Base-catalysed Condensation of (III) with Benzaldehyde—To a solution of 500 mg. of (III) in 10 cc. of EtOH and 1 cc. of H_2O, 300 mg. of BzH and 0.13 cc. of 10*N* NaOH were added. The mixture was allowed to stand overnight at room temperature, concentrated *in vacuo*, extracted with $CHCl_3$, and $CHCl_3$ solution was evaporated to afford an oil. A picrate, m.p. 142°(from MeOH), 170 mg. (44.4% yield), was identified as 1-methyl-2(1*H*)-pyridone picrate.

A solution of 1 g. of (III) and 1 g. of BzH in EtOH solution containing 10*N* NaOH was allowed to stand for 2 days in a refrigerator, acidified with $HClO_4$, and concentrated to a small volume to give white crystals, m.p. 212～215°(from H_2O), 100 mg. (15.6% yield), which was identified as 1-(β-hydroxyphenylethyl)pyridinium perchlorate,[7] m.p. 212～215°, by mixed fusion.

The author expresses his deep gratitude to Prof. M. Hamana for his kind instruction.

Summary

The order of the activity of the active methylene of 1-(2-pyridylmethyl)- (I), 1-(1-oxido-2-pyridylmethyl)pyridinium salt (II) and 1′-methyl-1,2′-methylenebis-pyridinium salt (III) was determined.

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153. Takeo Ueda, Shigeshi Toyoshima, Kiyoshi Takahashi, Masako Muraoka, Hiroko Koibuchi, and Yoshiko Seto: Researches on Chemotherapeutic Drugs against Viruses. XXXI.*2 Synthesis and Antiviral Effect of 3-(Alkoxyphenoxy)-1,2-propanediols on Respiratory Virus.

(*Pharmaceutical Institute, Keio-Gijuku University*[*1])

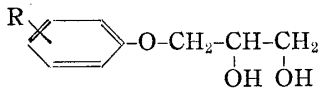
As can be seen from the summarized review written by Tamm[1] and by Horsfall and Tamm,[2] the search for effective chemotherapeutic drugs on respiratory viruses, particularly influenza virus, has been carried out by number of research workers, and many compounds have been reported to be effective on influenza virus in the experiment using chorioallantoic membrane culture or fertilized eggs, although all the substances except Caprochlorone[3] did not show any therapeutic effect on influenza in mice. On the contrary, search for chemotherapeutic agents on adenovirus has hardly been reported to date.

It was reported by Ueda[4] that 3-(alkyl- or alkanoylphenoxy)-1,2-propanediols, replacing the methyl group in Mephenesin with hydrophobic groups such as alkyl or alkanoyl group, exerted direct virus-inactivating action against poliomyelitis viruses, and moreover, certain compounds of these series were found to be fairly effective on the multiplication of the PR-8 strain of influenza virus in chorioallantoic membrane culture. It was considered, therefore, that the compounds formed by introduction of an active group into

*1 Shinano-machi, Shinjuku-ku; Tokyo (上田武雄, 豊島 滋, 高橋 廉, 村岡全子, 鯉淵弘子, 瀬戸淑子).

*2 This paper constitutes part of a series entitled "Researches on Chemotherapeutic Drugs against Viruses" by Takeo Ueda. Part XXX: This Bulletin, 8, 860(1960).

1) I. Tamm: "Viral and Rickettsial Infection of Man," 156(1952).
2) F. L. Horsfall, I. Tamm: Ann. Rev. Microbiol., **11**, 339(1957).
3) O. C. Liu, *et al.*: J. Immunol., **78**, 222(1957).
4) F. Ueda: This Bulletin, **7**, 823(1959).

TABLE I. 

Compd. No.	R	m.p. (°C)	b.p. (°C/mm. Hg)	Recrystn. solvent	Method	Yield (%)	Mol. formula	Analysis C (%) Calcd.	Analysis C (%) Found	Analysis H (%) Calcd.	Analysis H (%) Found
M-1	*m*-HO	93	208~210/3	Benzene	A	52	$C_9H_{12}O_4$	58.69	58.86	6.57	6.71
M-2	*m*-CH_3O	74~76	175/2.5	〃	〃	49	$C_{10}H_{14}O_4$	60.59	60.89	7.12	7.23
M-3	*m*-C_2H_5O	58~59	172~173/3	Me_2CO	〃	51	$C_{11}H_{16}O_4$	62.25	61.89	7.60	7.62
M-4	*m*-C_3H_7O	61~62	180~182/2.5	hydr. MeOH	〃	58	$C_{12}H_{18}O_4$	63.70	64.15	8.02	8.15
M-5	*m*-C_4H_9O	76~77	187~190/3	AcOEt	〃	37	$C_{13}H_{20}O_4$	64.98	64.91	8.39	8.37
M-6	*m*-$C_5H_{11}O$	74~75	188~190/2	〃	〃	39	$C_{14}H_{22}O_4$	66.11	65.07	8.72	8.73
M-7	*m*-$C_6H_{13}O$	78~80	197~199/2	〃	〃	50	$C_{15}H_{24}O_4$	67.13	67.22	9.02	9.08
M-8	*m*-$C_8H_{17}O$	81~83	219~220/2.5	〃	〃	39	$C_{17}H_{28}O_4$	68.89	69.25	9.52	9.57
M-9	*m*-$C_{10}H_{21}O$	92~93	223~225/2	〃	〃	49	$C_{19}H_{32}O_4$	70.33	70.67	9.94	10.13
M-10	*m*-$C_{12}H_{25}O$	95~96	226/2	〃	〃	50	$C_{21}H_{36}O_4$	71.55	71.65	10.30	10.44
P-1	*p*-HO	111~113	217~219/4	Benzene	〃	50	$C_9H_{12}O_4$	58.69	58.42	6.57	6.86
P-2	*p*-CH_3O	80~81	176~178/2.5	Benzene + Me_2CO	〃	35	$C_{10}H_{14}O_4$	60.59	60.82	7.12	7.36
P-3	*p*-C_2H_5O	83~85	172~174/2.5	EtOH	〃	68	$C_{11}H_{16}O_4$	62.25	61.86	7.60	7.75
P-4	*p*-C_3H_7O	90~92	182~183/2.5	Me_2CO	〃	58	$C_{12}H_{18}O_4$	63.70	63.37	8.02	7.96
P-5	*p*-C_4H_9O	88~90	182~184/2.5	〃	〃	52	$C_{13}H_{20}O_4$	64.98	64.91	8.39	8.39
P-6	*p*-$C_5H_{11}O$	83~84	200~202/3	〃	〃	54	$C_{14}H_{22}O_4$	66.11	66.01	8.72	8.69
P-7	*p*-$C_6H_{13}O$	71~73		〃	B	60	$C_{15}H_{24}O_4$	67.13	67.28	9.02	9.06
P-8	*p*-$C_8H_{17}O$	86~87		AcOEt	〃	76	$C_{17}H_{28}O_4$	68.89	68.88	9.52	9.50
P-9	*p*-$C_{10}H_{21}O$	96~98		EtOH	〃	75	$C_{19}H_{32}O_4$	70.33	70.39	9.94	10.06
P-10	*p*-$C_{12}H_{25}O$	101~103		〃	〃	73	$C_{21}H_{36}O_4$	71.55	71.16	10.30	10.33
O-1	*o*-HO	87~88	218~220/2.5	Benzene	A	56	$C_9H_{12}O_4$	58.69	58.81	6.57	6.54
O-2	*o*-CH_3O	80~82	157~159/4	Me_2CO		50	$C_{10}H_{14}O_4$	60.59	60.55	7.12	7.15
O-3	*o*-C_2H_5O	64~66	161~163/2.5	Me_2CO + hexane	C	58	$C_{11}H_{16}O_4$	62.25	62.79	7.60	7.67
O-4	*o*-C_3H_7O	60~62	170~172/2.5	Et_2O + petr. ether	〃	68	$C_{12}H_{18}O_4$	63.70	63.35	8.02	8.13
O-5	*o*-C_4H_9O	69~70	185~187/2.5	〃	〃	70	$C_{13}H_{20}O_4$	64.98	64.75	8.39	8.30
O-6	*o*-$C_5H_{11}O$	55~56	180~182/2.5	〃	〃	67	$C_{14}H_{22}O_4$	66.11	66.34	8.72	8.89
O-7	*o*-$C_6H_{13}O$	61~63	184~186/2.5	〃	〃	51	$C_{15}H_{24}O_4$	67.13	67.20	9.02	9.40
O-8	*o*-$C_8H_{17}O$	78~79		〃	〃	57	$C_{17}H_{28}O_4$	68.89	69.17	9.52	9.59
O-9	*o*-$C_{10}H_{21}O$	67~68		〃	〃	55	$C_{19}H_{32}O_4$	70.38	70.53	9.94	9.98
O-10	*o*-$C_{12}H_{25}O$	63~65		〃	〃	56	$C_{21}H_{36}O_4$	71.55	71.77	10.30	10.32

m- and *p*-compounds are colorless plates, and *o*-compounds colorless needles.

the structure of Mephenesin-like compounds might exert inhibitory effect on respiratory viruses. Thus, 3-(alkoxyphenoxy)-1,2-propanediols were synthesized and their effect on respiratory viruses was examined.

This paper is concerned with the synthesis of 3-(alkoxyphenoxy)-1,2-propanediols and their antiviral activity on the PR-8 strain of influenza type-A virus, and the type-1, -2, -3, -4, -6, and -7 strains of adenovirus.

(I) Synthesis of 3-(Alkoxyphenoxy)-1,2-propanediol

The compounds of 3-(*m*-alkoxyphenoxy)-1,2-propanodiol were prepared by the condensation of *m*-alkoxyphenol with 3-chloro-1,2-propanediol in the presence of sodium ethoxide. This method was found to be unsuitable for the synthesis of 3-(*p*-alkoxyphenoxy)-1,2-propanediol with higher alkyl group, because it produced a by-product which was difficult to separate from the reaction mixture. Therefore, the higher homologs of 3-(*p*-alkoxyphenoxy)-1,2-propanediol were synthesized by condensation of 3-(*p*-hydroxyphenoxy)-1,2-propanediol with alkyl halide in the presence of sodium ethoxide. 3-(*o*-Alkoxyphenoxy)-1,2-propanediol was prepared by the reaction of 3-(*o*-hydroxyphenoxy)-1,2-propanediol with alkyl halide in acetone solution, in the presence of anhydrous potassium carbonate, instead of sodium ethoxide.

Among the compounds of 3-(alkoxyphenoxy)-1,2-propanediol, *o*-,[5] *m*-,[6] and *p*-methoxy,[6] *o*-[7], *m*-[7], and *p*-ethoxy[6,7] *o*-propoxy,[7] and *o*-butoxy,[7,8] derivatives were already reported by several workers, but the higher alkoxyl derivatives have not been synthesized. The properties of the compounds are summarized in Table I.

(II) Antiviral Effect of 3-(Alkoxyphenoxy)-1,2-propanediols on Influenza Virus and Adeno-Virus

The primary screening test with the compounds shown in Table I was carried out using the PR-8 strain of influenza-A virus and the Type-1 strain of adenovirus.

1) Primary Screening Test on Influenza Virus—The experimental results are shown in Table II, from which it is seen that almost all compounds except 3-(*m*-decyloxyphenoxy)-1,2-propanediol did not show any inhibitory effect on the multiplication of influenza virus in chrioallantoic membrane. 3-(*m*-Decyloxyphenoxy)-1,2-propanediol was fairly effective on the PR-8 strain. The effect of these compounds, however, was not so good compared to other compounds[9] which have already been reported.

2) Primary Screening Test on Type-1 Adenovirus—Experimental results of the primary screening test on type-1 adenovirus are shown in Table III. It may be said from this Table that all of 3-(*p*-decyloxyphenoxy)-, 3-(*p*-dodecyloxyphenoxy)-, 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, 3-(*o*-decyloxyphenoxy)-, 3-(*o*-dodecyloxyphenoxy)-, 3-(*m*-butoxyphenoxy)-, 3-(*m*-pentyloxyphenoxy)-, 3-(*m*-hexyloxyphenoxy)-, 3-(*m*-octyloxyphenoxy),-3-(*m*-decyloxyphenoxy)-, and 3-(*m*-dodecyloxyphenoxy)-1,2-propanediols showed inhibitory action on the cytopathogenic effect caused by the infection of type-1 adenovirus in the HeLa.

3) Secondary Screening Test on Type-1 Adenovirus—The twelve compounds described above, selected from 3-(alkoxyphenoxy)-1,2-propanediols by the primary screening with adenovirus were examined in more detail for their effect on the type-1 strain of adenovirus. The secondary screening tests consisted of two parts, examination of direct virus-inactivating action and effect of these compunds on TCD_{50}*3 of the virus. All experiments

*3 TCD_{50}=50% Tissue culture dose.

5) R. R. Astoul : Span. Pat. 212,920(1954) (C. A., **49**, 8332(1955)).
6) H. L. Yale, *et al.* : J. Am. Chem. Soc., **72**, 3710(1950).
7) R. I. Metten, J. Docze : *Ibid.*, **72**, 4986(1950).
8) W. Bradly, *et al.* : Brit. Pat. 636,569(1950). British Drug Houses Ltd. (C. A., **44**, 7349(1950)).
9) T. Ueda, S. Toyoshima, S. Kanoh : Paper read at the Annual Meeting of the Pharmaceutical Society of Japan. April, 1958.

Table II. Antiviral Effect of 3-(Alkoxyphenoxy)-1,2-propanediols on Influenza Virus (PR-8 Strain 2×10^{-3})

Compd. No.	Max. non-toxic dose (M)	Hemagglutinin titer: Treated group (2^{-x}) x	Hemagglutinin titer: Untreated group (2^{-x}) x
P-1	$>5\times10^{-4}$	5.0	6.0
P-2	$>5\times10^{-4}$	8.0	7.0
P-3	$>5\times10^{-4}$	8.0	7.0
P-4	$>5\times10^{-4}$	8.0	7.0
P-5	10^{-4}	6.0	6.0
P-6	10^{-4}	6.0	6.0
P-7	10^{-4}	5.0	6.0
P-8	10^{-4}	5.5	6.5
P-9	10^{-4}	5.5	6.5
P-10	10^{-4}	7.0	6.5
M-5	10^{-4}	7.0	6.5
M-6	10^{-4}	6.0	6.5
M-7	10^{-4}	5.5	6.5
M-8	10^{-4}	4.5	6.5
M-9	10^{-4}	4.0	6.5
M-10	10^{-4}	5.0	6.5
O-1	$>5\times10^{-4}$	7.0	7.0
O-2	$>5\times10^{-4}$	6.0	6.0
O-3	$>5\times10^{-4}$	5.5	6.0
O-4	$>5\times10^{-4}$	7.0	6.0
O-7	10^{-3}	6.0	7.0
O-9	10^{-3}	7.0	7.0
O-10	10^{-3}	7.0	7.0

Table III. Antiviral Effect of 3-(Alkoxyphenoxy)-1,2-propanediol on Adenovirus

Compd. No.	Dose ($10^{-x}M$) x	Antiviral effect[a)]	Compd. No.	Dose ($10^{-x}M$) x	Antiviral effect[a)]
M-1	3	0/4	P-6	5	0/4
M-2	3	1/4	P-7	5	0/4
M-3	3	0/4	P-8	5	0/4
M-4	4	0/4	P-9	5	4/4
M-5	(2×10^{-5})	4/4	P-10	5	4/4
M-6	5	4/4	O-1	3	0/4
M-7	4	4/4	O-2	3	0/4
M-8	5	4/4	O-3	3	0/4
M-9	5	4/4	O-4	4	0/4
M-10	5	2/4	O-5	4	0/4
P-1	3	0/4	O-6	5	0/4
P-2	3	0/4	O-7	4	4/4
P-3	3	0/4	O-8	4	2/4
P-4	4	0/4	O-9	4	4/4
P-5	4	0/4	O-10	4	4/4

a) $\dfrac{\text{No. of tubes of no cytopathogenic effect}}{\text{Total no. of tubes}}$

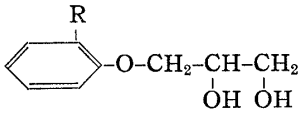
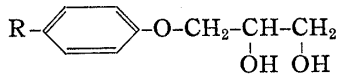
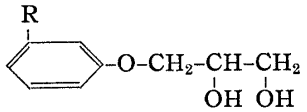
described in this section were carried out by using the FL strain cell of human amnion, from the reason described below.

Since the primary isolation of adenovirus from the patients was reported by Rowe,[10] the HeLa cell has been mainly used as the host cell for studies of the virus. However, it was reported by Ginsberg[11] that lactic acid, α-ketoglutaric acid, accumulated in the medium inoculated with adenovirus when the HeLa was used as the host cell. Takemoto and Lerner[12] recently reported the different responses of the FL-strain cell on adenovirus and said that adenovirus showed cytopathogenic effect and intracellular growth on the adeno-FL strain system, but that accumulation of organic acids as observed in the HeLa was not found. According to this finding, it was assumed that the FL-strain cell may be more suitable than the HeLa for examining the effect of compounds on the multiplication of adenovirus. Thus, the secondary screening test of twelve compounds selected from 3-(alkoxyphenoxy)-1,2-propanediol was carried out by using the adeno-FL strain system. The experimental results are shown in Tables IV, V, VI, and VII. The mean value and standard deviation of TCD_{50} of the type-1 strain of adenovirus employed are shown in Table IV. If TCD_{50} of the treated group was one in the order of 1/10 over or below that of the control, it was recognized as a significant value. From the comparison of data in Table V with those in Table III, it can easily be understood that the maximum nontoxic concentration of the twelve compounds on the FL is very similar to that shown in the case of the HeLa. As can be seen in Table VI, all of the twelve compounds did not possess any virus-inactivating action, but, it is seen from Table VII that TCD_{50} of the group treated with three compounds, 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, and 3-(*p*-dodecyloxyphenoxy)-1,2-propanediols is 1/10 that of the control.

Table IV. Titration of TCD_{50} of Type-1 Adenovirus

Expt. No.	TCD_{50} (10^{-x}) x	Expt. No.	TCD_{50} (10^{-x}) x
1	3.5	9	3.5
2	4.5	10	3.5
3	2.5	11	3.5
4	3.5	12	3.5
5	3.5	13	4.0
6	3.5		
7	3.5	Mean	3.5
8	3.5	Standard Deviation	0.41

Table V. Maximum Nontoxic Doses of 3-(Alkoxyphenoxy)-1,2-propanediols on FL-Strain Cell

Compound	R	Final concn. $10^{-3}M$	$10^{-4}M$	$10^{-5}M$	$10^{-6}M$	Max. nontoxic dose ($M\times10^{-x}$) x
	$C_6H_{13}O$	##	−	−	−	4
	$C_8H_{17}O$	##	##	−	−	5
	$C_{10}H_{21}O$	##	##	−	−	5
	$C_{12}H_{25}O$	##	##	−	−	5
	$C_{10}H_{21}O$	##	##	−	−	5
	$C_{12}H_{25}O$	##	+	−	−	5
	C_4H_9O	##	−	−	−	4
	$C_5H_{11}O$	##	+	−	−	5
	$C_6H_{13}O$	##	##	−	−	5
	$C_8H_{17}O$	##	##	−	−	5
	$C_{10}H_{21}O$	##	+	−	−	5
	$C_{12}H_{25}O$	##	−	−	−	4

10) W. R. Rowe : Proc. Soc. Exptl. Biol. Med., **84**, 570(1953).
11) T. N. Fisher, H. S. Ginsberg : *Ibid.*, **95**, 47(1957).
12) K. K. Takemoto, A. Lerner : *Ibid.*, **94**, 179(1957).

Table VI. Virus Inactivating Effect of 3-(Alkoxyphenoxy)-1,2-propanediols on Type-1 Adenovirus.

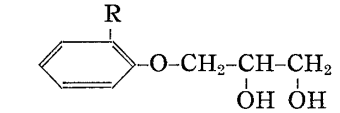
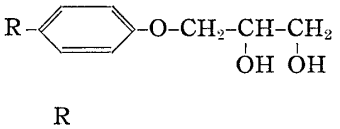
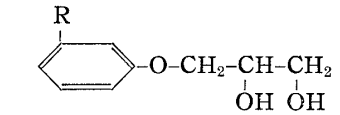
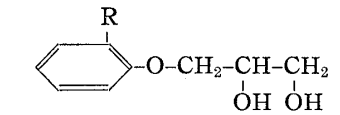
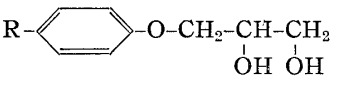
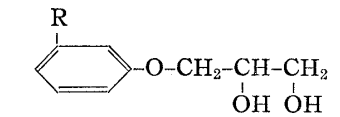
Compound	R	TCD_{50} (10^{-x}) Treated x	TCD_{50} (10^{-x}) Control x
	$C_6H_{13}O$	3.5	3.5
	$C_8H_{17}O$	3.5	
	$C_{10}H_{21}O$	3.5	
	$C_{12}H_{25}O$	3.5	
	$C_{10}H_{21}O$	3.5	
	$C_{12}H_{25}O$	3.5	
	C_4H_9O	3.5	
	$C_5H_{11}O$	3.5	
	$C_6H_{13}O$	3.5	
	$C_8H_{17}O$	3.5	
	$C_{10}H_{21}O$	3.5	
	$C_{12}H_{25}O$	3.5	

Table VII. Effect of 3-(Alkoxyphenoxy)-1,2-propanediols on Type-1 Adenovirus

Compound	R	TCD_{50} (10^{-x}) Treated x	TCD_{50} (10^{-x}) Control x
	$C_6H_{13}O$	2.5	3.5
	$C_8H_{17}O$	2.5	
	$C_{10}H_{21}O$	3	
	$C_{12}H_{25}O$	3.5	
	$C_{10}H_{21}O$	3.5	
	$C_{12}H_{25}O$	2.5	
	C_4H_9O	3.5	
	$C_5H_{11}O$	3.5	
	$C_6H_{13}O$	3.5	
	$C_8H_{17}O$	3.5	
	$C_{10}H_{21}O$	3.5	
	$C_{12}H_{25}O$	3.5	

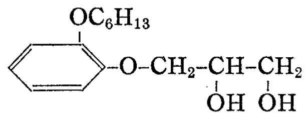
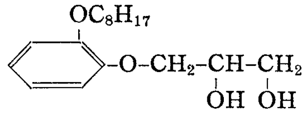
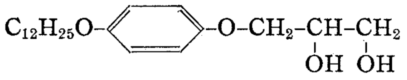
All compounds were examined by using the maximum nontoxic dose.

4) Inhibitory Effect on the Cytopathogenic Effect caused by Types-2, -3, -4, -6, and -7 Adenovirus—In the secondary screening test, 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, and 3-(*p*-dodecyloxyphenoxy)-1,2-propanediols were found to be effective on the cytopathogenic effect (CPE) caused by infection of the type-1 strain of adenovirus. From these results, further experiments on the inhibitory effect of these three compounds on the types-2, -3, -4, -6, and -7 strains of adenovirus were conducted. The experimental results are shown in Table VIII. As can be seen in Table VIII, TCD_{50} of type-4 and -6 strain in the group treated with 3-(*o*-hexyloxyphenoxy)-1,2-propanediol and that of the type-4 strain in the group treated with 3-(*o*-octyloxyphenoxy)-1,2-propanediol were 1/10 that of the control. 3-(*p*-Dodecyloxyphenoxy)-1,2-propanediol, however, did not show any effect on the cytopathogenic effect (CPE) caused by the five strains used.

5) Inhibitory Effect of 3-(*o*-Hexyloxyphenoxy)-, 3-(*o*-Octyloxyphenoxy)-, and 3-(*p*-Dodecyloxyphenoxy)-1,2-propanediol on the Multiplication of Type-1 Adenovirus—It was reported by Ginsberg[13] and Pereira[14] that the cytopathogenic effect (CPE) caused by adenovirus was due to the following two origins; (i) the host cell was destroyed by the multiplication

13) W. C. Wilcox, H. S. Ginsberg : Federation Proc., **18**, 604(1959).
14) H. G. Perira : Virology, **6**, 601(1958).

TABLE VIII. Effect of 3-(Alkoxyphenoxy)-1,2-propanediols on Type-2, 3, 4, 6, and 7 Adenovirus

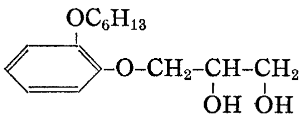
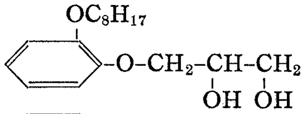
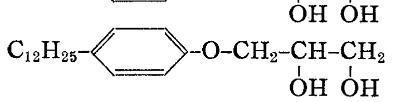
Compound	TCD_{50} (10^{-x}) Type-2 x	Type-3 x	Type-4 x	Type-6 x	Type-7 x
	3.5	3.5<	1.5	3.5	1.5
	3.5	3.5<	1.5	4.5<	1.5
	3.5	3.5<	2.5	4.5<	1.5
Virus control	3.5	3.5<	2.5	4.5<	1.5

of the virus and (ii) the cell destruction, particularly, the primary host-cell destruction by adenovirus was due to a kind of toxin-like substance produced by the virus (the so-called CPE factor).

Consequently, examination of whether the inhibitory effect of 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, and 3-(*p*-dodecyloxyphenoxy)-1,2-propanediols on the cytopathogenic effect caused by adenovirus was due to the inhibition of viral multiplication or the suppression of the so-called CPE factor, should be made to clarify their mode of action. This point was examined by the following procedures.

From the tubes in the first culture, which did not show any cytopathogenic effect with addition of 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, and 3-(*p*-dodecyloxyphenoxy)-1,2-propanediols, the medium was removed and diluted to 10^{-1}, 10^{-2}, and 10^{-3}, with the maintenance medium, and these dilutions were inoculated into the fresh monolayer cell-sheet of the FL-strain cell-established tubes. After incubation at 37° for 14 days, the viral growth was determined. Experimental results are shown in Table IX and it can be seen from this Table that no viral-growth pattern was found in the tubes inoculated with the dilutions of the medium of the groups treated with 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, and 3-(*p*-dodecyloxyphenoxy)-1,2-propanediols. From these results, it may be said that the above three compounds inhibited the multiplication of the type-1, -2, and -3 strains of adenovirus.

TABLE IX. Inhibitory Effect of 3-(Alkoxyphenoxy)-1,2-propanediols on the Multiplication of Type-1 Adenovirus

Compound	1st culture Virus dilution	CPE[a)]/total no. of tubes	2nd culture Virus dilution	CPE[a)]/total no. of tubes
	10^{-2}	0/4	10^{-2}	2/2
	10^{-3}	4/4	10^{-3}	2/2
	10^{-4}	4/4	10^{-4}	2/2
	10^{-2}	0/4	10^{-2}	2/2
	10^{-3}	4/4	10^{-3}	2/2
	10^{-4}	4/4	10^{-4}	2/2
	10^{-2}	0/4	10^{-2}	2/2
	10^{-3}	4/4	10^{-3}	2/2
	10^{-4}	4/4	10^{-4}	2/2
Virus Control	10^{-2}	0/4	10^{-2}	0/2
	10^{-3}	0/4	10^{-3}	2/2
	10^{-4}	4/4	10^{-4}	2/2

a) No. of tubes of no cytopathogenic effect.

Experimental

General Procedure for Synthesis of 3-(Alkoxyphenoxy)-1,2-propanediol—a) To a solution of EtONa (from 0.1 mole of metallic Na and 1.5 moles of EtOH) were added 0.1 mole of alkoxyphenol and 0.1 mole of 3-chloro-1,2-propanediol. After stirring on a steam bath for 6 hr., EtOH was distilled off, the residue was treated with water, and extracted with Et_2O. The Et_2O solution was washed with 10% NaOH and saturated NaCl solution, and dried over Na_2SO_4. After removal of Et_2O, the residue was distilled under a reduced pressure. The distillate was recrystallized from a suitable solvent.
b) To a solution of EtONa (from 0.1 mole of metallic Na and 1.5 moles of EtOH), 0.1 mole of 3-(hydroxyphenoxy)-1,2-propanediol, prepared according to the procedure (a), and 0.1 mole of alkyl bromide were added. After stirring on a steam bath for 6 hr., EtOH was distilled off, the residue was washed with water, and recrystallized from a suitable solvent.
c) Equimolar mixture of 0.1 mole of 3-(hydroxyphenoxy)-1,2-propanediol, prepared according to the procedure (a), and alkyl halide was stirred and refluxed with 0.05 mole of anhyd. K_2CO_3 in 40 cc. of dry Me_2CO for 7 hr. After removal of Me_2CO, the residue was poured into water and extracted with Et_2O. The Et_2O solution was dried over Na_2SO_4, evaporated, and the residue was distilled under a reduced pressure. The distillate was recrystallized from a suitable solvent.

3-(*p*-Alkoxyphenoxy)- and 3-(*o*-alkoxyphenoxy)-1,2-propanediols, having alkoxyl chain of 8~12 carbon atoms, were treated with water and petr. ether, and recrystallized from a solvent without distillation.

Test of Antiviral Activity

a) **Materials**

1) **Influenza Virus**—Egg-adapted PR-8 strain of influenza virus was kindly supplied by Prof. K. Ogasawara of the Bacteriological Department, University of Nagoya.

2) **Adenovirus**—Type-1, -2, -3, and -6 strains of adenovirus were kindly given by Prof. E. Hamamoto of the Pediatrics Department, Okayama University. Type-4 and -7 strains of adenovirus were supplied by the Institute for Infectious Diseases, University of Tokyo.

b) **Methods**

For the test of activity of the compounds against influenza virus, chorioallantoic-membrane tissue culture method was the same as that described in a previous paper of this series.[15]

For the test of antiviral activity on adenovirus, tissue culture method was employed using HeLa or FL strain cells.

1) **Methods using HeLa**—3×10^5 cells/cc. of HeLa cells were added into each tube and the tubes were incubated at 37° for 3 or 4 days until the monolayer-sheet of the cell was established on the glass surface. For the growth medium, the YLA medium added with 15% bovine serum was used. After establishment of the monolayer cell-sheet, the growth medium was removed, the cell-sheet was washed three times with phosphate buffer solution (pH 7.6). Immediately after 0.8 cc. of the maintenance medium and 0.1 cc. of the solution of test compounds were added into each tube, 0.1 cc. of the viral dilutions was inoculated into these tubes. For the maintenance medium, the YLA medium with 5% horse serum was used. The compound concentration used for the test of antiviral activity was the maximum non-toxic dose of the tested compound and the viral dilution employed for the antiviral screening test was $5\times TCD_{50}$ ($TCD_{50}=10^{-3.5}$). After inoculation, these test tubes were incubated for 7 days and the effectiveness determined from the daily microscopic examination of cytopathogenic effect of the virus.

2) **Method using FL**—The FL-strain cells were kindly given by Dr. N. Takatori of the National Institute of Health, Tokyo. 2×10^5 cells/cc. of the FL strain cells were added to each tube and incubated at 37° for 3~4 days. For the growth medium, the YLA medium added with 15% bovine serum was used.

a) Determination of the Maximum Nontoxic Dose of Compounds: After the monolayer sheet of the FL-strain cells was established, the growth medium was removed from the tube and 0.9 cc. of the maintenance medium (YLA medium added with 5% horse serum) and 0.1 cc. of the dilution of the test compound were added to each tube. After incubation at 37° for 4 days, the maximum nontoxic dose was determined by microscopic observation.

b) Virus Inactivating Action: Original suspension of the virus and solution of the test compound were mixed in a test tube, in which any cell was not present, and stood at 22°. After 24 hr., this mixture was diluted and the dilutions were added into the tube containing the monolayer sheet of the FL-strain cells and further incubated at 37° for 10 days.

c) Effect of Test Compounds on TCD_{50} of Adenoviruses: The FL strain cells (2×10^5 cells/cc.) were placed in a tube. After the monolayer cell-sheet was established, the growth medium was removed

15) F. Ueda, T. Ueda, S. Toyoshima: This Bulletin, **7**, 833(1959).

from the tube, and washed three times with a phosphate buffer solution (pH 7.6). 0.8 cc. of the maintenance medium (YLA medium added with 5% horse serum) and 0.1 cc. of 10 times the maximum nontoxic dose of the test compound were added to the tube and then 0.1 cc. of the viral dilution was inoculated. After incubation at 37° for 10 days, TCD_{50} was determined by microscopic observation.

d) Inhibitory Effect of Compounds on the Multiplication of Adenovirus: This method consisted of the following two stages: (i) The first stage was the same as the method described in (c). (ii) The medium in the tube, in which any cytopathogenic effect caused by the infection of adenovirus was not observed, was removed and intracellular virus was extracted by freezing and thawing 5 times. This extracted viral material was inoculated into the newly established cell-sheet test tubes and cytopathologic effect was observed daily at 37° for 21 days.

Summary

To find chemotherapeutic agents for influenza and adenoviruses, 3-(alkoxyphenoxy)-1,2-propanediols were synthesized. Among these derivatives, 3-(*m*-dodecyloxyphenoxy)-1,2-propanediol possessed a fairly inhibitory effect on the multiplication of the PR-8 strain of influenza virus in chorioallantoic membrane culture. By the primary screening test on the type-1 strain of adenovirus, 3-(*p*-decyloxyphenoxy)-, 3-(*p*-dodecyloxyphenoxy)-, 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, 3-(*o*-decyloxyphenoxy)-, 3-(*o*-dodecyloxyphenoxy)-, 3-(*m*-butoxyphenoxy)-, 3-(*m*-pentyloxyphenoxy)-, 3-(*m*-hexyloxyphenoxy)-, 3-(*m*-octyloxyphenoxy)-, 3-(*m*-decyloxyphenoxy)-, and 3-(*m*-dodecyloxyphenoxy)-1,2-propanediols were selected. By the secondary screening test, 3-(*o*-hexyloxyphenoxy)-, 3-(*o*-octyloxyphenoxy)-, and 3-(*p*-dodecyloxyphenoxy)-1,2-propanediols were found to be effective on $10 \times TCD_{50}$ of the type-1 of adenovirus. Among these three compounds, 3-(*o*-hexyloxyphenoxy)-1,2-propanediol was effective on the type-4 and -6 strains, and 3-(*o*-octyloxyphenoxy)-1,2-propanediol was effective on the type-4 strain virus, while 3-(*p*-dodecyloxyphenoxy)-1,2-propanediol did not show any effect on the type-2, -3, -4, -6, and -7 strains of adenovirus. These three compounds did not possess any direct action for inactivating adenovirus and it was suggested from the experiments that the effectiveness of the compounds was due to the inhibition of viral multiplication.

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