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Alkylated Pyrimidine Derivatives as Antiviral Agents. I. Syntheses and Antiviral Screening of Alkylpyrimidine and 5-Alkyluracil Nucleoside

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In order to find the effective antiviral agents, 5-alkyluracil, 3-alkyluracil, 5-alkylisocytosine, 5-alkyl-6-methylisocytosine, 3-alkyl-6-methyluracil, 5-alkyluracil-1-β-D-glucopyranoside and 5-alkyluracil-1-β-D-ribofuranoside were synthesized and screened as to their antiviral effect on both RNA and DNA containing viruses. For RNA viruses, type-1 Mahoney strain of polio virus and K-2211 strain of ECHO-28 virus were used. For DNA viruses, type-1 and type-12 strains of adeno virus and DV-96 strain of Vaccinia virus were used. Among these tested compounds, 5-butyluracil and 5-butyluracil-1-β-D-ribofuranoside were found to possess effect on both RNA and DNA viruses. Especially, the latter was more effective than the former. Moreover, the latter exerted a broader antiviral spectrum than that of 5-fluorodeoxyuridine.

It was reported by Salzman[2a] that 5-fluorodeoxyuridine (FUDR) inhibited the multiplication of vaccinia virus, but not that of polio virus. Ginsberg, *et al.*[2b] Simmons[2c] and Green, *et al.*[2d] also described that FUDR inhibited the growth of adeno virus. On the other hand, Seto, Toyoshima and Ueda[3] showed by using fluorescent antibody technique that the effect of FUDR on adeno virus was due to only a partial inhibition of the early step of viral multiplication, but not to a complete inhibition. It was shown by Kaufman[4] that FUDR inhibits thymidylic phosphorylase, and iododeoxyuridine, thymidylic phosphorylase and the specific DNA polymerase necessary to the synthesis of viral DNA.

On the basis of these findings, it is of interest to search for antiviral agents by preparing pyrimidine having alkyl group in lieu of halogen atom and screening as to its effect on several viruses. For this purpose, compounds of 5-alkyluracil, 3-alkyluracil, 5-alkylisocytosine, 5-alkyl-6-methylisocytosine and 3-alkyl-6-methyluracil were examined as to their effect of type-1 Mahoney strain of polio virus and K-2211 strain of ECHO virus as RNA viruses, and type-1 and type-12 strains of adeno virus and DV-96 strain of vaccinia virus as DNA viruses.

This paper is concerned with the synthesis and antiviral screening of alkylated pyrimidine and its related compounds.

Syntheses of Alkylated Uracil and Its Related Compounds

The derivatives of 5-alkyluracil were synthesized by the condensation of α-formylalkanoate prepared from ethylformate and methylester of fatty acid with thiourea in anhydrous ethereal solution, and treating the resulting 2-thio-5-alkyluracil with 10% aqueous solution of chloroacetic acid.

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3) Y. Seto, S. Toyoshima and T. Ueda, *Nature*, **201**, 219 (1962).

4) H.E. Kaufman, *Chemotherapia*, **7**, 1 (1963).

The derivatives of 5-alkylisocytosine were prepared by the condensation of α-formylalkanoate with guanidine in anhydrous ethereal solution.

The derivatives of 5-alkyl-6-methylisocytosine were synthesized from the corresponding ethylalkylacetoacetate and guanidine carbonate.

The derivatives of 3-alkyluracil were synthesized, according to the method of Whitehead[5] by the condensation of N-alkylurea (I) and diethyl ethoxymethylenemalonate (II) to form diethyl alkylureidomethylene malonate (III), which was cyclized with sodium ethoxide without the isolation, and by the hydrolysis of the resulting 3-alkyl-5-carboethoxyuracil (IV) under the decarboxylation with heating.

To prepare 3-alkyl-6-methyluracil, according to the synthetic method of Senda,[6] N-alkylurea (I) and ethylacetoacetate were reacted over sulfuric acid in a vacuum desiccator until the mixture became powdered, and the resulting ethyl 3-(3-alkylureido)crotonate (VI) was dissolved in hot aqueous solution of sodium hydroxide and the solution was acidified with hydrochloric acid to afford 3-alkyl-6-methyluracil (VII).

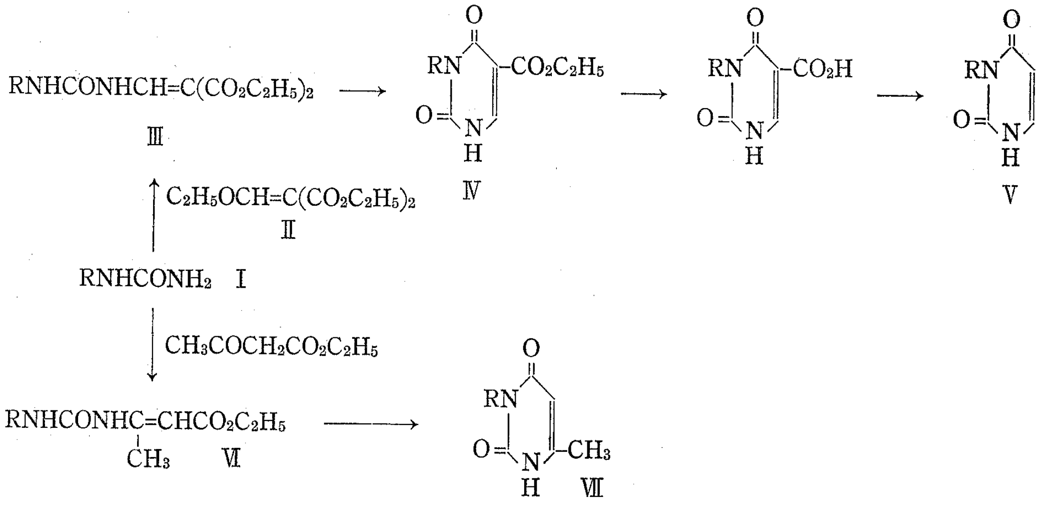

Chart 1

Syntheses of 5-Alkyluracil-1-β-D-glucopyranoside and 5-Alkyluracil-1-β-D-ribofuranoside

5-Alkyluracil-1-β-D-glucopyranoside and 5-alkyluracil-1-β-D-ribofuranoside were synthesized to potentiate the 5-alkyluracil by the formation of nucleoside. According to the synthetic method of Fox, *et al.*[7] 5-alkyluracil-1-β-D-glucopyranoside (XIII) and 5-alkyluracil-1-β-D-ribofuranoside (XIV) were prepared by the condensation of the mercury salts of 5-alkyluracil (VIII) with the corresponding tetra-O-acetyl-α-D-glucopyranosyl bromide (IX). The succeeding deacetylation and debenzoylation of 5-alkyluracil-1-tetra-O-acetyl-β-D-glucopyranoside (XI) and 5-alkyluracil-1-tri-O-benzoyl-β-D-ribofuranoside (XII) with methanolic ammonia or ethanolic hydrochloric acid by the method of Fox[7] were observed difficult, because of the imcompleteness of the reactions and the decomposition of the formed nucleosides. It was comfirmed by the authors that the deacetylation as well as the debenzoylation of XI and XII with sodium methoxide gave a good result to afford the desired nucleoside.

The characterization of the compounds synthesized were conducted through the elemental analysis and infrared absorption properties.

The properties of the compounds are summerized in Table I.

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[5] C.W. Whitehead, *J. Am. Chem. Soc.*, **74**, 4267 (1952).
[6] S. Senda and A. Suzui, *Chem. Pharm. Bull.* (Tokyo), **6**, 476 (1958).
[7] J.J. Fox, N. Yung, J. Davoll and G.B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

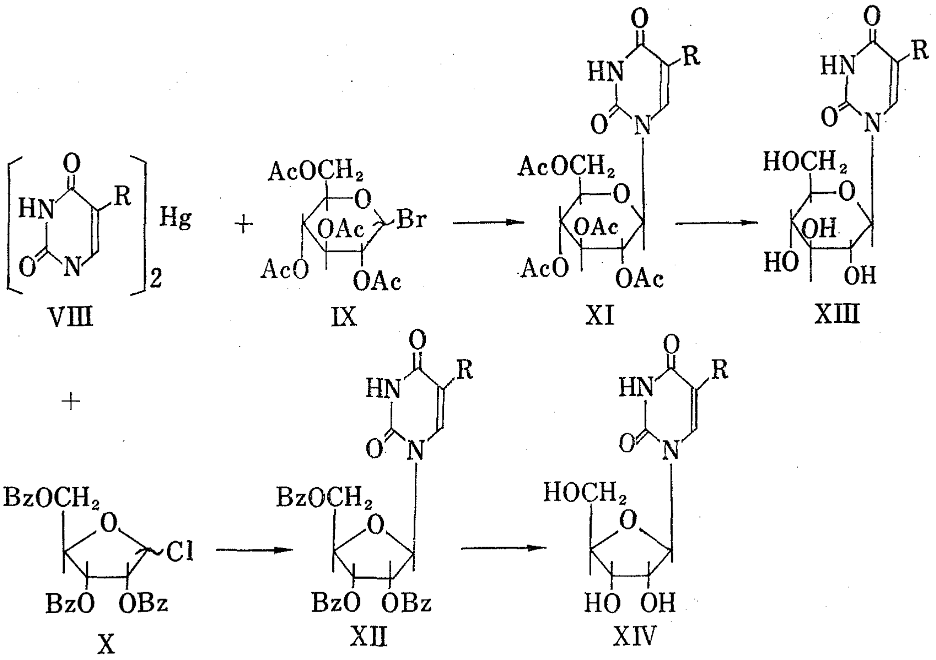

Chart 2

Table I. Alkylated Pyrimidine

	R	Appearance (recryst. solvt.)	mp (°C)	Formula	Analysis N (%) Calcd.	Found
Uracil	5-CH_3-	needles (MeOH)	315[a]	$C_5H_6O_2N_2$	22.22	20.20
	5-C_2H_5-	needles (MeOH)	300[b][c]	$C_6H_8O_2N_2$	19.99	19.87
	5-C_3H_7-	needles (MeOH)	292[c]	$C_7H_{10}O_2N_2$	18.17	17.93
	5-C_4H_9-	needles (MeOH)	291[c]	$C_8H_{12}O_2N_2$	16.66	16.44
	5-C_6H_{13}-	plates (MeOH)	273—274[c]	$C_{10}H_{16}O_2N_2$	14.28	14.31
	3-CH_3-	needles (EtOH)	176[d]	$C_5H_6O_2N_2$	22.22	22.06
	3-C_2H_5-	needles (EtOH)	173[e]	$C_6H_8O_2N_2$	19.99	20.07
	3-C_4H_9-	needles (EtOH)	147	$C_8H_{12}O_2N_2$	16.66	16.38
	3-C_6H_5-	needles (H_2O)	242[f]	$C_{10}H_8O_2N_2$	14.89	14.43
	3,6-Di-CH_3-	needles (H_2O)	260[g]	$C_6H_8O_2N_2$	19.99	19.68
	3-C_2H_5-6-CH_3-	needles (H_2O)	196[h]	$C_7H_{10}O_2N_2$	18.17	18.28
	3-C_3H_7-6-CH_3-	plates (H_2O)	185[i]	$C_8H_{12}O_2N_2$	16.66	16.79
	3-C_4H_9-6-CH_3-	plates (H_2O)	177[j]	$C_9H_{14}O_2N_2$	15.37	15.56
Isocytosine	5-CH_3-	needles (H_2O)	272—274[a]	$C_5H_7ON_3$	33.58	33.38
	5-C_2H_5-	needles (H_2O)	227—228	$C_6H_9ON_3$	30.20	30.20
	5-C_3H_7-	needles (H_2O)	238—239	$C_7H_{11}ON_3$	27.43	27.43
	5-C_4H_9-	needles (EtOH)	188—189	$C_8H_{13}ON_3$	25.13	25.19
	5-C_6H_{13}-	needles (EtOH)	207—208	$C_{10}H_{17}ON_3$	21.52	21.55
	5,6-Di-CH_3-	needles (EtOH)	303—306[k]	$C_6H_9ON_3$	30.20	30.10
	5-C_2H_5-6-CH_3-	needles (EtOH)	280—283	$C_7H_{11}ON_3$	27.43	27.37
	5-C_3H_7-6-CH_3-	needles (EtOH)	268—270	$C_8H_{13}ON_3$	25.13	25.43
	5-C_4H_9-6-CH_3-	needles (DMF)	237—239	$C_9H_{15}ON_3$	23.19	23.35
	5-C_6H_{13}-6-CH_3-	plates (DMF)	214—217	$C_{11}H_{19}ON_3$	20.08	20.05
	5-C_8H_{17}-6-CH_3-	plates (DMF)	178—180	$C_{13}H_{23}ON_3$	17.71	17.79

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a) H.W. Scherp, *J. Am. Chem. Soc.*, **68**, 912 (1946). Reported: 5-methyluracil, mp 314—315°; 5-methylisocytosine, mp 277—279°.

b) T.B. Johnson, *J. Biol. Chem.*, **2**, 111 (1906). mp 300°.

c) J.H. Burckhalter and H.C. Scaborough, *J. Am. Pharm. Assoc.*, **9**, 546 (1955). Reported: 5-ethyluracil, mp 300—303°; 5-propyluracil, mp 296—298°; 5-butyluracil, mp 291—293°; 5-hexyluracil, mp 272—273°.

d) lit. 5) mp 174—175°

e) T.B. Johnson and F.W. Heyl, *Am. Chem. J.*, **37**, 628 (1907). mp 173—174°

f) lit. 5) mp 244—246°

g) lit. 6) mp 262°

h) lit. 6) mp 193°

i) lit. 6) mp 183—184°

j) lit. 6) mp 176—177°

k) W. Baker, E.J. Prribyl, J.T. Sheeban,E.R. Spitamiller and W.A. Lott, *J. Am. Chem. Soc.*, **69**, 3072 (1947). mp 330°

Antiviral Effect of the Compounds Synthesized

The antiviral effect of the compounds synthesized were surveyed by the tissue cultures with type-1 Mahoney strain of polio virus, type-1 strain of adeno virus and type-12 strain of adeno virus.

The experimental results are shown in Table II.

Table II. Effect of Alkylpyrimidine Derivatives on Adeno Virus Type-12

Compound	Dose (M)	Antiviral effect	Control
5-CH_3-Uracil	5×10^{-3}	2[a]	3
5-C_2H_5-Uracil	5×10^{-4}	1	
5-C_3H_7-Uracil	5×10^{-4}	2	
5-C_4H_9-Uracil	5×10^{-4}	0.5	
5-C_6H_{13}-Uracil	5×10^{-4}	3	
3-CH_3-Uracil	10^{-4}	3	
3-C_2H_5-Uracil	10^{-4}	2.5	
3-C_4H_9-Uracil	10^{-4}	2	
3-C_6H_5-Uracil	10^{-4}	2.5	
3-CH_3-6-CH_3-Uracil	10^{-4}	3	
3-C_2H_5-6-CH_3-Uracil	10^{-4}	3	
3-C_3H_7-6-CH_3-Uracil	10^{-4}	3	
3-C_4H_9-6-CH_3-Uracil	10^{-5}	3	
5-CH_3-Isocytosine	10^{-3}	3.5	3
5-C_2H_5-Isocytosine	10^{-4}	3	
5-C_3H_7-Isocytosine	10^{-4}	3	
5-C_4H_9-Isocytosine	10^{-4}	3	
5-C_6H_{13}-Isocytosine	10^{-5}	3	
5-C_8H_{17}-Isocytosine	10^{-5}	3	
5-CH_3-6-CH_3-Isocytosine	10^{-3}	3	
5-C_2H_5-6-CH_3-Isocytosine	10^{-3}	3	
5-C_3H_7-6-CH_3-Isocytosine	10^{-4}	3	
5-C_4H_9-6-CH_3-Isocytosine	10^{-4}	3	
5-C_6H_{13}-6-CH_3-Isocytosine	10^{-4}	2.5	
5-C_8H_{17}-6-CH_3-Isocytosine	10^{-4}	3	
EUG	5×10^{-4}	1.5	3
EUR	5×10^{-4}	2	
BUR	5×10^{-4}	1.5	

a) CPE score: 0=0% CPE, 1=50% CPE, 2=75% CPE, 3=100% CPE, 4=strip down from glass surface

None of the compounds showed outstanding activity on the Mahoney strain of polio virus and the type-1 strain of adeno virus, and therefore no experimental results are reported here. The data presented in Table II indicate that 5-ethyluracil (EU), 5-butyluracil (BU), 5-ethyluracil-1-β-D-glucopyranoside (EUG) and 5-butyluracil-1-β-D-ribofuranoside (BUR) were effective on the type-12 strain of adeno virus among the compounds.

Next, the antiviral effect of EU, BU, EUG and BUR was examined more precisely on the type-1 strain of adeno virus, the DV-96 strain of vaccinia virus, the type-1 strain of polio virus and the K-2211 strain of ECHO-28 virus in tissue culture system.

The date obtained in the test of antiviral effect are shown in Table III.

As can be seen in the Table III, both of BU and BUR showed significant effect not only on the DNA viruses but also on the RNA viruses, especially on the K-2211 strain. Moreover, it may be said that the riboside of 5-butyluracil exerted the stronger effect than the free base on the viruses.

Table III. Effect of EU, BU, EUG and BUR on DNA containing Type-1 Strain of Adeno Virus and DV-96 Strain of Vaccinia Virus and RNA containing Mahoney Strain of Polio Virus and K-2211 Strain of ECHO-28 Virus

Compound	DNA virus				RNA virus			
	Type-1 Adeno virus		DV-96 Vaccinia virus		Mahoney Polio virus		K-2211 ECHO-28 virus	
	$-\log_{10}$ TCD_{50}	Inhibition %	$-\log_{10}$ TCD_{50}	Inhibition %	PFU	Inhibition %	PFU	Inhibition %
Control	4.0		7.5		2.9×10^{7}		1.4×10^{5}	
EU	4.5				3.0×10^{7}		1.6×10^{4}	88
BU	3.5	50			2.0×10^{7}	31	2.0×10^{4}	85.7
EUG	4.5				4.6×10^{7}		7.3×10^{4}	47.7
BUR	2.5	95	5.5	99	1.6×10^{7}	44.8	1.0×10^{5}	92.8

A comparison of the effect of BUR with that of FUDR on the Mahoney strain of polio virus, the type-1 strain of adeno virus and the DV-96 strain of vaccinia virus is presented in Table IV.

Table IV. Effect of 5-Fluorodeoxyuridine (FUDR) and 5-Butyluridine (BUR)

Compound	Mahoney Polio virus PFU inhibition %[a]	DV-96 Vaccinia virus	Type-1 Adeno virus
		$\Delta TCID_{50}$[b]	
FUDR	23.8	4	0
BUR	46.2	20	1.5

a) $\text{PFU inhibition }\% = \frac{\text{PFU control} - \text{PFU treated}}{\text{PFU control}} \times 100$

b) $\Delta TCID_{50} = -\log_{10}TCID_{50}\text{ control} - -\log_{10}TCID_{50}\text{ treated}$

It is evident from the table that BUR showed the inhibitory effect on all of the three viruses employed, while FUDR exerted the inhibition on the vaccinia virus but not on the other two viruses.

Discussion

Among pyrimidine derivatives, iododeoxyuridine and cytosine arabinoside are of interest as the structure for making more effective antiviral agents, because these compounds are, in practice, used topically to conjunctiva. The authors thereupon, conceived an idea to make antiviral agents by replacing halogen atom with alkyl group. From this view point, compounds of 3-alkyluracil, 5-alkyluracil, 5-alkylisocytosine, 5-alkyl-6-methylisocytosine and 3-alkyl-6-methyluracil were synthesized as described above.

As described in the experimental parts with antiviral screening, 5-ethyluracil and 5-butyluracil were found to have an effect on the type-1 strain of adeno virus and the DV-96 strain of vaccinia virus belonging to DNA viruses, and the type-1 strain of polio virus and the K-2211 strain of ECHO virus belonging to RNA viruses. These two agents were also effective on the type-12 strain of adeno virus.

Next, these effective agents were conceived to potentiate by combining with sugars to form nucleosides according to the improved method of Fox, *et al.*[7] and examined as to their effect on the viruses.

Among these nucleosides, 5-butyluridine was the most effective on the four kinds of viruses. The effectiveness of this agent was also found predominant over 5-butyluracil and 5-fluorodeoxyuridine. The antiviral spectrum of 5-butyluridine coincided with that of 5-butyluridine.

Compared with 5-fluorodeoxyuridine, 5-butyluridine had a broader spectrum, because the former was found inhibitory against only the vaccinia virus, while the latter, effective on both the RNA and DNA viruses. Thus, 5-butyluridine is of interest as an antiviral agent having a broad spectrum.

The mode of antiviral action of this agent is of interest. It is suggested by Kaufman[4] that the antiviral action of halodeoxyuridine should be ultimately due to the interference with viral DNA synthesis.

This speculation, however, may not pass extensively for the action of 5-butyluridine, since it inhibits the multiplication of both RNA and DNA viruses. The problems on the mode of action of 5-butyluracil and 5-butyluridine will be described in the next report.

Experimental

General Procedure for Synthesis of 5-Alkyluracil——a) 2-Thio-5-alkyluracil: To a suspension of sodium (8 g) in 75 ml of dry ether, a solution of methylester of alkanoic acid (0.43 mole) and ethylformate (0.58 mole) in 50 ml of dry ether was added dropwise at 0°, the mixture was stirred at 0° for 30 hr and at 25° forther 12 hr. After that, the reaction mixture was distilled off under reduced pressure without heating to remove ether and unreacted ester. Then to the residue, a solution of thiourea (0.22 mole) in 125 ml of abs. EtOH was added and a mixture was refluxed with stirring for 7 hr. After EtOH was removed by distillation, the residue was dissolved in H_2O (125 ml) and this solution was washed with ether. The solution was acidified with HCl. The resinous precipitate was collected and recrystallized from EtOH.

b) 5-Alkyluracil: A suspension of 2-thio-5-alkyluracil (0.01 mole) in 10% of aq. chloroacetic acid (19 ml) was refluxed for 6 hr. After cooling, the crude 5-alkyluracil was recrystallized from MeOH.

General Procedure for Synthesis of 5-Alkylisocytosine——According to the synthetic procedure for 2-thio-5-alkyluracil, the corresponding α-formylalkanoate was reacted with guanidine instead of thiourea. The treatment after this reaction were the same as for 2-thio-5-alkyluracil. These compounds having alkyl chain of C_1–C_3 were recrystallized from H_2O and the higher compounds of C_4–C_8 were recrystallized from EtOH.

General Procedure for Synthesis of 5-Alkyl-6-methylisocytosine——A mixture of guanidine carbonate (0.03 mole) and ethylacetoacetate (0.03 mole) was heated at 140—160° for 17 hr. After cooling, the reaction mixture was washed with ether dissolved with 10% NaOH and the acidified with AcOH. The precipitate was recrystallized from EtOH or N,N-dimethylformamide (DMF).

General Procedure for Synthesis of 3-Alkyluracil——a) 3-Alkyl-5-carboxyuracil: A mixture of N-alkylurea (0.05 mole) and diethyl ethoxymethylen malonate (0.05 mole) was heated at 120° for 24 hr. The syrupy mixture was dissolved in ethanolic EtONa (prepared from 0.05 atom of Na and 100 ml of abs. EtOH) and allowed to stand overnight at room temperature. After EtOH was distilled off under reduced pressure without heating, the residue was dissolved in ice water and acidified with 10% HCl. The precipitate was filtered and recrystallized from EtOH.

b) 3-Alkyl-5-carboxyuracil: A solution of 3-alkyl-5-carboethoxyuracil (0.01 mole) in 10% of NaOH (5 ml) was refluxed for 2 hr. After cooling, the mixture was acidified with 10% HCl. A precipitate was recrystallized from EtOH.

c) 3-Alkyluracil: 3-Alkyl-5-carboxyuracil (500 mg) was heated at 255° for 15 min. After cooling the solid was recrystallized from EtOH.

General Procedure for Synthesis of 3-Alkyl-6-methyluracil——A mixture of ethyl acetate (0.05 mole), abs. EtOH (1.2 ml), 1 drop of conc. HCl and N-alkylurea (0.05 mole) was reacted over conc. H_2SO_4 in a vacuum desiccator for 6 to 7 days until the mixture became powdered. The crude ethyl 3-(3-alkylureido)-crotonate (VI) was dissolved in hot 10% NaOH and acidified with HCl. The precipitate was collected and washed with H_2O, EtOH and ether and recrystallized from H_2O.

General Procedure for Synthesis of Di-5-alkyluracilmercury——To a solution of 5-alkyluracil (0.10 mole) in 400 ml of hot H_2O containing 4.0 g of NaOH was added an alcoholic solution of 13.5 g (0.005 mole) of mercuric chloride. After cooling, the white amorphous precipitate was filtrated and washed with H_2O, EtOH and ether. Di-5-ethyluracilmercury. *Anal.* Calcd. for $C_{12}H_{14}O_4N_4Hg$: N, 11.69. Found: N, 11.65: Di-5-butyluracilmercury. *Anal.* Calcd. for $C_{16}H_{22}O_4N_4Hg$: N, 10.47. Found: N, 10.50.

1-(Tetra-O-acetyl-β-D-glucopyranosyl)-5-ethyluracil——To 150 ml of dry toluene was added di-5-ethyluracilmercury (0.01 mole). The suspension was azeotropically dried by distillation of approximately one-fourth of the solvent under vigorous stirring. Tetra-O-acetyl-α-D-glucopyranosyl bromide (8.2 g, 0.02 mole) was added to the stirred suspension and refluxed for 2 hr. The warm turbid solution was filtered from unreacted material. After cooling, the filtrate was treated with petr. ether. The precipitate was collected and dissolved in 30 ml of $CHCl_3$. The $CHCl_3$ solution was washed with 30% KI solution, H_2O, successively, and dries over Na_2SO_4. After evaporation of $CHCl_3$ the residual sirup was dissolved in 100 ml of warm MeOH and the solvent removed under vacuum. MeOH addition and removal were repeated three times. The hygroscopic product was used to the next step without recrystallization.

1-β-D-Glucopyranosyl-5-ethyluracil——To a solution of uncrystallized 1-(tetra-O-acetyl-β-D-glucopyranosyl)-5-ethyluracil (2 mmole) in abs. MeOH (20 ml) was added methanolic MeONa (prepared from 4 mg atom of Na and 20 ml of abs. MeOH) at 0°. After 2 hr at 0°, the reaction mixture was diluted with an equal volume of H_2O and was passed through Dowex-50 W-X8 (H^+) column. The column was washed with 80% aq. MeOH and the eluate was concentrated *in vacuo*. The residue was azeotropically dried with 5 ml of benzene for several times until white crystals was obtained. The crystallin material was washed well with ether and then recrystallized from EtOH–ether mp 213—215°. *Anal.* Calcd. for $C_{12}H_{18}O_7N_2$: C, 47.68; H, 6.00; N, 9.27. Found: C, 47.59; H, 6.18; N, 9.27.

General Procedure for Synthesis of 1-(Tri-O-benzoyl-β-D-ribofuranosyl)-5-alkyluracil——To 200 ml of anhyd. ether was added 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose (0.02 mole), and the solution was saturated with HCl at 0°. After 6 days keeping at 3—5°, the solvent was removed *in vacuo* and the residual sirup was dried three times by azeotropical distillation with 50 ml of anhyd. benzene *in vacuo*. A benzene solution (30 ml) of the halogenose (2,3,5-tri-O-benzoyl-D-ribosylchloride) was added to an azeotropically dried suspension of 0.01 mole of di-5-alkyluracilmercury in hot xylene. After refluxing for 1 hr, the hot reaction mixture was filtered from the insoluble material. After cooling, the filtrate was treated with petr. ether. The white precipitate that occurred was dissolved in $CHCl_3$ and treated in a similar manner to that used in the preparation of 1-(tetra-O-acetyl-β-D-glucopyranosyl)-5-ethyluracil. Removal of solvent *in vacuo* left a viscous sirup which was dissolved in 15 ml of hot AcOEt and petr. ether was added to incipient cloudiness. The precipitate was collected and washed with ether and recrystallized from AcOEt.

1-(2,3,5-Tri-O-benzoyl-D-ribofuranosyl)-5-ethyluracil: colorless needles, mp 154—155°. *Anal.* Calcd. for $C_{32}H_{28}O_9N_2$: C, 65.75; H, 4.83; N, 4.79. Found: C, 65.42; H, 4.75; N, 5.05.

1-(2,3,5-Tri-O-benzoyl-D-ribofuranosyl)-5-butyluracil: colorless needles, mp 157—158°. *Anal.* Calcd. for $C_{34}H_{32}O_9N_2$: C, 66.66; H, 5.27; N, 4.57. Found: C, 66.35; H, 5.46; N, 4.58.

General Procedure for Synthesis of 1-β-D-Ribofuranosyl-5-alkyluracil——A mixture of 1-(2,3,5-tri-O-benzoyl-D-ribofuranosyl)-5-alkyluracil (1 mmole) in abs. MeOH (5 ml) and MeONa (4 mmole) was refluxed gently for 2 hr. After cooling, the reaction mixture was diluted with an equal volume of H_2O and was passed through Dowex-50 W-X8 (H^+) column and treated in a similar manner to that used in the preparation of 1-β-D-glucopyranosyl-5-ethyluracil. Then the obtained material was recrystallized from EtOH.

1-β-D-Ribofuranosyl-5-ethyluracil: colorless plates, mp 158—159°. *Anal.* Calcd. for $C_{11}H_{16}O_6N_2$: C, 48.52; H, 5.92;N, 10.26. Found: C, 48.79; H, 5.78; N, 10.28.

1-β-D-Ribofuranosyl-5-butyluracil: colorless plates, mp 161—163°. *Anal.* Calcd. for $C_{13}H_{20}O_6N_2$: C, 51.99; H, 6.71; N, 9.33. Found: C, 51.58; H, 6.59; N, 9.34.

Screening as to the Effect of 5-Alkyluracil, 3-Alkyluracil, 3-Alkyl-6-methyluracil, 5-Alkylisocytosine, 5-Alkyl-6-methylisocytosine, 1-β-D-Glucopyranosyl-5-ethyluracil, 1-β-D-Ribofranosyl-5-ethyluracil and 1-β-D-Ribofranosyl-5-butyluracil——A) Viral Materials: The following viruses were employed for the experiments: adeno viruses (type-1 and type-12 strain), polio virus (type-1 Mahoney strain), ECHO-28 virus (K-2211 strain) and Vaccinia virus (DV-96 strain).

B) Host cell: Hep. No. 2 cells were employed.

C) Media: As the growth medium, the YLA medium supplemented with 15% bovine serum was employed and as the maintenance medium, the YLA medium supplemented with 5% bovine serum of 5% horse serum and the Eagle's medium supplemented with 15% horse serum were used.

D) General Procedure for the 1st Screening Test: 2×10^5 cells/ml of the Hep. No. 2 cells were added into tubes, and then these tubes were incubated at 37° for 3 or 4 days to obtain the mono sheet. After the establishment of it, the growth medium was removed from these tubes and the cell–sheet was washed three times with phosphate buffered saline (PBS) (pH 7.6) and then used for the experiments. Immediatly after 0.8 ml of the maintenance medium and 0.1 ml of the solution of a tested compound were added into each tube, 0.1 ml of the viral dilution was inoculated into the tube. Prior to the antiviral test of each compound the maximum non-toxic dose of the compound on the Hep. No. 2 cell was determined, and this dose was employed for antiviral test. After viral inoculation, these test tubes were incubated at 37° for 7 days and the effectiveness was determined from the daily microscopic examination of the cytopathogenic effect produced by the virus.

For the 1st screening test, adeno type-1, type-12 viruses and polio virus type-1 Mahoney strain were used.

The inoculum size of each viral material and the observation days to evaluate the effectiveness of a tested compound were as follows:

1) Adeno virus type-1: The viral inoculum size employed was $10\times TCD_{50}$ ($TCD_{50}=10^{-3.5}$/ml). The other experimental procedures were the same to those in D. After the observation for 7 days, the effectiveness

was determined. 2) Adeno virus type-12: The viral inoculum size employed was $10 \times TCD_{50}$ ($TCD_{50}=10^{-2.5}$/ml). The other experimental procedures were the same as the described in D. After the observation for 8 days, the effectiveness of a tested compound was evaluated. 3) Polio virus type-1 Mahoney strain: $100 \times TCD_{50}$ of the Mahoney strain was inoculated and the all tubes were incubated at 37° for 7 days.

E) Experimental Procedures for the 2nd Screening Test: For the 2nd screening test, the two RNA containing viruses, polio virus (type-1 Mahoney strain) and ECHO-28 virus (K-2211 strain) and the two DNA containing viruses, vaccinia virus (DV-96 strain) and adeno virus type-1 were employed. For the evaluation of the inhibitory effect of a tested compound toward RNA containing viruses plaque assay technique was employed, and the assay of the effect on DNA containing viruses was carried out by using the $TCID_{50}$ (Tissue Culture Infective Dosis 50) estimating dilution method.

1) Plapue Assay.Technique for the Assay of the Effect on a Tested Compound on either of Polio and ECHO Viruses: In plaque assay technique, a small bottle was used for the culture. After the establishment of the mono-sheet of the Hep. No. 2 cell, the cell sheet was washed three times with PBS. The viral material was introdused into the bottle and the bottle was incubated for one hr. In this procedure, the temperature of incubation were 37° for polio virus and 33° for ECHO-28 virus. Then, the agar overlay medium was added in which each of the maximum non-toxic concentration of the tested compound solution was added. In the control group, the same amount of PBS as that of the tested compound solution was added to the agar overlay medium. After standing at 22° for 15 min, these bottles were incubated. After incubation, at 37° for 4 days in polio virus experiments and at 33° for 7 days in ECHO-28 virus experiments, PFU of both of the control and the treated groups were calculated.

2) $TCID_{50}$ Estimating Dilution Method for the Assay of the Effect of a Tested Compound on DNA Containing Virus. After the establishment of the mono sheet of Hep. No. 2 cells, the growth medium was removed from each tube, and the cell-sheet was washed with PBS (pH 7.4). Immediately later, 0.8 ml of the maintenance medium, the YLA medium supplemented with 5% horse serum for the cultivation of adeno virus and the same basal medium added with 5% bovine serum for vaccinia virus, and 0.1 ml of 10 maximum non-toxic concentration of a tested compound were added into each tube of the treated group. To the tubes of the control group, 0.1 ml of PBS was added instead of the compound solution. Soon later, 0.1 ml of each of 10 folds dilutions of each viral material was inoculated to the tubes of both of the control group and the treated one. Then all the tubes were incubated at 37° for 10 days to cultivate adeno virus, and for 7 days to cultivate vaccinia virus. After the cultivation, the $TCID_{50}$ of the both of the control group and the treated one was calculated by Reed and Meunch's[8] method.

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[8] L. D. Read and H. Meunch, Simple method of estimating 50% endopoint, *Am. J. Hyg.*, 27, 493 (1938).