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122. Morio Ikehara, Tohru Ueda, and Kazuyoshi Ikeda : Studies on Coenzyme Analogs. XI.*1 Synthesis of N-Methyl- and N,N-Dimethylcytidine 5'-Phosphate.

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In the course of the studies on interaction between several phosphatases[1~4] authors obtained some evidences that substitution of amino group of adenine caused marked differences in the hydrolytic nature of 5'-phosphoryl residues. Therefore, particular examination was made as to the C_4-position of the pyrimidine moiety of nucleoside 5'-mono- and -tri-phosphates by substituting NH_2 group of cytidine with $NHCH_3$ and with $N(CH_3)_2$ groups.

The synthesis of N-alkyl substituted cytidine via the route of 4-alkoxy derivatives was reported by two independent workers,[5,6] the former employed the technique of Hilbert-Johnson and the latter used the mercury salt method. The method of Fox *et al.*[7] to thiolate the protected nucleoside was used in this investigation to obtain N-alkyl cytidine in the sufficient yield. In the application of this method for the synthesis of N-alkylated cytidine 5'-phosphate via nucleoside, the method of protection of 5'-OH group had to differ from that of 2'- and 3'-positions for stepwise removal. Another requirement was that both groups should resist P_2S_5 thiolation reaction. From this standpoint 5'-acyl(benzoyl or acetyl), which may be removed by alkaline hydrolysis, and 2',3'-isopropylidene group, which may be hydrolyzed by acidic condition, were employed as the protecting groups. The 5'-acyl group had a further advantage in that it could be removed prior to the methylation of the thiol group and that it, therefore, afforded a suitable intermediate for mild nucleophilic substitution.

The starting compound, 2',3'-O-isopropylideneuridine (II), was obtained in 87% yield by condensation of uridine (I) with acetone in the presence of *p*-toluene sulfonic acid.[8,9] The properties of (II) were almost identical with those described by Levene.[10] The benzoylation of 5'-OH group of (II) was achieved with 90% yield using 1.1~1.5 moles of benzoyl chloride in pyridine at 65~70° for 10 hours.*3 When the resulting compound, 5'-O-benzoyl-2',3'-O-isopropylideneuridine (III), was thiolated in pyridine with P_2S_5 in absolutely anhydrous condition, reddish resinous substance was obtained as the

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*3 Thiolation of 5'-O-acetyl-2',3'-O-isopropylideneuridine yielded a non-crystalline material. The benzoylation by this procedure gave no dibenzoyl derivative as it could be predicted from Fox's study[7] of the benzoylation of uridine.

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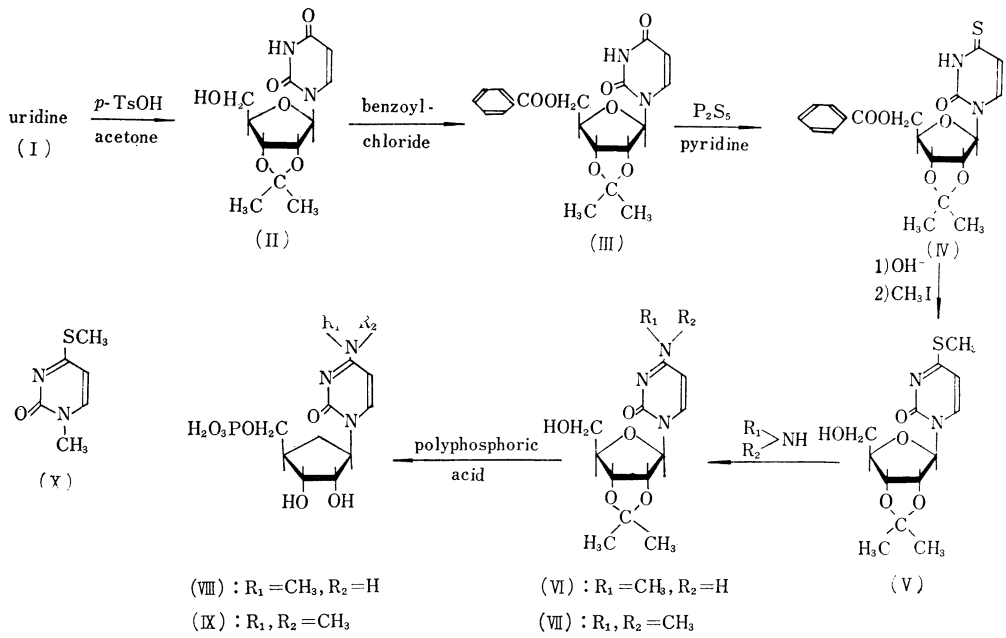

sole product.[11] The relationship between the amount of water added and the yield of product was then studied. As shown in Table I, the formula consisting of 1.0 g. of (III), 2.3 g. of P_2S_5, and 0.57 cc. of water in 50 cc. of pyridine gave the highest yield and afforded most easily purifiable material. The stoichiometric ratios among above reactants could not be determined.

2′,3′-O-Isopropylidene-5′-O-benzoyl-4-thiouridine (IV) was then debenzoylated by dissolving it in dilute alkali at room temperature or slightly warming it to give a translucent solution. Methyliodide was added to either of these solutions for direct methylation. 4-Methylthio derivative (V), m.p. 214°, was obtained in 85~90% yield and its structure was elucidated by its optical behaviors. The ultraviolet absorption spectral curve of (V) had λ_{max} at 301 mμ (shoulder at 270 mμ) in neutral and alkaline media (see Fig. 1) was closely resembled that of 1-methyl-4-methylthio-2-oxo-1,2-dihydropyrimidine[12] (λ_{max} 302 and $\lambda_{shoulder}$ 267 mμ in water) and not of 1,3-dimethyl-4-thiouracil.[13] Moreover in acidic solution λ_{max} of (V) shifted to 329 mμ similar to that of the former compound. From these evidences (V) appeared to be 4-methylthio- rather than 3-methyl-4-thio derivative. The optical behaviors of (V) was also consistent with those

Table I.

Raw material (III) (g.)	P_2S_5 (g.)	Pyridine (cc.)	H_2O (cc.)	Yield of (IV) (mg.)
1.0	2.3	50	0.15	200
1.0	2.3	50	0.25	400
1.0	2.3	50	0.40	600
1.0	2.3	50	0.45	680
1.0	2.3	50	0.50	800
1.0	2.3	50	0.57	810
1.0	2.3	50	0.60	640
1.0	2.3	50	0.70	630
1.0	2·3	50	0.80	620

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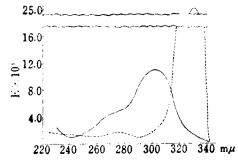

Fig. 1. Absorption Spectra of 2′,3′-O-Isopropylidene-4-methylthiouridine (V)

—— H_2O
---- *N* HCl

of 4-thiomethyl derivative in as much as in acidic solution, λ_{max} at 260~270 mμ and decreasing λ_{max} at 301 mμ.

2′,3′-O-Isopropylidene-4-methylthiouridine (VI) was then allowed to react with methylamine or dimethylamine in absolute alcohol at room temperature. This reaction was effected much more smoothly as expected in the reaction of 4-thio derivative (IV).[7] Monomethyl (VI) and dimethyl (VII) derivatives possessed identical absorption spectra as those of N-methylcytidine[7] and of N,N-dimethylcytosine-β-D-glucoside[14] respectively.

The isopropylidene nucleoside, (VI) and (VII), were then phosphorylated by the polyphosphoric acid method[15,16] with a slight modification. Both 5′-monophosphates were obtained in the form of barium salt of 89.1 and 86% purity as determined by ion-exchanger chromatography. Paper chromatography and paper electrophoresis showed only one spot for each compound (see Table II).

2′,3′-O-Isopropylidene-N,N-dimethylcytidine (VII) 80% m.p. 156°, was derived to N,N-dimethylcytidine, m.p. 155~156°, by acetic acid hydrolysis.[17] Mixed melting point test for these two compounds gave a decreased reading. The paper chromatogram showed that the latter compound had the different Rf values from that of starting material, (VIII) (see Experimatal). These findings, by melting point test and chromatography, in correlation with results obtained in metaperiodate test and optical behavior studies confirmed the structure of N,N-dimethylcytidine.*3

The interaction between these two nucleotides and snake venom 5′-nucleotidase will be reported in another communication.

Table II.

Paper chromatography (Isopropanol-conc. NH_4OH-H_2O; 7:1:3)		
Rf values :	Cytidine 5′-phosphate	0.21
	N-Methylcytidine 5′-phosphate	0.30
	N,N-Dimethylcytidine 5′-phosphate	0.34
Paper electrophoresis : Borate buffer, pH 6.0, subjecting of a potential of 15 volts per cm. (15~17 m. Amp.) for 1.5 hr.		
Mobilities :	Cytidine 5′-phosphate N-Methylcytidine 5′-phosphate N,N-Dimethylcytidine 5′-phosphate	8.5 cm.

Experimental

2′,3′-O-Isopropylideneuridine (II)——30 mg. of *p*-toluenesulfonic acid was dissolved in 300 cc. of Me_2CO and then 3.0 g. of uridine was added during a vigorous stirring of the mixture at room temperature. After 45 min. of reaction time, 60 g. of powdered $NaHCO_3$ was added portionwise and stirred for an additional hour. The precipitate was removed by filtration and washed with hot Me_2CO several times. The filtrate and the washings were combined and evaporated *in vacuo*. The

*4 During this communication was submitted for publication, J. J. Fox, *et al*[18] reported m.p. 158~160° for N,N-dimethylcytidine.

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residue was recrystallized from MeOH or Me_2CO-hexane, m.p. 160°. Mixed m.p. with uridine gave a depressed reading. The yield was 3.0 g. (87%).

2',3'-O-Isopropylidene-5'-O-benzoyluridine (III)——4.0 g. of (II) was dissolved in 60 cc. of dry pyridine. 1.8 cc. of benzoylchloride was added under stirring. Reaction temperature was kept constant from 65° to 70° for 10 hr. Pyridine hydrochloride, thus precipitated, was removed by filtration and discarded. The filtrate was evaporated to a 10 cc. volume under reduced pressure and then poured into ice-water with vigorous stirring to give a white solid. This material was collected by filtration and dried. The filtrate was neutralized with $NaHCO_3$, concentrated *in vacuo* to ca. 200 cc. and kept in an ice-box overnight. The precipitate, thus produced, was collected and dried. Both of the dry material were combined and recrystallized from benzene, m.p. 142°, 5.0 g. (91%). Mixed m.p. with 2',3',5'-tri-O-benzoyluridine showed depression. *Anal.* Calcd. for $C_{19}H_{20}N_2O_7$: C, 59.28; H, 5.19; N, 7.12. Found : C, 59.39; H, 4.92; N, 7.25.

2',3'-O-Isopropylidene-5'-O-benzoyl-4-thiouridine (IV)——1.0 g. of (III) was dissolved in 50 cc. of dry pyridine, to which was added 2.3 g. of P_2S_5 and 0.57 cc. of H_2O. The mixture was refluxed for 6 hr. during which time it was constantly agitated. Upper pyridine-layer was decanted and the lower layer was washed twice with pyridine. The pyridine-layers were then combined and evaporated *in vacuo*. The concentrate was poured into ice-water and extracted with $CHCl_3$. $CHCl_3$ layer was washed twice with H_2O, filtered and then dried over Na_2SO_4. The evaporation of $CHCl_3$ extract under reduced pressure give a solid, which was recrystallized from hot EtOH. 810 mg. (78%) of yellow needles were obtained, m.p. 196°. *Anal.* Calcd. for $C_{19}H_{20}N_2O_6S$: C, 56.42; H, 4.98; N, 6.93. Found: C, 56.40; H, 4.96; N, 6.79.

2',3'-O-Isopropylidene-4-methylthiouridine (V)——2.0 g. of (IV) was added to 30 cc. of 0.5*N* NaOH and the mixture was stirred until it became clear (ca. 30 min. at room temperature or ca. 10 min. at 30°). Into this solution, 700 mg. of methyl iodide and 30 cc. of H_2O were added and stirred again for 1.5 hr. Further addition of 300 mg. of methyl iodide was made and stirring was continued for few hour. This yielded a precipitate which was collected by filtration, washed thoroughly with H_2O and dried. Recrystallization from EtOH furnished white needles, m.p. 214° (1.4 g. 90%). UV : λ_{max} 301 mμ (ε 11,000), λ_{min} 240 mμ, $\lambda_{shoulder}$ 270 mμ in neutral or alkaline solution. $\lambda_{max}^{N\,HCl}$ 329, 273 mμ, $\lambda_{min}^{N\,HCl}$ 288, 245 mμ. *Anal.* Calcd. for $C_{13}H_{18}N_2O_5S$: C, 49.67; H, 5.57; N, 8.91. Found : C, 49.90; H, 5.68; N, 8.84.

2',3'-O-Isopropylidene N-methylcytidine (VI)——700 mg. of (V) was suspended in 30 cc. of dehyd. EtOH and into which methylamine was bubbled at room temperature until the whole solution became clear. After standing overnight, the solvent was removed *in vacuo*, and the glassy residue was recrystallized from Me_2CO-hexane. White crystal, m.p. 161~162°, (500 mg. 80%). UV λ_{max} mμ: 271 (ε 13,400), 237 (H_2O); 281 (0.5*N* HCl). λ_{min} mμ : 248, 227 (H_2O); 241 (0.5*N* HCl). *Anal.* Calcd. for $C_{13}H_{19}N_3O_5$: C, 52.51; H, 6.44; N, 14.13. Found : C, 52.62; H, 6.30; N, 13.91.

2',3'-O-Isopropylidene N,N-dimethylcytidine (VII)——500 mg. of (V) was suspended in 20 cc. of dehyd. EtOH and the mixture was bubbled with dimethylamine at room temperature. Residue obtained by a procedure described for (VI) was recrystallized from EtOH-hexane. White crystal, m.p. 156°(420 mg. 84.8%). UV λ_{max} mμ : 278 (ε 17,500) (H_2O), 285 (0.5*N* HCl); λ_{min} mμ : 238 (H_2O), 245 (0.5*N* HCl). *Anal.* Calcd. for $C_{14}H_{21}N_3O_5$: C, 54.01; H, 6.80; N, 13.50. Found : C, 54.13; H, 6.70; N, 13.37.

N,N-Dimethylcytidine——200 mg. of (VII) was dissolved in 10 cc. of 80% AcOH and heated on a boiling water-bath for 1.5 hr. Concentration and evaporation of AcOH by co-distillation with dehyd. EtOH gave a glassy residue. Trituration with EtOH and recrystallization from EtOH gave white crystal, m.p. 155~156°(140 mg., 80%). Mixed m.p. test with (VII) gave m.p. 135~142°. It consumed metaperiodate and had Rf 0.89 (H_2O adjusted to pH 10) and 0.29 (BuOH-H_2O=84:16). UV : $\lambda_{max}^{H_2O}$ 278 mμ, $\lambda_{min}^{H_2O}$ 238 mμ. *Anal.* Calcd. for $C_{11}H_{17}O_5N_3$: C, 48.70; H, 6.31; N, 15.49. Found : C, 48.73; H, 6.28; N, 15.70.

N-Methylcytidine 5'-phosphate (VIII)——100 mg. of (VI) was mixed thoroughly with 1.3 g. of 85% H_3PO_4 and 1.0 g. of P_2O_5. The mixture was heated at 60° for 2.5 hr. under an anhydrous condition. Then 10 cc. of hot H_2O was added to the mixture which was heated again for 15 min. on water-bath. The entire batch was adjusted to pH 6.5 with hot $Ba(OH)_2$ solution. The resulting precipitate was washed with hot-water. The filtrate and washings were combined and concentrated to 10 cc. under reduced pressure. The pH of the concentrate was adjusted to 7.4 with $Ba(OH)_2$. The filtrate and washings were combined and evaporated to 2.5 cc. *in vacuo*. To the residual liquid was added 2 volumes of EtOH. The precipitate was centrifuged and dried with EtOH-Et_2O. The yield was 50 mg. of white powder. UV λ_{max} mμ : 272, 237 (H_2O); 281 (0.1*N* HCl), λ_{min} mμ : 248, 227 (H_2O); 242 (0.1*N* HCl). *Anal.* Calcd. for $C_{10}H_{14}N_3O_8PBa$: P, 6.55. Found : P, 6.38. Purity analyzed by ion-exchange chromatography (Dowex-1 formate) was 89.1%.

N,N-Dimethylcytidine 5'-phosphate (IX)——200 mg. of (VII) was phosphorylated with 1.3 g. of 85% H_3PO_4 and 1.0 g. of P_2O_5 as described above. White powder, 116 mg., was obtained. UV : $\lambda_{max}^{H_2O}$ 278

mμ, $\lambda_{min}^{H_2O}$ 238 mμ. *Anal.* Calcd. for $C_{11}H_{16}N_3O_8PBr$: P, 6.37. Found : P. 6.08. Analysis by ion-exchange chromatography gave 86% purity containing 12.3% of nucleoside.

1-Methyl-4-methylthiouracil (X)——Uracil was thiolated with P_2S_5 in pyridine. 4-Thiouracil, thus obtained, conformed to original descriptions.[19] It was methylated by Wheeler's method.[11] 1.0 g. of 4-Thiouracil was dissolved in 10 cc. of dehyd. EtOH, containing 500 mg. of Na, followed by the addition of 2.8 g. of CH_3I. Solidified reaction mixture was dissolved in 20 cc. of dehyd. EtOH and heated. Then 1.0 g. of CH_3I was added to the solution which was heated again for 2 hr. The clear reaction mixture was evaporated to dryness *in vacuo* and the residue was extracted three times with 50 cc. of $CHCl_3$. The evaporation of the combined extracts gave a solid which was dissolved in EtOH, treated with charcoal and recrystallized from benzene. White crystal, m.p. 123～124° (900 mg.). UV : $\lambda_{max}^{H_2O}$ 302, $\lambda_{shoulder}^{H_2O}$ 267 mμ. *Anal.* Calcd. for $C_6H_8N_2OS$: C, 46.13; H, 5.16; N, 17.93. Found : C, 45.91; H, 5.15; N, 18.09.

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Summary

Uridine was converted to 2′,3′-O-isopropylidene-5′-O-benzoyluridine and derived to 2′,3′-O-isopropylidene-4-methylthiouridine by successive thiolation and methylation. The latter compound was reacted with either CH_3NH_2 or $(CH_3)_2NH$ to produce 2′,3′-O-isopropylidene-N-methyl- or N,N-dimethylcytidine respectively. N-Methyl- and N,N-dimethylcytidine 5′-phosphate were synthesized by the poly-phosphoric acid method followed by the acidic removal of isopropylidene groups.

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123. Yuriko Kato : Formation of a Micelle-like Structure in Aqueous Solution of Glycols.*2

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Behavior of ionic surfactants in aqueous solution has been reported by McBain[1] and numerous workers, who agreed on the following point : A surfactant in a low concentration dissolves in water by monomolecular dispersion but, in a higher concentration beyond the critical micelle concentration, the surfactant molecules aggregate to form an associated body generally called a micelle. At this concentration, various properties of the solution undergo a drastic change. Only fragmentary reports are available for nonionic surfactants, such as those by Gonik and McBain,[2] and by Goto,

*1 Hongo, Tokyo (加藤百合子).

*2 Paper presented at the Tokyo Local Meeting of the Pharmaceutical Society of Japan, January, 1961.

1) J.W. McBain : "Colloid Science," 240 (1950). D.C. Heath & Co., Boston.

2) E. Gonick, J.W. McBain : J. Am. Chem. Soc., **69**, 334 (1947).