). *Anal.* Calcd. for $C_9H_{12}O_5N_2$: C, 47.37; H, 5.30; N, 12.28. Found: C, 47.37; H, 5.20; N, 11.84. IR $\lambda_{max}^{KBr}$ $cm^{-1}$: 3400, 1650, 1590, 1530.

The absorption maxima in UV region ($\lambda_{max}^{H_2O}$ (pH −0.4) mμ(ε): 225 (10,980), 258 (4,310); $\lambda_{max}^{H_2O}$ (pH 6.0) mμ(ε): 218 (7,060), 270 (4,530)) indicate that this product is 3-N-ribofuranosyl-4-oxo-3,4-dihydropyrimidine and not 1-N-ribofuranosyl-4-oxo-1,4-dihydropyrimidine or 4-O-ribofuranosyl-4-hydroxypyrimidine. When treated with metaperiodate by the usual procedure,[6] (Ⅲ) consumed 0.95 mole of periodate per mole of nucleoside within 10 min. No further consumption of the oxidant was observed for the ensuing 48 hr.

**3-β-D-Ribofuranosyl-2-oxo-2,3-dihydropyrimidine** (Ⅳ)——2,3,5-Tri-O-benzoyl-D-ribofuranosyl chloride obtained from 15.1 g. of 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose was added to 200 cc. of an azeotropically dried suspension of 9.90 g. of monochloromercury salt of 2-hydroxypyrimidine in xylene. The mixture was heated in an oil bath heated at 145° for 50 min., the precipitate that appeared was removed from the warm solution by filtration, and xylene was evaporated in a diminished pressure. Petr. ether was added to the residue, the precipitate formed was taken up in $CHCl_3$, and $CHCl_3$ solution was washed successively with 30% KI solution and water. The solvent from the $CHCl_3$ layer after drying was removed and the residue was treated with EtOH, by which a yellow resinous precipitate formed, followed by an amorphous white sediment. Recrystallization of the yellow resinous matter from EtOH gave an amorphous powder (A) with m.p. ca. 120° (decomp.) and recrystallization of the white sediment from EtOH gave a powdery product (B) of m.p. 154~158°. Yield, 36%. The analysis of (B) was in good agreement with that of (Ⅸ), 2′,3′,5′-tri-O-benzoyl derivative of (Ⅳ). $[\alpha]_D^{13.7}$ +71.71° (c=1.29, $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{24}O_8N_2$: C, 66.66; H, 4.48; N, 5.18. Found: C, 66.28; H, 4.24; N, 5.11. Rf 0.92 (BuOH-$H_2O$=86:14). IR $\lambda_{max}^{KBr}$ $cm^{-1}$: 1730, 1680, 1540, 1280. UV $\lambda_{max}^{EtOH}$ mμ(ε): 230 (34,560), 276 (4,200), 282 (4,240), 310 (3,890). Product (A), $[\alpha]_D^{22.8}$ −3.91° (c=1.38, $CHCl_3$). *Anal.* Found: C, 65.02; H, 4.50. UV $\lambda_{max}^{EtOH}$ mμ(ε): 230 (34,560), 276 (3,880), 282 (3,360), 305 (1,680).

A solution of 1.0 g. of (Ⅸ) dissolved in 50 cc. of MeOH previously saturated with $NH_3$ at 0° was kept at 10~15° for 22 hr. in a seald tube. After removal of MeOH by distillation and subsequent steam-distillation, the residue was extracted with $CHCl_3$. The aqueous solution was concentrated to a syrup which was purified through a cellulose column, eluting with 86% hydr. BuOH. From the combined fractions, which exhibited an absorption maximum at 303 mμ (at pH 6.0), the solvents were removed by distillation and the residue was recrystallized from hydr. EtOH to colorless hygroscopic needles, m.p. 156.5~157° (decomp.) (by Hans Bock's Monoscop Ⅳ). Yield, 57%. $[\alpha]_D^{7.8}$ +61.68° (c=1.43, $H_2O$), Rf 0.17 (BuOH-$H_2O$=86:14). *Anal.* Calcd. for $C_9H_{12}O_5N_2$: C, 47.37; H, 5.30; N, 12.28. Found: C, 47.19; H, 5.05; N, 12.21. IR $\lambda_{max}^{KBr}$ $cm^{-1}$: 3480, 1660, 1550. When treated with metaperiodate, (Ⅳ) consumed 1.05 moles of the oxidant per mole within 5 min.

By comparison of absorption maxima of this product in UV range at both pH 0.3 and 6.0 with those of 3-N-methyl-2-oxo-2,3-dihydropyrimidine[5] (Ⅻ) and 2-methoxypyrimidine[5] (XIV), it is seen that the product (Ⅳ) is N-ribofuranoside and not O-ribofuranoside (Table Ⅱ).

Table Ⅱ.

| Compound | pH | Absorption maxima mμ(ε) |
|---|---|---|
| (Ⅳ) | 0.3 | <208 (>8,370), 313 (7,520) |
| | 6.0 | 212 ( 9,000), 303 (5,320) |
| (Ⅻ) | 0.3 | <215 (>6,360), 313 (7,102) |
| | 6.0 | 215 ( 10,000), 320 (5,400) |
| (XIV) | 0 | 273~274 (4,900), 309 ( 708) |
| | 6.98 | 264 (4,780) |

6) B. Lythgeoe, A. R. Todd: J. Chem. Soc., **1944**, 592.