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151. Fumio Ueda, Takeo Ueda, and Shigeshi Toyoshima : Researches on Chemotherapeutic Drugs against Viruses. XXIV.[1] Studies on the Syntheses and Antiviral Effect of 2-Alkylphenoxyalkanoic Acids.

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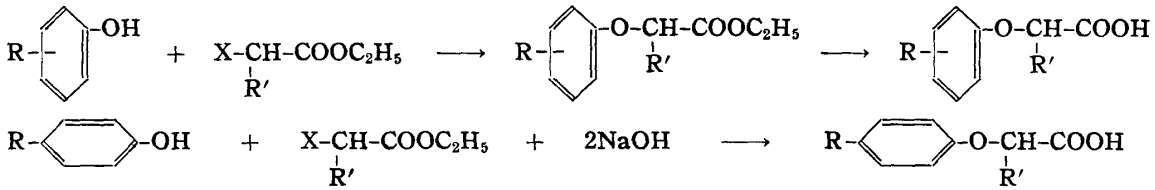
As described in the preceding paper,[1] the compounds of the 3-alkylphenoxy-1,2-propanediol series were inactive against Japanese B encephalitis virus *in vivo*, but some compounds of this series were inhibitory against poliomyelitis virus *in vitro*. On the basis of these findings, it seemed to be of interest in the search for chemotherapeutic drugs against poliomyelitis virus to prepare alkylphenoxy derivatives, in which the propanediol group has been replaced by other hydrophilic groups, and to examine their antiviral activity. On the other hand, several 2-hydroxylated fatty acids have been reported by Fauconnier[2] to possess virucidal activity.

Taking these findings into consideration, a number of alkanoic acids containing alkylphenoxy group in 2-position were synthesized and their antiviral activities examined. This paper describes the synthesis and antiviral activity of alkylphenoxyacetic acid, -propionic acid, -butyric acid, and -valeric acid.

Some of the alkylphenoxyacetic acids were already reported by Sandulesco, *et al.*[3] but compounds of 2-alkylphenoxy-propionic, -butyric, and -valeric acid series have not been reported. A synthetic method for alkylphenoxyacetic acid was not described at all in any literature. Aryloxyacetic acid is usually prepared by combining phenol with haloacetic acid in the presence of sodium hydroxide. However, this method was not always suitable for the syntheses of *o*-alkylphenoxyalkanoic acid, as *o*-alkylphenol is not soluble in sodium hydroxide solution. 2-(*o*-Alkylphenoxy)alkanoic acids were obtained easily by the following method. Through reaction of *o*-alkylphonols and ethyl 2-haloalkanoate with sodium ethoxide in dehydrated ethanol, ethyl 2-(*o*-alkylphenoxy)alkanoates were obtained, which were readily hydrolyzed to 2-(*o*-alkylphenoxy)alkanoic acids by warming in aqueous or ethanolic solution of alkali hydroxide. The compounds of 2-(*p*-alkylphenoxy)alkanoic acid series were also synthesized by this method. On the other hand, condensation of *p*-alkylphenol with ethyl 2-haloalkanoate and hydrolysis of the produced ethyl 2-(*p*-alkylphenoxy)alkanoate was effected by means of sodium hydroxide in aqueous solution. The whole process of the reaction is illustrated in Chart 1.



R : $CH_3(CH_2)_n$, n : 5, 7, 9, 11 R′ : H, $CH_3(CH_2)_n$, n : 0, 1, 2, 3 X : Cl, Br

The compounds thus obtained are summarized in Table I. The compounds were obtained as colorless crystals, sparingly soluble in water and soluble in alkali solution with formation of the alkali salt.

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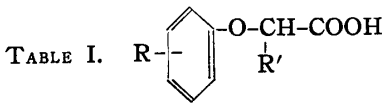
1) Part XXIII. F. Ueda : This Bulletin, **7**, 823(1959).
2) B. Fauconnier : Ann. inst. Pasteur, **89**, 101(1955)(C. A., **49**, 13355(1955)).
3) G. Sandulesco, A. Girard : Bull. soc. chim., (4)**47**, 1300(1930)(C. A., **25**, 1228(1931)).

Table I. 

Compd. No.	R	R′	b.p. (°C/mm. Hg)	m.p. (°C)
1	*o*-$CH_3(CH_2)_5$	H	184/4	89～90[a]
2	*o*-$CH_3(CH_2)_7$	H	206/6	85～86[b]
3	*o*-$CH_3(CH_2)_9$	H	203/4	85～86
4	*o*-$CH_3(CH_2)_{11}$	H	199/1	87～88
5	*p*-$CH_3(CH_2)_7$	H	198/1.5	65～66
6	*p*-$CH_3(CH_2)_9$	H	213/1.5	73～74
7	*o*-$CH_3(CH_2)_7$	CH_3	193/5	50～51
8	*o*-$CH_3(CH_2)_9$	CH_3	201/4	61～62
9	*p*-$CH_3(CH_2)_7$	CH_3	208/4.5	43～45
10	*p*-$CH_3(CH_2)_9$	CH_3	207/3	43～45
11	*o*-$CH_3(CH_2)_7$	CH_3CH_2	185/1	50～51
12	*o*-$CH_3(CH_2)_9$	CH_3CH_2	208/1	52～53
13	*p*-$CH_3(CH_2)_7$	CH_3CH_2	205/1.5	42～43
14	*p*-$CH_3(CH_2)_9$	CH_3CH_2	228/4	36～37
15	*o*-$CH_3(CH_2)_5$	$CH_3(CH_2)_2$	192/5	67～68
16	*o*-$CH_3(CH_2)_7$	$CH_3(CH_2)_2$	216/5	55～56
17	*o*-$CH_3(CH_2)_9$	$CH_3(CH_2)_2$	204/4	59～60
18	*p*-$CH_3(CH_2)_5$	$CH_3(CH_2)_2$	181/3	35～36
19	*p*-$CH_3(CH_2)_7$	$CH_3(CH_2)_2$	204/3	36～37

All are colorless needle crystals.
Reported m.p.[3] *a*) 89.5～90 *b*) 88～89.5

These synthesized compounds were examined for their antiviral activity *in vivo* on the Nakayama strain of Japanese B encephalitis virus, *in vitro* on the PR-8 strain of influenza A virus, and the Lansing strain of poliomyelitis virus. The procedure of screening tests are described in the experimental section. The results are shown in Tables II～VI.

Table II shows that *o*-hexylphenoxyacetic acid has weak activity *in vivo* against the Nakayama strain, but the other compounds are ineffective when tested as mentioned above. From Table III, it may be noted that 2-(*o*-decylphenoxy)butyric, 2-(*p*-octyl-

Table II. Antiviral Activity against Japanese B Encephalitis Virus

Compd. No.	Dose (mg./kg.)	Treated group[a]	Untreated group[a]	χ^2 [b]
1	50	9/40	3/40	2.45
2	50	3/40	3/40	—
3	35	6/40	3/40	0.52
4	45	3/40	3/40	—
5	50	5/40	3/40	—
6	55	4/40	3/40	—
7	40	4/40	3/40	—
8	38	4/40	3/40	—
9	38	5/40	3/40	—
10	38	6/40	3/40	0.52
11	27	3/40	4/40	—
12	18	4/40	4/40	—
13	70	3/40	3/40	—
14	40	3/40	3/40	—
15	38	4/40	3/40	—
16	40	3/40	3/40	—
17	20	4/40	3/40	—
18	45	3/40	2/40	—
19	45	3/40	2/40	—

a) The numerator represents the number of mice that survived and the denominator, total number injected.
b) $P(\chi^2>3.84)=0.05$

Table III. Antiviral Activity against Influenza A Virus

Compd. concn.(M) / Compd. No.	Hemaglutinin titer 2^n				
	$10^{-3.5}$	$10^{-4.0}$	$10^{-4.5}$	$10^{-5.0}$	Untreated group
1	n = 6.0	7.0	7.0	8.0	8.0
2	6.0	7.0	7.0	7.0	
3	3.0	4.0	7.0	8.0	
4	2.5	5.0	6.0	8.0	
5	7.0	7.0	8.0	8.0	
6	6.0	6.0	7.0	7.0	
7	3.0	5.0	5.0	6.0	
8	4.0	5.0	6.0	6.0	
9	7.0	7.0	7.0	7.0	
10	6.0	6.0	6.0	6.0	
11	4.5	6.0	7.0	8.0	
12	1.0	4.0	5.0	5.0	
13	1.5	4.0	4.0	4.0	
14	1.0	4.0	4.0	6.0	
15	2.0	6.0	7.0	7.0	
16	1.5	1.5	7.0	7.0	
17	1.0	1.0	8.0	8.0	
18	4.0	8.0	8.0	8.0	
19	1.0	6.0	8.0	8.0	

Table IV. Antiviral Activity against Poliomyelitis Virus

Compd. No.	LD_{50}		
	Treated group (Compd. concn.)		Untreated group
	500 γ/cc.	200 γ/cc.	
1	$10^{-2.29}$	$10^{-3.0}$	$10^{-3.5}$
2	$10^{-3.0}$	$10^{-3.0}$	
3	$10^{-3.0}$	$10^{-3.0}$	
4	$10^{-2.34}$	$10^{-3.0}$	
5	$10^{-2.94}$	$10^{-3.0}$	
6	$10^{-2.75}$	$10^{-3.0}$	
7	$10^{-3.0}$	$10^{-3.0}$	
8	$10^{-2.4}$	$10^{-3.0}$	
9	$10^{-2.5}$	$10^{-3.0}$	
10	$10^{-3.0}$	$10^{-3.0}$	
11	$10^{-2.42}$	$10^{-3.0}$	
12	$10^{-2.0}$	$10^{-3.0}$	
13	$10^{-2.12}$	$10^{-3.0}$	
14	$10^{-2.75}$	$10^{-3.0}$	
15	$10^{-2.2}$	$10^{-3.0}$	
16	$10^{-2.4}$	$10^{-3.0}$	
17	$10^{-2.0}$	$10^{-2.7}$	
18	$10^{-3.0}$	$10^{-3.0}$	
19	$10^{-3.0}$	$10^{-3.0}$	

phenoxy)butyric, 2-(*p*-decylphenoxy)butyric, 2-(*o*-hexylphenoxy)valeric, 2-(*o*-octylphenoxy)valeric, 2-(*o*-decylphenoxy)valeric, and 2-(*p*-octylphenoxy)valeric acids were active *in vitro* on the PR-8 strain. From Table IV, it is seen that *o*-hexylphenoxyacetic, *o*-decylphenoxyacetic, 2-(*o*-decylphenoxy)propionic, 2-(*p*-octylphenoxy)propionic, 2-(*o*-octylphenoxy)butyric, 2-(*o*-decylphenoxy)butyric, 2-(*p*-octylphenoxy)butyric, 2-(*o*-hexylphenoxy)valeric, 2-(*o*-octylphenoxy)valeric, and 2-(*o*-decylphenoxy)valeric acids were effective *in vitro* on the Lansing strain in the concentration of 500 γ/cc.

From the results thus obtained, it may be said that the compounds of this series are of interest for seeking a more effective antiviral drug against influenza and poliomyelitis virus.

In the studies reported by Stock and Francis,[4] it was shown that higher fatty acids have virucidal activity against influenza virus. It was later reported by Liu, *et al.*[5] that *l*-4-phenyl-4-(2-chlorobenzyl)-5-oxohexanoic acid was effective against influenza A and B virus.

At the present stage, it is difficult to discuss the relationship between antiviral activity and chemical structure of antiviral compounds. However, it seems that the presence of carboxyl group is advisable in finding compounds with antiviral activity against influenza virus. Although this assumption requires further studies, it may be said that the present work contributes to the syntheses of compounds having antiviral activities.

Experimental

General Method for Syntheses of 2-Alkylphenoxy-alkanoic Acid—a) To a solution of EtONa from 0.5 g. (0.022 mole) of metallic Na and 10 cc. of EtOH, 0.02 mole of alkylphenol and then 0.024 mole of ethyl 2-haloalkanoate were added. After stirring on a steam bath for 6 hr., EtOH was distilled off, the residue was treated with water, and extracted with ether. The combined ether extract was washed with water and ether removed. To the residue, a solution of 1.0 g. of NaOH in 20 cc. of water was added, the mixture was warmed on a steam bath with rapid stirring until the oily layer disappeared. When cool, the mixture was washed with ether and the aqueous layer was acidified with 10% HCl. The separated oily substance was extracted with ether, washed with water, and dried over Na_2SO_4. On evaporation of ether, the residue was purified by distillation under reduced pressure. The yield was 50~60%.

b) To a solution of 1.6 g. (0.04 mole) of NaOH in 5 cc. of water, a solution of 0.02 mole of *p*-alkylphenol and 0.02 mole of ethyl 2-haloalkanoate in 20 cc. of EtOH were added. The mixture was warmed on a steam bath for 6 hr. with stirring. After removal of EtOH, the residue was poured into 30 cc. of water and washed with ether. The aqueous layer was acidified with 10% HCl, the liberated oil was extracted with ether, washed with water, and dried over Na_2SO_4. On evaporation of ether, the residue was distilled under diminished pressure. The yield was 50~60%.

o-Alkyl derivatives were prepared by method (a) and *p*-alkyl derivatives by method (a) or (b). The analytical data are given in Table V.

Table V.

Compd. No.	Mol. formula	C (%)		H (%)	
		Calcd.	Found	Calcd.	Found
1	$C_{14}H_{20}O_3$	71.16	70.94	8.53	8.69
2	$C_{16}H_{24}O_3$	72.69	72.97	9.15	9.30
3	$C_{18}H_{28}O_3$	73.93	73.59	9.65	9.58
4	$C_{20}H_{32}O_3$	74.96	75.06	10.06	9.82
5	$C_{16}H_{24}O_3$	72.69	72.89	9.15	9.04
6	$C_{18}H_{28}O_3$	73.93	74.23	9.65	9.96
7	$C_{17}H_{26}O_3$	73.34	73.11	9.41	9.28
8	$C_{19}H_{30}O_3$	74.47	74.28	9.87	10.00
9	$C_{17}H_{26}O_3$	73.34	73.00	9.41	9.41
10	$C_{19}H_{30}O_3$	74.47	74.78	9.87	10.16
11	$C_{18}H_{28}O_3$	73.93	74.13	9.65	9.77
12	$C_{20}H_{32}O_3$	74.96	74.73	10 06	10.10
13	$C_{18}H_{28}O_3$	73.93	73.61	9.65	9.73
14	$C_{20}H_{32}O_3$	74.96	75.22	10.06	10.33
15	$C_{17}H_{26}O_3$	73.34	72.98	9.41	9.41
16	$C_{19}H_{30}O_3$	74.47	74.00	9.87	9.60
17	$C_{21}H_{34}O_3$	75.40	75.26	10.25	10.24
18	$C_{17}H_{26}O_3$	73.34	73.16	9.41	9.51
19	$C_{19}H_{30}O_3$	74.47	74.52	9.87	10.03

4) C. C. Stock, T. J. Francis : J. Exptl. Med., **71**, 661(1940). J. Immunol., **47**, 303(1943).
5) O. C. Liu, R. G. Maloberger, J. E. Carter, A. N. DeSanctis, F. P. Weiner, B. Hampil : J. Immunol., **78**, 214(1957).

Method of Screening Test for Antiviral Activity

1) **Japanese B Encephalitis Virus**—The experimental procedures were the same as those described in the preceding paper.[1)]

2) **Influenza Virus**—The PR-8 egg-adapted strain of influenza virus (Type A)*[2] was employed. Chorioallantoic membrane of 15-day embryonated egg was cut into pieces of 1.0 cm. in diameter. 0.1 cc. of 10^{-2} dilution of the virus was placed in a test tube containing 0.8 cc. of Hanks solution and a piece of the cut chorioallantoic membrane, 0.1 cc. of a sterillized solution of a compound was added to the test tube immediately after the viral inoculation. After shaking the culture at 37° for 18 hr., the viral content of the fluid in the test tube was estimated by chicken-cell agglutination. For chicken-cell agglutination, Horsfall's method was employed.

3) **Poliomyelitis Virus**—The experimental procedures were the same as those described in the preceding paper.[1)]

Summary

In order to study the antiviral effect of alkanoic acid containing alkylphenoxy group in 2-position, 2-alkylphenoxyacetic, -propionic, -butyric, and -valeric acids were synthesized. By the condensation of alkylphenol with ethyl 2-haloalkanoate, ethyl 2-alkylphenoxy-alkanoate was obtained, which was hydrolysed to 2-alkylphenoxyalkanoic acid.

o-Hexylphenoxyacetic acid had antiviral activity against Japanese B encephalitis virus *in vivo*. Several compounds in this series were found to be effective against the PR-8 strain of influenza A virus and the Lansing strain of poliomyelitis virus *in vitro*.

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*[2] The strain was given by Dr. K. Ando, the retired director of the National Institute of Health, Tokyo.