period of 1 hr, while the solution was maintained at pH 8 by addition of 1 N Na_2CO_3. After stirring for an additional 2 hr, the solution was washed with ether and then acidified with 10% citric acid. The resulting precipitate was extracted with AcOEt, which was washed with H_2O-NaCl, dried over Na_2SO_4 and then evaporated. The oily residue was converted to the corresponding DCHA salt in the usual manner; yield 25.0 g.

Z(OMe)-His-NHNH$_2$——*p*-Methoxybenzyl azidoformate (6.8 g) was added to a mixture of H-His-OMe hydrochloride (5.7 g) and triethylamine (4.1 ml) in chloroform (60 ml) and the solution was stirred at room temperature for 48 hr. The chloroform solution was washed with ether, dried over Na_2SO_4 and then evaporated. The residue was dissolved in EtOH and 90% hydrazine hydrate (5 ml) was added. The solid formed on standing overnight was recrystallized from EtOH; yield 6.7 g.

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Alkylated Pyrimidine Derivatives as Antiviral Agents. III.[1] Synthesis and Antiviral Effect of 5-Ethyluracil Nucleosides

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In the previous papers,[1,3] the authors have described that 5-ethyluracil (EU), 5-butyluracil (BU), 5-ethyluracil-1-β-D-ribofuranoside (EUR) and 5-butyluracil-1-β-D-ribofuranoside (BUR) exerted the inhibitory effect on Mahoney strain of poliomyelitis virus and type-1 strain of adeno virus and that the nucleosides of 5-alkyluracil were found to exert the effect more markedly than the free base on the viruses.

On the other hand, it has been reported that 5-ethyldeoxyuridine was the effective inhibitor for herpes simplex virus[4] and for vaccinia virus.[5]

On the basis of these findings, it is of interest to search for more effective antiviral agents by preparing additional 5-ethyluracil nucleoside analogues.

This paper is concerned with the synthesis and antiviral effect of 5-ethyluracil-1-β-D-galactopyranoside (EUGa) and 5-ethyluracil-1-β-D-xylopyranoside (EUXp).

According to the synthetic method of Fox,[6] EUGa and EUXp were prepared by the condensation of the mercury salt of 5-ethyluracil with corresponding tetra-O-acetyl-α-D-galactopyranosyl bromide[7] and tri-O-acetyl-α-D-xylopyranosyl bromide,[8] respectively. The deacetylation of the resulting 1-(tetra-O-acetyl-β-D-galactopyranosyl)-5-ethyluracil and 1-(tri-O-acetyl-β-D-xylopyranosyl)-5-ethyluracil with sodium methoxide gave the objective nucleosides EUGa and EUXp in 80—95% yield.

The antiviral effect of EUGa and EUXp were surveyed on the Mahoney strain of poliomyelitis virus, belonging to RNA virus and the type-1 of adeno virus, belonging to DNA virus in the Hep. No. 2 cells system.

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At first, a comparison of the effect of EUGa with that of EU and EUR on the Mahoney strain of poliomyelitis virus was attempted. For this experiment, one input multiplicity of the Mahoney strain of poliomyelitis virus was added to the tubes in which the monolayer sheet of Hep. No. 2 cells had been established. The tubes were incubated at 37° for 1 hr and unabsorbed virus was removed from the tubes. After that the maintenance medium containing one-fourth of maximum non-toxic dose of the agent for the cells was added to the tubes. After the tubes were incubated at 37° for 4 days, the viral amounts in the cultures were determined according to the plaque assay method.[1)]

As can be seen in the Table I, EUGa showed an effect of 85% inhibition.

Next, the effect of EUGa and EUXp was examined at a shorter incubation preiod using the Mahoney strain of poliomyelitis virus. The agent was added to the cultures 1 hr after the viral inoculation and then the tube was incubated for 24 hr. The viral amounts in the cultures were determined by the plaque assay technique.[1)]

The experimental results shown in Table II indicate that EUGa and EUXp showed the effect of 87% and 75% inhibition, respectively.

Table I. Effect of EU, EUR, and EUGa on Multiplication of Mahoney Strain of Poliomyelitis Virus

Compound	Dose (M)	Viral amount[a)]	
		PFU	Inhibition (%)[b)]
Control		1.34×10^{8}	
EU	2.5×10^{-4}	1.52×10^{8}	
EUR	5×10^{-4}	1.38×10^{8}	
EUGa	5×10^{-4}	2.00×10^{7}	85

a) Viral amount was estimated 4 days after viral inoculation.

b) Inhibition % was calculated by $\frac{\text{(PFU of control)}-\text{(PFU of agent)}}{\text{(PFU of control)}}$.

Table II. Effect of EUGa and EUXp on Multiplication of Mahoney Strain of Poliomyelitis Virus

Compound	Dose (M)	Viral amount[a)]	
		PFU	Inhibition (%)[b)]
Control		1.3×10^{7}	
EUGa	5×10^{-4}	1.6×10^{6}	87
EUXp	5×10^{-4}	3.2×10^{6}	75

a) Viral amount was estimated 24 hr after viral inoculation.

b) Inhibition % was calculated by $\frac{\text{(PFU of control)}-\text{(PFU of agent)}}{\text{(PFU of control)}}$.

In the other experiments, the inhibitory action of EUGa and EUXp was investigated employing the type-1 of adeno virus. For the experiment, 10^{-1} dilution ($TCID_{50}=10^{-2.5}$/ml) of the type-1 of adeno virus was inoculated into the tubes in which the monolayer sheet of Hep. No. 2 cells had been established and then the tubes were incubated at 37° for 6 hr. After that the tubes were added with agents in maximum non-toxic dose for the cell and reincubated at 37° for 24 hr. After the incubation, the viral amount in the cultures was determined in term of $TCID_{50}$.[1)] From the experiments, EUGa and EUXp did not show any inhibitory effect on the type-1 of adeno virus.

From the above results it was found that EUGa and EUXp inhibited the multiplication of Mahoney strain of poliomyelitis virus, but not that of the type-1 of adeno virus in Hep. No. 2 cells.

On the contrary, EUR have been revealed a comparatively wide antiviral spectrum on both RNA and DNA viruses.

The specific activity of EUGa and EUXp may be caused by conversion of galactose or xylose in lieu of ribose on the carbohydrate moeity of 5-ethyluracil glucoside.

Among pyrimidine nucleoside derivatives, 1-β-D-arabinosyl cytosine(Ara-C) is well known[9] as a remarkable inhibitor on DNA viruses and can, therefore, be used to distinguish DNA viruses from RNA viruses. However, any agent as specific inhibitor on RNA viruses, was not found out, yet.

It is notable that EUGa and EUXp have the specific inhibitory effect on poliomyelitis virus, as a RNA viruses.

The antiviral effect of EUGa and EUXp on the RNA viruses other than the poliomyelitis virus are under investigation, and will be reported in the future.

Experimental

5-Ethyluracil was prepared according to the method of Burckhalter and Scarborough.[10]

Di-5-ethyluracilmercury was prepared according to the method of Fox, *et al.* for dithyminylmercury. To a solution of 5-ethyluracil (14 g, 0.1 mole) in 400 ml of hot H_2O containing NaOH (4.0 g) was added an alcoholic solution of mercuric chloride (13.5 g, 0.05 mole). After cooling, the white amorphous precipitate was filtered and washed with H_2O, EtOH and ether. The dried material weighed 20 g (84%) mp>320°. *Anal.* Calcd. for $C_{12}H_{14}O_4N_4Hg$: C, 30.09; H, 2.95; N, 11.70. Found: C, 30.15; H, 2.91; N. 11.65.

1-(Tetra-O-acetyl-β-D-galactopyranosyl)-5-ethyluracil——To 150 ml of dry toluene was added di-5-ethyluracilmercury (4.8 g 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-galactopyranosyl bromide[7] (8.2 g, 0.02 mole) was added to the stirred suspension and refluxed for 2 hr. The warm solution was filtered from unreacted material. After cooling, the filtrate was treated with petr. ether. The precipitate was collected and dissolved in $CHCl_3$. The solution was washed with 30% KI solution, H_2O, succesively, and dried over Na_2SO_4. After evaporation of $CHCl_3$, residue was recrystallized from MeOH. Colorless needles 2.25 g (24%). mp 211—215°. *Anal.* Calcd. for $C_{20}H_{26}O_{11}N_2$: C, 51.06; H, 5.57; N, 5.96. Found: C, 51.32; H, 5.76; N, 6.09.

1-β-D-Galactopyranosyl-5-ethyluracil——1-(Tetra-O-acetyl-β-D-galactopyranosyl)-5-ethyluracil (1 g, 2.1 m mole) was added to methanolic MeONa prepared from Na (0.19 g) and abs. MeOH (5 ml) 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 MeOH and the eluate was concentrated *in vacuo*. The residue was azeotropically dried with benzene for several times until crystals was obtained. The crystalline material was recrystallized from EtOH–ether. Colorless needles, mp 182—184°. 0.6 g (95%). *Anal.* Calcd. for $C_{12}H_{18}O_7N_2$: C, 47.68; H, 6.00; N, 9.27. Found: C, 47.45; H, 6.14; N, 9.24. UV absorption properties: $\lambda_{max}^{H^+}$ 265.5 nm (ε 9800); $\lambda_{max}^{pH_7}$ 265 nm (ε 9800); $\lambda_{max}^{OH^-}$ 265.5 nm (ε 7300).

1-β-D-Xylopyranosyl-5-ethyluracil——1-(Tri-O-acetyl-β-D-xylopyranosyl)-5-ethyluracil was prepared from tri-O-acetyl-D-xylopyranosyl bromide[8] (8.8 g, 0.026 mole) and di-5-ethyluracilmercury (6.24 g, 0.013 mole) using the same procedure as with 1-(tetra-O-acetyl-β-D-galactopyranosyl)-5-ethyluracil with the exception that 200 ml of xylene was used in place of toluene. The sirupy product was used to the next step without recrystallization. A mixture of uncrystallized acetyl derivative (2 g) in abs. MeOH (20 ml) and MeONa (8 m mole) was refluxed for 2 hr. The reaction mixture 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-galactopyranosyl-5-ethyluracil. Recrystallization from EtOH gave 0.8 g of colorless needles mp 231—233°. *Anal.* Calcd. for $C_{11}H_{16}O_6N_2$: C, 48.52; H, 5.92; N, 10.29. Found: C, 48.37; H, 6.00; N, 10.27. UV absorption properties $\lambda_{max}^{H^+}$ 265.5 nm (ε 9600); $\lambda_{max}^{pH_7}$ 265.5 nm (ε 9600); $\lambda_{max}^{OH^-}$ 265.5 nm (ε 7000).

Screening test of EUGa and EUXp on the Viruses——A) Viral Materials: Mahoney strain of poliomyelitis virus was employed as a representative of RNA virus and the type-1 of adeno virus, as a representative of DNA virus.

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

C) Media: For the growth medium, yeast extract-lactalbumin hydrolysate (YLA) medium supplemented with 15% of bovine serum was employed. For the maintenance medium to cultivate poliomyelitis virus, YLA medium supplemented with 5% of bovine serum was employed and for the cultivation of adeno virus, YLA medium supplemented with 5% of horse serum was employed.

D) General Procedure: The monolayer of Hep. No. 2 cells was prepared by inoculating 2×10^5 cells

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in 1 ml per tube. After a tube was incubated at 37° for 3 or 4 days to obtain the monolayer cell sheet, growth medium was removed from the tube. The tube was washed three times with phosphate buffered saline (pH 7.6) and 0.1 ml of the dilution of viral material and 0.9 ml of the maintenance medium were added. Each tube was incubated at 37°. The experimental details were described in each saction.

E) Assay of the Infectivity of Tested Viral Materials: The two methods, the plaque assay technique[1] to estimate plaque forming unit (PFU) for the Mahoney strain of poliomyelitis virus and the $TCID_{50}$ estimating dilution method[1] for the type-1 of adeno virus were used.