[Chem. Pharm. Bull. 12 (7) 828 ~ 835]

UDC 612.398.145 : 547'118.5

119. Tyunosin Ukita, Ryoya Funakoshi,*1 and Yuji Hirose*2 : Organic Phosphates. XX*3. Syntheses of 1-(3-Cyanoethylphosphoryl-5-trityl-D-ribofuranosyl)-2(1*H*)-pyrimidinone, -6(1*H*)-pyrimidinone, and -2(1*H*)-pyridone and their Properties as Substrate of Bovine Pancreatic Ribonuclease.

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In the previous communication,[1] the present authors reported the syntheses of some unnatural pyrimidine ribonucleosides, 1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (I) and -6(1*H*)-pyrimidinone (II). In this paper the syntheses of an additional unnatural nucleoside, 1-β-D-ribofuranosyl-2(1*H*)-pyridone (III), a further conversion of these nucleosides to 5'-trityl-2'(+3')-cyanoethylphosphates, (Ib+Ic), (IIb+IIc), (IIIb+IIIc) and the property of these derivatives as substrates of bovine pancreatic ribonuclease (RNase-A) will be reported.

2-Pyridinol was reacted in aqueous sodium hydroxide with equimolar amount of mercuric chloride to obtain a chloromercuri-salt (VI) in a yield of 97.0%. This salt lacked an absorption at 1660 cm^{-1} which is characteristic of carbonyl group of N_1-substituted pyridone. The salt was boiled in xylene with 2,3,5-tri-O-benzoylribosyl chloride at 160° for 15 minutes to furnish 2-O-(2,3,5-tri-O-benzoylribofuranosyl)pyridine (VII), m.p. 87~89° (yield, 65.5%). This product was verified as to be an O-glycoside because it did not reveal an absorption at 1660 cm^{-1} in infrared spectrum characteristic of carbonyl group of pyridone, and was easily hydrolyzed in diluted acid.

No evidence was observed to produce N-(2,3,5-tri-O-benzoylribofuranosyl)-2(1*H*)-pyridone (VIII) even by three hours' prolonged boiling in above reaction.

In order to obtain the desired N-riboside (VIII) by rearrangement of its ribofuranosyl group, several reactions were performed on the O-riboside. The compound (VII) was

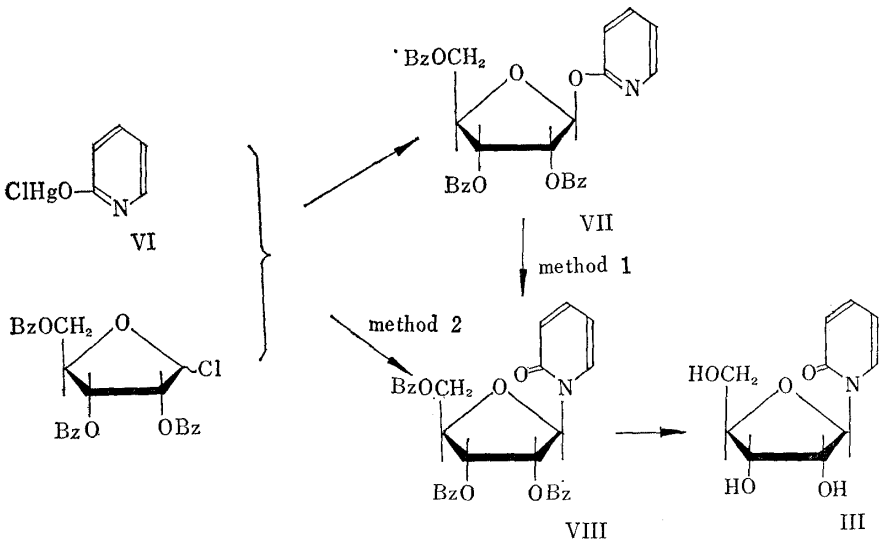

Chart 1.

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*3 Part XIX. T. Ukita, N. Imura, K. Nagasawa, N. Aimi : This Bulletin, **10**, 1113 (1962).

1) R. Funakoshi, M. Irie, T. Ukita : *Ibid.*, **9**, 406 (1961).

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treated in acetonylacetone with sodium iodide at 100° for 6 hours[2] or Ⅶ was treated with sodium iodide and acetic acid in the same solvent, but no rearrangement of the sugar moiety occurred. A similar result has been reported by Ulbricht[3] in the case of the N-acetylcytosine-O-glucoside treated by the former reaction condition.

The desired rearrangement was found to occur when Ⅶ was boiled in xylene with two molar equivalent of mercuric bromide at 160° for 3 hours, this method was primarily reported by Wagner[4] in the rearrangement of 2-O-(2,3,4,6-tetra-O-acetylglucopyranosyl)pyridine to N-(2,3,4,6-tetra-O-acetylglucopyranosyl)-2(1*H*)-pyridone. By this reaction, Ⅷ, m.p. 138~139°, was obtained (yield, 62.0%) and revealed a typical absorption of N_1-substituted pyridone-carbonyl group at 1660 cm^{-1} in infrared spectrum and a typical ultraviolet spectrum for N_1-substituted pyridone-2.

The condensation of chloromercuri-salt of the base (Ⅵ) with tribenzoylribosylchloride was performed with co-existence of mercuric bromide, thus, these materials were boiled in xylene at 160° for 3 hours, and the product (Ⅷ) was obtained by one step reaction in a yield of 45.2%.

The N-riboside (Ⅷ) was debenzoylated by keeping the compound in ammonia-saturated ethanol for 48 hours to furnish N-(D-ribofuranosyl)-2(1*H*)-pyridone (Ⅲ), m.p. 149~150°, which on oxidation with periodate consumed one equimolar amount of the reagent. Though this product (Ⅲ) showed a large positive specific rotation, $[\alpha]_D^{20.2}$ +115°, the configuration of its glycosidic linkage could be assigned as β-type, because a N-glucoside of pyrimidine derivative 1-(2,3,4,6-tetra-O-acetyl-D-glucopyranosyl)-4-ethoxy-2(1*H*)-pyrimidinone, obtained from the corresponding O-glucoside by a similar rearrangement reaction was found to have β-configuration[4b] and further because, as will be mentioned below, a phosphoryl nucleoside ester obtained from Ⅲ was active as a substrate of RNase-A which is known to hydrolyse naturally occurring pyimidine nucleoside 3'-phosphodiester having β-configuration at the glycosidic linkage.

The nucleosides, Ⅰ, Ⅱ and Ⅲ, were tritylated by ordinary procedure to the corresponding 5'-trityl compound, Ⅰa : m.p. 161~162°, Ⅱa : m.p. 158~162°, and Ⅲa : m.p. 87° with respective yield of 67.0, 71.8, and 71.2%. Each 5'-trityl compound was treated with cyanoethylphosphate[5] and DCC (dicyclohexylcarbodiimide) in pyridine and the corresponding products, the mixtures of 2'- and 3'-cyanoethylphosphoryl-5'-tritylribonucleosides, (Ⅰb+Ⅰc), (Ⅱb+Ⅱc), (Ⅲb+Ⅲc) were isolated as analytically pure cyclohexylammonium salts by paper chromatography using solvents of isopropyl alcohol-ammonia-water (28:1:10).

By the similar series of reactions, uridine and adenosine were converted to their corresponding 2'(+3')-cyanoethylphosphoryl-5'-trityl derivatives, (Ⅳb+Ⅳc), and (Ⅴb+Ⅴc), which were used in the following experiments of enzymatic hydrolysis as standard compounds.

Different from the uridine derivatives, (Ⅳb+Ⅳc), which have naturally occurring pyrimidine base in nucleoside residue, the similar derivatives having unnatural pyrimidine base, (Ⅰb+Ⅰc) and (Ⅱb+Ⅱc), were found unstable against the treatment with alkali, thus in *N* sodium hydroxide at 100° after 5 minutes the characteristic absorption maxima in ultraviolet spectra of Ⅰb+Ⅰc (at 303 mμ) and Ⅱb+Ⅱc (at 268 mμ) changed irreversibly.*5

*5 Shugar and Fox reported the similar instability of 1,3-dimethyluracil to alkaline treatment (D. Shugar, J. J. Fox : Biochim. Biophys. Acta, **9**, 199 (1952)).

2) T. L. V. Ulbricht : J. Chem. Soc., **1961**, 3345.
3) *Idem* : Proceedings Chem. Soc., **1962**, 298.
4) *a*) G. Wagner, H. Pischel : Archiv. der Pharmazie, **295**, 373 (1962). *b*) T. Ukita, H. Hayatsu, Y. Tomita : This Bulletin, **11**, 1068 (1963).
5) G. M. Tener : J. Am. Chem. Soc., **83**, 159 (1961).

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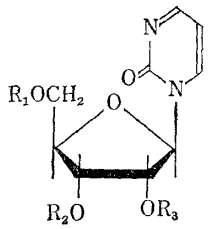
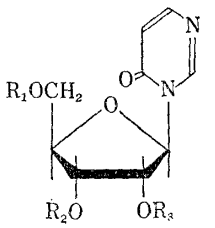
The similar instability was observed also for 1-methyl-2(1*H*)-pyrimidinone as well as for 1-methyl-6(1*H*)-pyrimidinone.

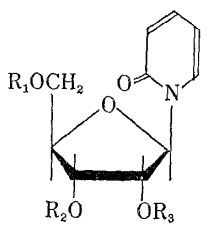
The instability to above alkaline condition of these compounds made it difficult to give the corresponding phosphomonoesters by chemical hydrolysis of their cyanoethyl groups and to derive the monoesters to 2′,3′-cyclic phosphates which are useful compounds for the research on substrate specificity of cyclic phosphodiesterase activity of RNase-A.



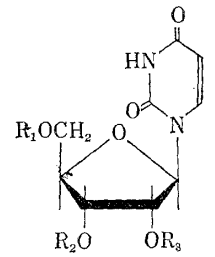
I : $R_1=R_2=R_3=H$
Ia : $R_1=C(C_6H_5)_3$, $R_2=R_3=H$
Ib : $R_1=C(C_6H_5)_3$, $R_2=H$, $R_3=PO(OH)(OCH_2CH_2CN)$
Ic : $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)(OCH_2CH_2CN)$
Ic′: $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)_2$



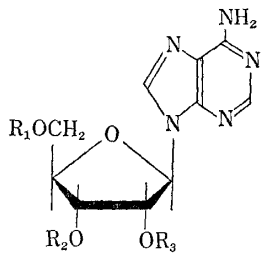
II : $R_1=R_2=R_3=H$
IIa : $R_1=C(C_6H_5)_3$, $R_2=R_3=H$
IIb : $R_1=C(C_6H_5)_3$, $R_2=H$, $R_3=PO(OH)(OCH_2CH_2CN)$
IIc : $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)(OCH_2CH_2CN)$
IIc′: $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)_2$



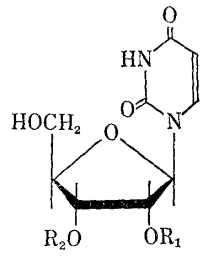
III : $R_1=R_2=R_3=H$
IIIa : $R_1=C(C_6H_5)_3$, $R_2=R_3=H$
IIIb : $R_1=C(C_6H_5)_3$, $R_2=H$, $R_3=PO(OH)(OCH_2CH_2CN)$
IIIc : $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)(OCH_2CH_2CN)$
IIIc′: $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)_2$



IVa : $R_1=C(C_6H_5)_3$, $R_2=R_3=H$
IVb : $R_1=C(C_6H_5)_3$, $R_2=H$, $R_3=PO(OH)(OCH_2CH_2CN)$
IVc : $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)(OCH_2CH_2CN)$
IVc′: $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)_2$



Va : $R_1=C(C_6H_5)_3$, $R_2=R_3=H$
Vb : $R_1=C(C_6H_5)_3$, $R_2=H$, $R_3=PO(OH)(OCH_2CH_2CN)$
Vc : $R_1=C(C_6H_5)_3$, $R_3=H$, $R_2=PO(OH)(OCH_2CH_2CN)$



Xb : $R_1=PO(OH)(OCH_2CH_2CN)$, $R_2=H$
Xc : $R_1=H$, $R_2=PO(OH)(OCH_2CH_2CN)$
X : $R_1=H$, $R_2=PO(OH)(OCH_2CH_3)$

Chart 2.

The quantitative determination of each 3′-cyanoethylphosphoryl 5′-trityl isomers, (Ic), (IIc), (IIIc) and (IVc), contained in respective mixtures, (Ib+Ic), (IIb+IIc), (IIIb+IIIc) and (IVb+IVc), was performed as follows: the mixed cyanoethyl nucleotides were completely digested with RNase-A which is known to convert the 3′-phosphodiesters of naturally occurring pyrimidine ribonucleosides to the corresponding 3′-phosphomonoesters and is inert to the 2′-isomeric phosphodiesters, and after separation by paper chromatography, the products were quantitatively analyzed by spectrophotometric estimation. The results of the analyses revealed that each content of 3′-isomer in Ib+Ic, IIb+IIc, and IIIb+IIIc was 53, 54, and 51%, respectively.

Furthermore, the fact that these products, 3′-phosphomonoesters (Ic′), (IIc′), and (IIIc′), isolated from the incubation mixtures, on dephosphorylation with alkaline phosphatase gave the corresponding mother nucleosides, (Ia), (IIa) and (IIIa), showed that the digestion of Ic, IIc and IIIc by RNase-A caused no change at the ribonucleoside structure but mere hydrolysis of the cyanoethyl group of their 3′-cyanoethylphosphoryl residue.

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In order to know the relative activity of Ic, IIc and IIIc as a substrate of RNase-A to that of IVc and Vc, each Ib+Ic, IIb+IIc, IIIb+IIIc, IVb+IVc and Vb+Vc was incubated with RNase-A in phosphate buffer (pH 7.6) at 37° with each substrate/enzyme ratio given in Table III and the amounts of the 3′-phosphomonoesters, (Ic′), (IIc′), (IIIc′) and (IVc′), produced were plotted against incubation times to give curves summarized in Fig. 1.

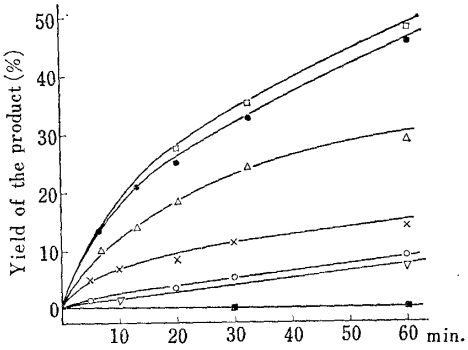

Fig. 1. Formation of 3′-Phosphomonoesters by RNase-A Hydrolyses of 3′-Phosphodiesters
Curves represent the amounts of 3′-phosphomonoesters from the substrate 3′-phosphodiesters.
Each symbol represents respective substrate:
△: Ic ○: IIc ▽: IIIc ●: IVc
■: Vc □: IXc ×: X

Fig. 1 includes the results of the similar experiment for 2′(+3′)-cyanoethylphosphoryl uridine, (IXb+IXc), and 3′-ethylphosphoryl uridine (X) which were respectively prepared from IVb+IVc by hydrolysis of trityl group with 80% acetic acid (50°, 40 min.) and by enzymatic ethanolysis of uridine 2′,3′-cyclic phosphate with RNase-A.[6]

From the results shown in Fig. 1, there is no remarkable difference in the rates of formation of the corresponding 3′-phosphomonoesters from IVc and IXc, that is, the substitution of a trityl group at the 5′-position of IXc does not markedly influence the susceptibility of the substrate to the enzyme.*[6] On the other hand, the remarkable difference in formation of the product from IXc and X represents that the cyanoethyl group esterified at 3′-phosphoryl group of uridine is more hydrolysable by RNase-A than the corresponding ethyl group.

As for the phosphodiesters of ribonucleosides having unnatural pyrimidine base, the amounts of the hydrolysis products of Ic, IIc and IIIc after 20 minutes' incubation with RNase-A, was respectively 75, 16, and 12% of that of the corresponding derivative of uridine (IVc). Thus the removal of a hydrogen at N_3-position and an oxo-group at 4-position of uracil residue of IVc caused no fatal influence on its property as a substrate of the enzyme, while a further replacement of N_3-nitrogen of Ic with -CH= (the compound (IIIc)) or replacement of -CH= at the 5-position of IIIc with nitrogen (the compound (IIc)) reduced remarkably the susceptibility of the substrate towards the hydrolysis by the enzyme. The compound (Vc) gave no hydrolysis product even after 24 hours' incubation with RNase-A.

From the above observations, at pH value used for the enzyme assay, the imino group at N_3-position in 3′-phosphodiester of uridine seems to be more important than the carbonyl-group at C_4-position for its affinity to the active site of RNase-A.

Experimental*[7]

Paper Chromatography——Ascending chromatography unless otherwise mentioned on Toyo Roshi No. 51 paper was performed, with solvents : solvent 1, BuOH-H_2O (86:14), solvent 2, iso-PrOH-conc. NH_4OH-H_2O (28:1:10). Rf value obtained was represented by Rf_1 or Rf_2 corresponding to the solvent used.

*[6] Several reports have been presented concerning the modification of 5′-carbinol group of 3′-phosphodiester of naturally occurring pyrimidine ribonucleoside and activities of the products as substrate of the RNase, *i.e.* the replacement of the 5′-CH_2OH group with CH_3 (R. Funakoshi, H. Hayatsu, T. Ukita : 10th Nucleic Acid Symposium, Kanazawa, Japan, May, 1962), $BrCH_2$ (A. M. Michelson : J. Chem. Soc., **1962**, 979), CH_3COOCH_2 (J. Smrt, F. Šorm : Coll. Czech. Chem. Commun., **27**, 73 (1962)) and $-SCH_2$ (A. M. Michelson : J. Chem. Soc., **1962**, 979) showed no remarkable decrease in the susceptibility of the compounds towards the RNase digestion.

*[7] All melting points are uncorrected.

6) G. R. Barker : J. Chem. Soc., **1957**, 3786.

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The spots of nucleosides or nucleotides were detected by scanning the chromatogram over ultraviolet lamp. The phosphorus containing compounds were detected with Hanes and Isherwood[7] reagent and followed irradiation of ultraviolet ray.[8] The quantitative determination of phosphorus was performed by Allen's method.[9]

Bovine Pancreatic Ribonuclease (RNase-A)——The enzyme was obtained in this laboratory according to the procedure of Kunitz[10] and purified by the method of Hirs, Moore and Stein.[11]

2-Pyridinol-monochloromercury (VI)——In 20 ml. of H_2O containing 0.8 g. of NaOH, 1.90 g. of 2-pyridone was dissolved.*8 To the solution under stirring was added 5.4 g. of $HgCl_2$ dissolved in 120 ml. of EtOH, whereupon precipitation occurred. The mixture was allowed to cool while stirring. The precipitate was filtered and washed repeatedly with cold H_2O until the filtrate was free from halide ion. After subsequent washing with EtOH and Et_2O, the precipitate was dried to furnish 2-hydroxypyridine-monochloromercury in a yield (6.38 g.) of 97% calculated from $HgCl_2$. *Anal.* Calcd. for $C_5H_4ONClHg$: N, 4.42. Found: N, 4.29. UV λ_{max}^{EtOH} mμ: 228, 305.

2-(2,3,5-Tri-O-benzoyl-D-ribofuranosyl)-2-pyridinol (VII)——To 150 ml. of anhyd. Et_2O was added 6 g. of 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose, and the solution was saturated with HCl at 0°. After 4 days' keeping at 3~5°, the solvent was removed from the solution *in vacuo*, and the residual syrup was dried three times by azeotropical distillation with 30 ml. of anhyd. benzene *in vacuo*. A benzene solution of the halogenose (2,3,5-tri-O-benzoyl-D-ribosyl chloride) was added to a vigorously stirred azeotropically dried suspension of 2-hydroxypyridinemonochloromercury in hot xylene, which had been maintained at 145° in an oil bath. After refluxing at 160° (bath temp.) for 15 min., the reaction mixture was cooled and filtered from the insoluble material. The filtrate was evaporated *in vacuo* to 20 ml. and added with 300 ml. of petr. ether. The white precipitate that occurred was collected, dissolved in $CHCl_3$ and washed with 30% KI solution and H_2O, successively. After removal of solvent from the solution, the residual syrup was recrystallized from 25 ml. of EtOH to obtain 2.80 g. (65.5%) of the product. m.p. 87~89°, $[\alpha]_D^{20.2}$ +10.0° (c=1.00, $CHCl_3$), UV λ_{max}^{EtOH} mμ (ε): 230 (38300), 268 (4590). *Anal.* Calcd. for $C_{31}H_{25}O_8N$: C, 68.90; H, 4.85; N, 2.60. Found: C, 68.42; H, 4.81; N, 2.43. Rf_1, 0.95.

1-(2,3,5-Tri-O-benzoyl-D-ribofuranosyl)-2(1*H*)-pyridone (VIII)——Method 1: A suspension of 1.5 g. of above described tribenzoyl-O-riboside (VII) and 3.4 g. of dried $HgBr_2$ in 100 ml. of dried xylene was refluxed for 3 hr. at 160° under stirring. After cooling the suspension was filtered and the filtrate was evaporated to 30 ml. To the solution 300 ml. of petr. ether was added, and the precipitate that occurred was taken up in 20 ml. of $CHCl_3$ and washed successively with 30% KI solution and H_2O. The dried $CHCl_3$ solution was evaporated to dryness and the residue was crystallized from EtOH to give 1.55 g. of VIII, m.p. 136~139°, yield, 62%. On further recrystallization from EtOH, the melting point rised to 138~139° (sintered at 136°). *Anal.* Calcd. for $C_{31}H_{25}O_8N$: C, 68.90; H, 4.85; N, 2.60. Found: C, 68.67; H, 4.80; N, 2.58. $[\alpha]_D^{20.2}$ +59.8° (c=1.02, $CHCl_3$), UV λ_{max}^{EtOH} mμ (ε): 230 (47900), 276 (5180), 282.5 (5700), 303 (5420). IR ν_{max}^{KBr} cm^{-1}: 1680. Rf_1, 0.93.

Method 2: To 100 ml. of dry xylene was added 3.96 g. of powdered 2-hydroxypyridine-monochloromercury (VI). The suspension was azeotropically dried by distilling approximately one-fourth of the solvent under vigorous stirring. To the boiling suspension was added under stirring tri-O-benzoyl-D-ribofuranosylchloride. After 5 min. reaction, 8 g. of $HgBr_2$ was added and refluxing was continued for an additional 3 hr. at 150~160° (bath temp.). The solution was cooled, evaporated, added with petr. ether, and the precipitate that occurred was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with 30% KI solution, H_2O, succesively. After evaporation of $CHCl_3$, the residue was crystallized from EtOH to give the product melted at 138~139°. A mixed melting point of this product with VIII obtained by the method 1 showed no depression.

1-D-Ribofuranosyl-2(1*H*)-pyridone (III)——A solution of 1.0 g. of VIII dissolved in 50 ml. of MeOH, previously saturated with NH_3 at 0°, was kept in a sealed tube at room temperature for 2 days. After removal of MeOH by distillation and ethyl benzoate by steam distillation, the residue was washed with $CHCl_3$. The aqueous layer was condensed to a syrup, the syrup was purified by passing through a cellulose column. On elution from the column with 86% aq. BuOH, was obtained 420 mg. of the desired product. The yield of the crude product was 88.2%. The product was recrystallized from EtOH to give white crystals which melted at 149~150°. $[\alpha]_D^{20.2}$ +115° (c=1.23, H_2O). *Anal.* Calcd. for $C_{10}H_{13}O_5N$:

*8 When the solution was made slightly excess in alkali, the precipitate become yellowish. (cf. J. J. Fox, N. Yung, I. Wempen, I. L. Doerr: J. Am. Chem. Soc., **79**, 5060 (1957)). This was avoided by adding additional trace amounts of pyridone.

7) C. S. Hanes, F. A. Isherwood: Nature, **164**, 1107 (1949).
8) R. S. Bandurski, B. Axelrod: J. Biol. Chem., **193**, 405 (1951).
9) R. J. L. Allen: Biochem. J., **34**, 858 (1940).
10) M. Kunitz: J. Gen. Physiol., **24**, 15 (1940).
11) C. H. W. Hirs, S. Moore, W. H. Stein: J. Biol. Chem., **200**, 493 (1953).

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C, 52.80; H, 5.70; N, 6.16. Found: C, 52.68; H, 5.84; N, 6.06. UV λ_{max}^{EtOH} mμ (ε): 228 (6170), 303 (5800). IR ν_{max}^{KBr} cm^{-1}: 1660. Rf_1, 0.74.

General Method in Tritylation of the Nucleosides, (I), (II) and (III)——Levene and Tipson's procedure[12] was employed with a slight modification. A solution of 2 mmoles of well dried nucleosides (I), (II), (III) and 2.2 mmoles of triphenyl methyl chloride in 8 ml. of dry pyridine was set aside at 37° for 2 days and then warmed on a boiling water bath for 2 hr. with exclusion of moisture. The solution was cooled and poured into 6 times volume of ice water with stirring. The precipitated gum was washed with H_2O and dissolved in EtOH, and the solution filtered and the filtrate evaporated to dryness. The process was repeated until the last trace of pyridine was removed. The analytically pure product was obtained by precipitation with Et_2O from ethanolic solution or by keeping cool a concentrated ethanolic solution of the residue in the yields given in Table I.

Table I. Yields of 5′-Tritylated Nucleosides

Nucleoside 1-β-D-ribofuranosyl-	5′-Tritylated product	Yield (%)
2(1*H*)-pyrimidinone (I)	Ia	67
6(1*H*)-pyrimidinone (II)	IIa	71
2(1*H*)-pyridone (III)	IIIa	71

1-(5-Trityl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (Ia): *Anal.* Calcd. for $C_{28}H_{26}O_5N_2$: C, 71.50; H, 5.54; N, 5.95. Found: C, 71.95; H, 5.89; N, 5.44. UV λ_{max}^{EtOH} mμ: 310, Rf_1, 0.95.
1-(5-Trityl-β-D-ribofuranosyl)-6(1*H*)-pyrimidinone (IIa): *Anal.* Calcd. for $C_{28}H_{26}O_5N_2$: C, 71.50; H, 5.54; N, 5.95. Found: C, 72.02; H, 6.03; N, 5.19. UV λ_{max}^{EtOH} mμ (ε): 270 (4210), Rf_1, 0.97.
1-(5-Trityl-β-D-ribofuranosyl)-2(1*H*)-pyridone (IIIa): *Anal.* Calcd. for $C_{29}H_{27}O_5N$: C, 74.18; H, 5.80; N, 2.98. Found: C, 74.32; H, 6.39; N, 2.98. UV λ_{max}^{EtOH} mμ (ε): 303 (5460). IR ν_{max}^{KBr} cm^{-1}: 1660, Rf_1, 0.95.

A 60% EtOH solution of IIIa consumed 0.85 mole (10 min.), 0.95 mole (35 min.), 0.98 mole (80 min.) and 1.04 mole (22 hr.) of periodate. Spots of Ia, IIa and IIIa on the chromatograms are all positive to periodate reaction.

General Method in Cyanoethylphosphorylation of 5′-Tritylated Nucleosides, (Ia), (IIa), (IIIa), (IVa) and (Va)——A solution of 1 mmole of the 5′-tritylated nucleosides, (Ia), (IIa) or (IIIa), and 2 mmole of 2-cyanoethylphosphate in 10 ml. of anhyd. pyridine was evaporated to dryness *in vacuo*.[5] The residue was dissolved in 5 ml. of anhyd. pyridine and the solvent was azeotropically distilled. The process was repeated twice more and to a solution of the residue dissolved in 5 ml. of dry pyridine was added 8 mmole of DCC. The mixture was kept at 37° for 2 days with exclusion of moisture. To the reaction mixture were added 6 ml. of H_2O and 5 ml. of Dowex-50 resin (cyclohexylammonium form) and the mixture was left at room temperature for 1 hr. After filtration, the residue was washed four times with 5 ml. of H_2O by vigorous shaking until the final ultraviolet absorbing material was eluted. The filtrate and the washings were combined and concentrated to 1 ml. after addition of 0.5 ml. of Dowex-50 resin (cyclohexylammonium form). The concentrate was applied to paper chromatography run with solvent 2 using 5 sheets of Toyo Roshi No. 26 (16×40 cm.). Spaces of the chromatograms corresponding to the Rf_2 value given in the following text were extracted with H_2O containing a small amount of Dowex-50 resin (cyclohexylammonium form). After filtration, the filtrate was lyophilized and dried over P_2O_5 to furnish the cyanophosphorylated product in the yield given in Table II.

Table II. Yields of Cyanoethylphosphates of 5′-Tritylnucleosides

5′-Tritylnucleoside	Cyanoethylphosphate	Yield (%)
Ia	Ib + Ic	67.1
IIa	IIb + IIc	43.7
IIIa	IIIb + IIIc	86.7
IVa	IVb + IVc	74.0
Va	Vb + Vc	73.3

1-(5-Trityl-2(+3)-cyanoethylphosphoro-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (cyclohexylammonium salt) (Ib+Ic): *Anal.* Calcd. for $C_{37}H_{43}O_8N_4P \cdot 4H_2O$: C, 57.40; H, 6.60; N, 7.24; P, 4.03. Found: C, 56.72; H, 6.81; N, 7.68; P, 3.95; Rf_2, 0.85.

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1-(5-Trityl-2(+3)-cyanoethylphosphoro-β-D-ribofuranosyl)-6(1*H*)-pyrimidinone (cyclohexylammonium salt) (IIb+IIc): *Anal.* Calcd. for $C_{37}H_{43}O_8N_4P \cdot \frac{1}{4}H_2O$: C, 63.00; H, 6.16; N, 7.95; P, 4.54. Found: C, 63.09; H, 6.36; N, 7.67; P, 4.48. Rf_2: 0.90.

1-(5-Trityl-2(+3)-cyanoethylphosphoro-D-ribofuranosyl)-2(1*H*)-pyridone (cyclohexylammonium salt) (IIIb+IIIc): *Anal.* Calcd. for $C_{38}H_{44}O_8N_3P \cdot 2H_2O$: C, 61.90; H, 6.55; N, 5.70; P, 4.25. Found: C, 61.37; H, 7.32; N, 5.67; P, 4.08. Rf_2: 0.85.

5'-Trityluridine-2'(+3')-cyanoethylphosphate (cyclohexylammonium salt) (IVb+IVc): *Anal.* Calcd. for $C_{37}H_{43}O_9N_4P \cdot 3H_2O$: C, 57.50; H, 6.35; N, 7.25; P, 4.04. Found: C, 57.06; H, 6.31; N, 7.87; P, 4.00. Rf_2: 0.80. 5'-O-Trityladenosine-2'(+3')-cyanoethylphosphate (cyclohexylammonium salt) (Vb+Vc): *Anal.* Calcd. for $C_{38}H_{43}O_7N_7P \cdot 7H_2O$: C, 52.60; H, 6.69; N, 11.30; P, 3.69. Found: C, 52.22; H, 7.41; N, 11.00; P, 3.50. Rf_2: 0.90.

Uridine-2'(+3')-cyanoethylphosphate (IXb+IXc)——One hundred fifty milligrams of 5'-O-trityluridine-2'(+3')-cyanoethylphosphate (IVb+IVc) was dissolved in 10 ml. of 80% AcOH and the solution was incubated at 50° for 40 min. The mixture was then applied to chromatography to obtain IXb+IXc (Rf_2 0.35) in a yield of 84.4% based on ultraviolet absorption. As side products, uridine 2',3'-cyclic phosphate (Rf_2 0.35) and uridylic acid (Rf_2 0.11) were also isolated and the products were identified with respective authentic specimens.

Uridine-3'-ethylphosphate (X)——In 2 ml. of 50% EtOH-0.2*M* phosphate buffer (pH 7.6), 94.1 mg. of Ba salt of uridine 2',3'-cyclic phosphate and 8.9 mg. of bovine pancreatic RNase were dissolved and the solution incubated at 37° for 125 min. By treatment of the reaction mixture as reported by Barker,[6] uridine 3'-ethylphosphate was obtained in a yield of 35%.

Behaviour of 1-(5-Trityl-2(+3)-cyanoethylphosphoro-β-D-ribofuranosyl)-2(1*H*)-pyrimidine (Ib+Ic) and 1-(5-Trityl-2(+3)-cyanoethylphosphoro-β-D-ribofuranosyl)-6(1*H*)-pyrimidinone (IIb+IIc) to Alkali——Treatment of Ib+Ic with *N* NaOH for 5 min. on boiling water bath in the purpose of removing cyanoethyl group resulted in an irreversible change in ultraviolet absorptions. This indicates occurrence of an irreversible change in the structures of base moieties. On similar treatment of I and II and the corresponding N-methyl compounds, similar changes in ultraviolet absorptions were observed. On keeping I or II with *N* NaOH at 18°, the absorption maxima changed with elapse of time as follows: in the case of I, λ_{max} mμ (time), 303 (0 time) → 315 (5 min.) → 275, 316 (2 hr.) → 273, 316 (3 hr.) → 267 (24 hr.). In the case of II, λ_{max} mμ (time), 218, 270 (0) → 272 (24 hr.). In both cases, remarkable rises in absorption coefficient were observed.

Behaviours of I, II, III and Uridine to Acid Hydrolysis——Ten milligrams of each compound was dissolved in 2*N* HCl solution and kept on a boiling water bath. With the interval of time, aliquots of the solution were withdrawn and neutralized with ammonium carbonate. The hydrolysates were applied to paper chromatography with solvent 1, and the spots corresponding to the starting materials and the hydrolysis products (base) were eluted with H_2O and the eluates were applied to spectrophotometer for quantitative determination. The results were shown in Fig. 2.

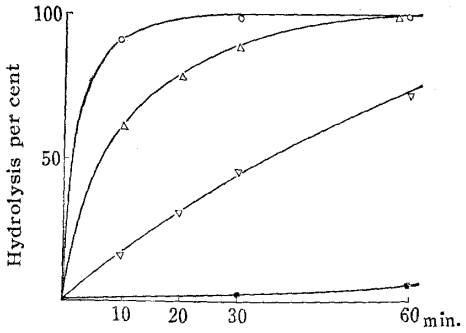

Fig. 2. Hydrolysis Rates of Ribonucleosides (I, II, III) and uridine with 2*N* Hydrochloric Acid at 100°

Each symbol represents respective case of: △: I, ○: II, ▽: III and ●: uridine.

Behaviours of the Nucleoside Phosphodiesters to RNase-A.——Each of the mixed isomers, 2'- and 3'-cyanoethyl phosphates of the 5'-tritylated nucleosides, (Ib+Ic), (IIb+IIc), (IIIb+IIIc), (IVb+IVc), (Vb+Vc), (IXb+IXc) and (X), synthesized above was incubated with RNase-A in 1 ml. of 0.2*M* phosphate buffer (pH 7.6) at 37° in substrate/enzyme mole ratios given in Table III (the amount of the substrates used was 10 mg. or near about). One tenth milliliter of the aliquot was withdrawn at intervals, 0.1 ml. of pyridine was added to break the enzyme activity and submitted to paper chromatography run in solvent 2. No separate spot other than the starting material and the final product was observed on the chromatograms throughout the intervals. The spots of the starting material and the product, (Ic'), (IIc'), (IIIc'), (IVc') and 3'-uridylic acid, were extracted with H_2O and absorbancies at the wave lengths of the each absorption maximum were determined. The results are summarized in Fig. 1.

Table III. Mole Ratio of Substrate and Ribonuclease incubated

Substrate	Ic	IIc	IIIc	IVc	Vc	IXc	X
Mole ratio of substrate/RNase-A	860	770	670	930	19	650	640

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The absorbancies of the products appeared from Ib+Ic, IIb+IIc, IIIb+IIIc, IVb+IVc and Kb+Kc, after 2 days' incubation were 53, 54, 51, 53 and 54%, respectively, of those of the corresponding starting materials. In Fig. 1 yield of the product at intervals was given by per cent of the amount of the product obtained after 2 days' incubation, which was taken as an ultimate value, because as is described below, after 2 days no further hydrolysis was observed when an aqueous extract of the spot remaining at the original Rf_2 was incubated with the enzyme.

In the case of the incubation of IVb+IVc with RNaes-A, for instance, after 2 days, the paper chromatography of the hydrolysate revealed two phosphorus positive and ultraviolet absorbing (at 260 mμ) spots. One had Rf_2 value of 0.80 and another that of 0.54. The extract of Rf_2 0.80 spot gave no additional amount of the product by further incubation with RNase-A, thus the extract must contain only 2'-cyanoethyl phosphate of 5'-trityluridine (IVb), which is not susceptible to the digestion by the enzyme. The product having Rf_2 0.54 must be 3'-phosphate of 5'-trityluridine (IVc'). Alkaline treatment of IVb gave 2'(+3')-phosphate of 5'-trityl uridine which gave Rf_2 0.54 and was chromatographically identified with 5'-trityluridylic acid obtained by alkaline hydrolysis of IVb+IVc. Furthermore, the phosphomonoester enzymatically obtained was converted to uridylic acid by refluxing with 80% AcOH for 20 min. and to 5'-trityl uridine by incubation with alkaline phosphomonoesterase.

The authors are indebted to Takeda Pharmaceutical Industries Co., Ltd. for his gift of the starting materials. Thanks are due to Mr. D. Ohata of the Tokyo Research laboratory, Yoshitomi Pharmaceutical Industries Co., Ltd. for carrying out the microanalyses.

Summary

On condensation of chloromercuri-salt of 2-pyridinol and 2,3,5-tri-O-benzoylribofuranosyl chloride, 2-O-(2,3,5-tri-O-benzoylribofuranosyl)-pyridine (VII) was obtained, the ribosyl group of which migrated to furnish N_1-(2,3,5-tri-O-benzoylribofuranosyl)-2(1*H*)-pyridone (VIII) on treatment with mercuric bromide.

1-β-D-Ribofuranosyl-2(1*H*)-pyridone (III) obtained from VIII by alkaline hydrolysis and two other nucleosides, 1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (I) and -6(1*H*)-pyrimidinone (II) were converted to respective 5'-trityl-3'-cyanoethylphosphates, (Ic), (IIc) and (IIIc), and the property of these nucleotides as substrates of bovine pancreatic ribonuclease was investigated.

The result was that Ic revealed about three fourth activity as substrate of the enzyme compared with 5'-trityl-3'-cyanoethylphosphate of uridine (IVc), but IIc and IIIc were much less active than Ic. The relation between structure of base group of these nucleotides and their properties as substrate of RNase-A was discussed.

(Received March 23, 1964)

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