Calcd. for $C_{10}H_{10}ONBr$: C, 50.02; H, 4.20; N, 5.83; Br, 33.28. Found : C, 50.29; H, 4.25; N, 5.66; Br, 33.13.

α-Methyl-β-bromocinnamic Acid (X)——A mixture of α-methyl-β-bromocinnamamide (0.505 g.) and conc. HCl (15 ml.) was refluxed for 8 hr, After cooling, the mixture was diluted with H_2O, and made alkaline with 10% NaOH solution. The mixture was extracted with Et_2O to remove unchanged IX, and filtered. The filtrate was acidified with conc. HCl and extracted with Et_2O. The ethereal layer was washed with H_2O, treated with small amount of Norite, filtered and dried over Na_2SO_4. After evaporation of Et_2O, the residue was crystallized from benzene to give colorless prisms of α-methyl-β-bromocinnamic acid (X), m.p. 129~130°. Yield, 0.130 g. X showed depression in m.p. (96~108°) on admixture with authentic α-methyl-β-bromocinamic acid (XI) of m.p. 128~129°, which was prepared by Körner's method[7] and considered to be *cis*-α-methyl-β-bromocinnamic acid. (See Chart III.) UV : λ_{max} 255 mμ (log ε 3.74). IR : ν_{max} 1700 cm^{-1} (-COOH) (Nujol). *Anal.* Calcd. for $C_{10}H_9O_2Br$: C, 49.82; H, 3.76; Br, 33.15. Found : C, 49.74; H, 3.68; Br, 33.58.

The authors express their deep gratitude to Mr. M. Matsui, Director of this laboratory, for his encouragement throughout this work. The measurement of infrared and ultraviolet spectra were carried out by Mr. H. Higuchi, Misses N. Sawamoto and Y. Nakajima. Microanalyses were made by Dr. T. Onoe, Messrs. K. Ono, H. Nagashima and Misses K. Saito, N. Gonda and H. Masuda.

Summary

The addition of cyanogen bromide to an acetylenic bond was studied. Phenylacetylene reacted with cyanogen bromide in the presence of aluminum bromide to afford *trans*-β-bromocinnamonitrile. Similarly, *p*-bromo- and *p*-methoxyphenylacetylene, tolane and methylphenylacetylene yielded corresponding β-bromocinnamonitriles. Nevertheless, *p*-nitrophenylacetylene did not react with cyanogen bromide under the same conditions.

(Received June 27, 1964)

[Chem. Pharm. Bull. 12(12)1451~1457(1964)] *UDC 547.541.07 : 615.778.25*

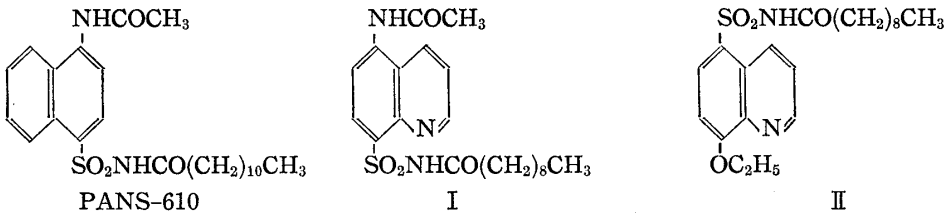
198. Tadakazu Tsuji, Junzo Kawabata, Sachiko Kobayashi, and Takeo Ueda : Syntheses and Antiviral Effect of *p*-Alkylbenzenesulfonamide Derivatives.

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

T. Ueda, *et al.*[1] found that N-dodecanoyl-4-acetamido-1-naphthalenesulfonamide (PANS-610) showed a significant chemotherapeutic effect on Japanese encephalitis virus in mice and human. After that, T. Itoh,[2] one of our group examined compounds of N^1-alkanoyl-N^4-alkanoylsulfanilamide, *p*-alkylbenzenesulfonic acid and N-alkanoyl-4-alkylbenzenesulfonamide related to PANS-610 as to their effect on the Nakayama strain of Japanese encephalitis virus in mice, but could not find any agent more effective than PANS-610. Also, Kawabata, *et al.*[3] found that N-decanoyl-5-acetamido-8-quinolinesulfonamide (I) and N-decanoyl-8-ethoxy-5-quinolinesulfonamide (II) exerted effects stronger than PANS-610 among derivatives of quinolinesulfonamide.

*1 Shinano-machi, Shinjuku-ku, Tokyo (辻 忠和, 川畑潤三, 小林佐知子, 上田武雄).

1) T. Ueda, S. Toyoshima : Keio J. of Med., **5**, 123 (1956).
2) T. Itoh, S. Toyoshima, T. Ueda : Papers read at the Annual Meeting of the Pharmaceutical Society of Japan (1954).
3) J. Kawabata, H. Koibuchi, S. Toyoshima : This Bulletin, 8, 788 (1960); J. Kawabata, H. Koibuchi, T. Itoh, S. Toyoshima : *Ibid.*, 8, 930 (1960).



Furthermore, PANS-610 was found to be effective on viral hapatitis, New Castle disease etc.[4] These findings suggested that additional compounds related to PANS-610 should be investigated as to their effects on pathogenic viruses.

In connection of N-alkanoyl-*p*-alkylbenzenesulfonamide, alkyl- and alkoxy-benzenesulfonamide derivatives of other types were synthesized and examined as to their effects on viruses of poliomyelitis, influenza and Japanese encephalitis.

This report is concerned with the syntheses and antiviral effect of 4-alkyl- and 4-alkoxy-benzenesulfonamide derivatives.

Syntheses of *p*-Alkylbenenesulfonamide Derivatives

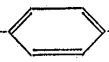
Firstly, *p*-alkylbenzenesulfonamides were synthesized. The properties of the higher members of this series, which were reported by Itoh,[2] were shown in Table I. *p*-Toluenesulfonamide was found effective on poliomyelitis virus among compounds of the above series. On the basis of this finding, the syntheses of N-alkyl, N-phenyl, and N-(4-alkylphenyl) derivatives of *p*-toluenesulfonamide were conceived. Several unknown compounds of this series were listed in Table I.

TABLE I. R-C6H4-SO2NHR'

R	R'	Crystal form	m.p. (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
C_6H_{13}[2]	H	thin plates	92~94	$C_{12}H_{19}O_2NS$						
C_8H_{17}[2]	〃	〃	94~96	$C_{14}H_{27}O_2NS$						
$C_{10}H_{21}$[2]	〃	〃	87~89	$C_{16}H_{27}O_2NS$						
$C_{18}H_{37}$[2]	〃	〃	100~101	$C_{24}H_{43}O_2NS$						
CH_3	C_8H_{17}	fine needles	59~61	$C_{15}H_{25}O_2NS$	63.58	8.89	4.94	63.41	8.92	5.01
〃	$C_{10}H_{21}$	〃	65~67.5	$C_{17}H_{29}O_2NS$	65.56	9.39	4.50	65.34	9.41	4.70
〃	$C_{12}H_{25}$	〃	69~71	$C_{19}H_{33}O_2NS$	67.22	9.80	4.13	67.50	9.98	4.18
〃	$C_{14}H_{29}$	〃	87~88	$C_{21}H_{37}O_2NS$	68.63	10.15	3.81	68.02	10.64	3.53
〃	C_6H_{13}-C6H4	prisms	80~81	$C_{19}H_{25}O_2NS$	68.86	7.60	4.23	69.00	7.08	4.29
〃	C_8H_{17}-C6H4	plates	90~92	$C_{21}H_{25}O_2NS$	70.17	8.13	3.90	70.41	8.07	3.99

Alkoxyl group attached to benzene ring was found to be important to exert an antiviral effect.[5] Taking this finding into consideration, attempts were made to replace alkyl group in alkylbenzenesulfonamide with alkoxyl group. Thus, *p*-alkoxybenzenesulfonamide, N-phenyl-*p*-alkoxybenzenesulfonamide and N-(4-alkoxyphenyl)-*p*-toluenesulfonamide were synthesized. The unknown compounds among these three series, were listed in Table II.

4) T. Ueda, S. Toyoshima : Keio J. of Med., **5**, 159 (1956).
5) F. Ueda : This Bulletin, **7**, 824 (1959).

TABLE II. R-  -SO_2NHR'

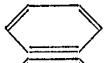
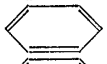
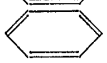
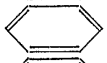
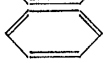
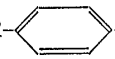
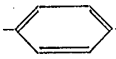
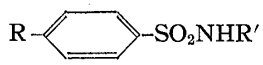
R	R′	Crystal form	m.p. (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
$C_5H_{11}O$	H	plates	98～98.5	$C_{11}H_{17}O_3NS$	54.31	7.04	5.76	54.45	7.19	5.71
$C_6H_{13}O$	〃	needles	99～101	$C_{12}H_{19}O_3NS$	56.02	7.44	5.44	56.43	7.24	5.58
$C_8H_{17}O$	〃	fine needles	102～103	$C_{14}H_{23}O_3NS$	58.93	8.13	4.91	59.13	8.15	4.98
$C_{10}H_{21}O$	〃	〃	100～102	$C_{16}H_{27}O_3NS$	61.29	8.68	4.47	61.65	8.75	4.56
C_3H_7O		needles	105～106	$C_{15}H_{17}O_3NS$	61.85	5.88	4.81	61.87	5.93	4.83
C_4H_9O		plates	94.5～95.5	$C_{16}H_{19}O_3NS$	62.94	6.27	4.59	62.92	6.22	4.58
$C_5H_{11}O$		needles	93～95	$C_{17}H_{21}O_3NS$	63.93	6.63	4.39	64.08	6.68	4.21
$C_6H_{13}O$		fine needles	99～100	$C_{18}H_{23}O_3NS$	64.85	6.95	4.20	64.65	6.99	4.25
$C_8H_{17}O$		〃	100～101	$C_{20}H_{27}O_3NS$	66.45	7.53	3.88	66.52	7.59	3.76
CH_3	C_4H_9O	prisms	97～98	$C_{17}H_{21}O_3NS$	63.93	6.63	4.39	64.05	6.79	4.40
〃	$C_5H_{11}O$	needles	105～107	$C_{18}H_{23}O_3NS$	64.86	6.95	4.20	64.69	7.00	4.48
〃	$C_6H_{13}O$	〃	96～98	$C_{19}H_{25}O_3NS$	65.69	7.25	4.03	65.88	7.36	4.05

TABLE III. R-  -SO_2NH-  -$CONHR'$

R	R′	Crystal form	m.p. (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
H	H	needles	218～219	$C_{13}H_{12}O_3N_2S$	56.52	4.34	10.14	56.81	4.39	10.01
〃	CH_3	prisms	194～195.5	$C_{14}H_{14}O_3N_2S$	57.93	4.86	9.65	57.85	4.73	9.77
〃	C_2H_5	〃	176～178	$C_{15}H_{16}O_3N_2S$	59.20	5.30	9.21	59.37	5.08	9.33
〃	C_3H_7	〃	194～195	$C_{16}H_{18}O_3N_2S$	60.37	5.70	8.80	60.51	5.69	9.00
CH_3	H	needles	181～183	$C_{14}H_{14}O_3N_2S$	57.93	4.86	9.65	58.10	4.71	9.57
〃	CH_3	prisms	226～227	$C_{15}H_{16}O_3N_2S$	59.20	5.30	9.21	58.93	5.26	9.07
〃	C_2H_5	〃	225～227	$C_{16}H_{18}O_3N_2S$	60.37	5.70	8.80	60.58	5.58	8.96
〃	C_3H_7	〃	215～216	$C_{17}H_{20}O_3N_2S$	61.43	6.07	8.43	61.65	5.98	8.30
C_2H_5	H	needles	210～212	$C_{15}H_{16}O_3N_2S$	59.20	5.30	9.21	59.43	5.52	9.20
〃	CH_3	fine needles	167～169	$C_{16}H_{18}O_3N_2S$	60.37	5.70	8.80	60.45	5.96	8.82
〃	C_2H_5	〃	171～173	$C_{17}H_{20}O_3N_2S$	61.43	6.07	8.43	61.07	5.76	8.58
〃	C_3H_7	needles	176～178	$C_{18}H_{22}O_3N_2S$	62.41	6.40	8.09	62.63	6.20	8.25
C_3H_7	H	〃	201～203	$C_{16}H_{18}O_3N_2S$	60.37	5.70	8.80	60.13	5.52	8.94
〃	CH_3	〃	169～171.5	$C_{17}H_{20}O_3N_2S$	61.43	6.07	8.43	61.15	6.02	8.56
〃	C_2H_5	plates	178～179.5	$C_{18}H_{22}O_3N_2S$	62.41	6.40	8.09	61.99	6.12	8.14
〃	C_3H_7	needles	161～162.5	$C_{19}H_{24}O_3N_2S$	63.32	6.71	7.77	63.26	6.63	7.80
iso-C_3H_7	H	〃	203～206	$C_{16}H_{18}O_3N_2S$	60.37	5.70	8.80	60.46	5.49	8.92
〃	CH_3	prisms	197～200	$C_{17}H_{20}O_3N_2S$	61.43	6.07	8.43	61.01	5.92	8.67
〃	C_2H_5	needles	142～144.5	$C_{18}H_{22}O_3N_2S$	62.41	6.40	8.09	62.42	6.15	8.24
〃	C_3H_7	〃	173～174.5	$C_{19}H_{24}O_3N_2S$	63.32	6.71	7.77	63.46	7.00	7.83
C_4H_9	H	fine needles	179～181	$C_{17}H_{20}O_3N_2S$	61.43	6.07	8.43	61.67	5.99	8.42
〃	CH_3	needles	132～134	$C_{18}H_{22}O_3N_2S$	62.41	6.40	8.09	62.91	6.13	8.29
〃	C_2H_5	plates	147～149.5	$C_{19}H_{24}O_3N_2S$	63.32	6.71	7.70	63.35	6.62	7.59
〃	C_3H_7	〃	144～146	$C_{20}H_{26}O_3N_2S$	64.15	7.00	7.48	64.63	7.27	7.73

Table IV. Antiviral Activities on Poliomyelitis Virus



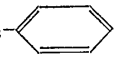
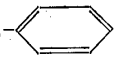
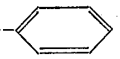
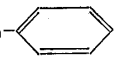
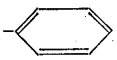
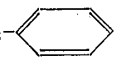
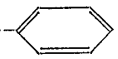
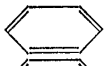
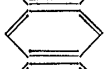
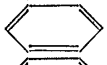
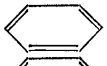
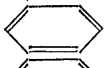
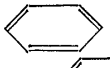
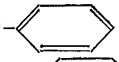
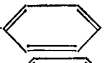
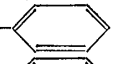
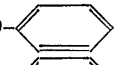
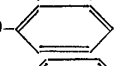
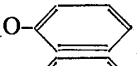
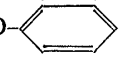
Compound R	Compound R′	Concentration of compound (γ/ml.)	log LD_{50} Treated group	log LD_{50} Untreated group	Effect
H	H	500	>3.0	4.0	−
		200	>3.0	4.0	
CH_3	〃	500	<2.0	4.0	+
		200	2.8	4.0	
C_2H_5	〃	500	<2.0	4.0	+
		200	2.2	4.0	
C_3H_7	〃	500	<2.0	4.0	+
		200	2.5	4.0	
C_4H_9	〃	500	<2.0	4.0	+
		200	>3.0	4.0	
C_6H_{13}	〃	500	2.8	4.0	−
		200	>3.0	4.0	
C_8H_{17}	〃	500	2.8	4.0	−
		200	>3.0	4.0	
$C_{10}H_{21}$	〃	500	3.0	4.0	−
		200	3.0	4.0	
$C_{18}H_{37}$	〃	500	>3.0	4.0	−
		200	3.0	4.0	
CH_3	CH_3	500	<2.0	3.7	+
		200	2.8	3.7	
〃	C_2H_5	500	<2.0	3.7	+
		200	2.6	3.7	
〃	C_3H_7	500	<2.0	3.7	+
		200	2.8	3.7	
〃	C_4H_9	500	<2.0	3.7	+
		200	3.0	3.7	
〃	C_6H_{13}	500	3.0	3.7	−
		200	>3.0	3.7	
〃	C_8H_{17}	500	3.0	3.7	−
		200	3.0	3.7	
〃	$C_{10}H_{21}$	500	>3.0	3.7	−
		200	3.0	3.7	
〃	$C_{12}H_{25}$	500	3.0	3.7	−
		200	3.0	3.7	
〃	$C_{14}H_{29}$	500	3.0	3.7	−
		200	3.0	3.7	
〃		500	<2.0	3.6	+
		200	2.2	3.6	
〃	CH_3– 	500	2.2	3.6	−
		200	3.5	3.6	
〃	C_2H_5– 	500	<2.0	3.6	+
		200	3.0	3.6	
〃	C_3H_7– 	500	<2.0	3.6	+
		200	3.4	3.6	
〃	C_4H_9– 	500	2.2	3.6	−
		200	3.4	3.6	
〃	C_5H_{11}– 	500	2.6	3.6	−
		200	3.6	3.6	
〃	C_6H_{13}– 	500	3.2	3.6	−
		200	>3.5	3.6	
〃	C_8H_{17}– 	500	>3.0	3.6	−
		200	>3.0	3.6	

Table V. Antiviral Activities on Poliomyelitis Virus

R-  -SO_2NHR'

Compound R	Compound R′	Concentration of compound (γ/ml.)	log LD_{50} Treated group	log LD_{50} Untreated group	Effect
CH_3O	H	500	2.2	4.0	+
		200	3.0	4.0	
C_2H_5O	〃	500	2.4	4.0	+
		200	2.8	4.0	
C_3H_7O	〃	500	2.6	4.0	−
		200	>3.0	4.0	
C_4H_9O	〃	500	2.6	4.0	−
		200	>3.0	4.0	
$C_5H_{11}O$	〃	500	3.0	4.0	−
		200	>3.0	4.0	
$C_6H_{13}O$	〃	500	3.0	4.0	−
		200	>3.0	4.0	
$C_8H_{17}O$	〃	500	3.0	4.0	−
		200	>3.0	4.0	
$C_{10}H_{21}O$	〃	500	3.0	4.0	−
		200	>3.0	4.0	
CH_3O		500	2.6	3.8	−
		200	>3.0	3.8	
C_2H_5O		500	2.8	3.8	−
		200	>3.0	3.8	
C_3H_7O		500	3.0	3.8	−
		200	>3.0	3.8	
C_4H_9O		500	>3.0	3.8	−
		200	>3.0	3.8	
$C_5H_{11}O$		500	>3.0	3.8	−
		200	3.0	3.8	
$C_6H_{13}O$		500	>3.0	3.8	−
		200	3.0	3.8	
$C_8H_{17}O$		500	>3.0	3.8	−
		200	3.0	3.8	
CH_3	HO- 	500	2.4	4.2	+
		200	2.8	4.2	
〃	CH_3O- 	500	2.2	4.2	+
		200	2.6	4.2	
〃	C_2H_5O- 	500	>2.8	4.2	−
		200	>3.0	4.2	
〃	C_3H_7O- 	500	>2.8	4.2	−
		200	>3.0	4.2	
〃	C_4H_9O- 	500	>2.8	4.2	−
		200	>3.0	4.2	
〃	$C_5H_{11}O$- 	500	>3.0	4.2	−
		200	>3.0	4.2	
〃	$C_6H_{13}O$- 	500	>3.0	4.2	−
		200	>3.0	4.2	

Recently, our group found that carboxamido group contributed to exert an antiviral activity in the study of antiviral agents on common cold virus.[6] Suggested by this finding, the introduction of carboxamido group into the structure of *p*-alkylbenzenesulfonamide was conceived. *p*-(*p*-Alkylbenzenesulfonamido)-N-alkylbenzamide obtained hereof were listed in Table Ⅲ.

Results of Antiviral Tests

p-Alkylbenzenesulfonamide derivatives of the seven series were tested as to their

6) M. Furukawa, S. Toyoshima, T. Ueda : This Bulletin, **11**, 1249 (1963).

activities on viruses of Japanese encephalitis (Nakayama strain), influenza A (PR-8 strain) and poliomyelitis (Lansing strain). All of these compounds were found ineffective on the Nakayama strain and on the PR-8 strain. Therefore, these data are omitted in this report. On the other hand, several compounds exerted *in vitro* activity on the Lansing strain virus. The experimental results were shown in Table Ⅳ and Ⅴ. The data of ineffective *p*-(*p*-alkylbenzenesulfonamido)-N-alkylbenzamide series was omitted.

As can be seen from the tables, the compounds having lower alkyl and alkoxyl groups were, in general, found effective on poliomyelitis virus. Some of these compounds showed activity approximately equal to that of hydroquinone discovered by Horsfall.[7] Since benzenesulfonamide lacked any activity on poliomyelitis virus, the effect of active compounds should be due to the introduction of alkyl and alkoxyl groups.

In alkylbenzenesulfonamide derivatives, the introduction of lower alkyl group into benzenesulfonamide gives rise to *in vitro* activity on poliomyelitis virus, while the introduction of alkoxyl group in lieu of alkyl partakes in decrease of the antiviral activity. The N-substitution of alkylbenzenesulfonamide with alkyl, alkylphenyl or alkoxyphenyl was found not to support the increase of antiviral activity of the basal compound.

The above effective compounds were examined as to their *in vivo* effect on the Lansing strain virus infection in mice. As the results, *p*-toluenesulfonamide and N-phenyl-*p*-toluenesulfonamide showed the significant *in vivo* effect on the multiplication of virus in mice, but all of other compounds did not exert any effect. The latter compound was selected as the best among the two effective compounds in the balance of effectiveness, toxicity, hemolytic action and other pharmacological property. The effectiveness of N-phenyl-*p*-toluenesulfonamide was shown in Table Ⅵ. LD_{50} of N-phenyl-*p*-toluene-

Table Ⅵ. *in vivo* Effect of N-Phenyl-*p*-toluenesulfonamide

Dose (mg./kg.)	Hours after viral inoculation	Treated group	Untreated group	$\chi^{2\,b)}$	Effect
22	24	19/30[a)]	9/30	10.04	+
14	24	18/30	9/30	5.45	+
11	24	11/30	9/30	0.3	−
22	48	23/30	9/30	13.13	+
14	48	17/30	9/30	4.34	+
11	48	16/30	9/30	3.36	−
22	72	19/30	9/30	10.04	+
14	72	14/30	9/30	1.76	−
11	72	10/30	9/30	0.08	−
22	96	17/30	9/30	4.34	+
14	96	17/30	9/30	4.34	+
11	96	14/30	9/30	1.76	−
14	24, 48[c)]	18/30	8/30	6.79	+
14	24, 48, 72[c)]	15/30	8/30	3.45	−
14	24, 48, 72, 96[c)]	17/30	8/30	5.55	+
11	24, 48, 72[c)]	18/30	10/30	4.29	+
11	24, 48, 72, 96[c)]	17/30	10/30	3.30	−
11	24, 48, 72, 96, 120[c)]	18/30	10/30	4.29	+

a) $\frac{\text{number of survived mice}}{\text{number of total mice employed}}$
b) $P(\chi^2 > 3.84) = 0.05$
c) The compound was administered repeatedly.

sulfonamide was 44 mg./kg. in the intravenous administration. As can be understood in Table Ⅵ, N-phenyl-*p*-toluenesulfonamide was found effective on the virus by the intravenous injection with the single dose of 22 mg./kg. 96 hours later after the viral

7) H. L. Horsfall, I. Tamm : Ann. Rev. Microbiol., **11**, 339 (1957).

inoculation, and with the single dose of 14 mg./kg. 48 hours later after the viral inoculation. It was considered that this compound was ineffective with the single dose of 11 mg./kg. However, if 11 mg./kg. dose of this compound was administered repeatedly, once daily for 3 to 5 days, it exerted the *in vivo* activity.

On the other hand, the authors found that hydroquinone having an *in vitro* activity, did not show any *in vivo* activity on the Lansing strain virus in mice. This finding is of interest that there existed an antiviral agent having both *in vitro* and *in vivo* effects among the series of alkylbenzenesulfonamide.

Nextly, all compounds of *p*-(*p*-alkylbenzenesulfonyl)-N-alkylbenzamide did not show any antiviral activity, but some compounds of this series were found to have hyperglycemic activities. The study on this line will be published in a medical journal in the future.

Experimental

Syntheses of *p*-Alkylbenzenesulfonamide Derivatives——All compounds were synthesized by the reaction of *p*-alkyl- or *p*-alkoxy-benzenesulfonyl chloride with amine or ammonia, according to the known method.[8~10] These compounds were recrystallized from dil. EtOH.

Mothods of Screening Test for Antiviral Activity——(1) *in vivo* Test on Japanese encephalitis virus: 0.3 ml. of $2\times LD_{50}$ of the Nakayama strain ($LD_{50}=10^{-2}$) was inoculated intraperitoneally into groups of mice and 72 hr. later, $1/2\,LD_{50}$ of the test compound was injected intravenously into these mice as a single dose. After daily observation for two weeks, the ratio of the number of survived and total mice used was recorded.

(2) *in vitro* Test on influenza A virus: The cholioallantoic membrane of fertilized eggs was cut into pieces with diameter of 0.1 cm. and each piece of these cut membranes was added to a test tube containing 0.8 ml. of Hanks' solution. Then 0.1 ml. of 10^{-2} of the egg-adapted PR-8 strain and 0.1 ml. of a dilution of the test compound were added into these tubes. After shaking the culture at 37° for 18 hr., the medium was removed and its HA value was estimated by using the pattern method. For the control group, 0.1 ml. of phosphate buffer solution (pH 7.6) was added instead of the dilution of a compound.

(3) *in vitro* Test on poliomyelitis virus: The Lansing strain of poliomyelitis virus was employed. The D. M. K. strain of mice, 7~9 g. in body weight, was used for the experiments. Various viral dilutions of the Lansing strain were prepared. Each 0.1 ml. of the dilution was placed in a test tube containing 0.1 ml. of a sterilized solution of a compound and 0.8 ml. of Lush's solution. After incubation at 37° for 1 hr., 0.03 ml. of each of this mixture was inoculated intracerebrally into each mouse. After daily observation for 3 weeks, LD_{50} of the treated and untreated groups was calculated by the method of Reed and Muench.[11] log LD_{50} of the treated group was compared to that of the untreated group. If Δ log LD_{50} was over 1.5 (compound concentration; 500 γ/ml.), the compound was considered to be effective.

(4) *in vivo* Test on poliomyelitis virus: The viral dilution, $4\times LD_{50}$, was inoculated intracerebrally into groups of 30 mice and 24~96 hr. later, the mice received the dose of 11~22 mg./kg. ($1/3\sim 1/2\times LD_{50}$) of the compound intravenously. After daily observation for 21 days, χ^2 was calculated from the survival ratio of the treated group to the untreated group. If χ^2 was over 3.8 ($P=0.05$), it was recognized as a significant value.

Summary

Seven series of compounds, *i.e.* *p*-alkylbezenesulfonamide, N-alkyl and N-(4-alkylphenyl) derivatives of *p*-toluenesulfonamide, *p*-alkoxybenzenesulfonamide, N-phenyl-*p*-alkoxybenzenesulfonamide, N-(4-alkoxyphenyl)-*p*-toluenesulfonamide and *p*-(*p*-alkylbenzenesulfonamido)-N-alkylbenzamide, were synthesized and examined as to their antiviral activities on viruses of Japanese encephalitis, influenza A and poliomyelitis. Several compounds were found effective for the *in vitro* test on Lansing strain of poliomyelitis virus. Among these effective compounds, N-phenyl-*p*-toluenesulfonamide was found to possess the promising *in vivo* activity on Lansing strain virus.

(Received June 29, 1964)

8) F. Reverdin: Ber., **42**, 1526 (1909).
9) W. Markwald, D. Huelshoff: *Ibid.*, **32**, 561 (1899).
10) E. H. Huntress, F. H. Caster: J. Am. Chem. Soc., **62**, 603 (1940).
11) L. J. Reed, H. Muench: Am. J. Hyg., **27**, 493 (1938).