CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 10 No. 1 **January 1962**

UDC 615.778-012+615.778-092

1. Atsushi Takada and Hiroko Nishimura : Researches on Chemotherapeutic Drugs against Viruses. XXXIV.*1 Syntheses and Antiviral Activity of 10-(ω-Dialkylaminoalkyl)-alkylphenothiazine and N-Substituted 10-Glycyl-alkylphenothiazine.

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As recently reported by this research group[1] several antiviral compounds were obtained by the introduction of alkyl group into the structure of neurotropic drugs. Based on these findings, the present work was taken up to find new antiviral compounds by introducing alkyl group into the structure of 10-(ω-dialkylaminoalkyl)-phenothiazine, a known tranquilizing agent, and 10-glycylphenothiazine, an antispasmodic agent. Thus, alkylated derivatives of 10-(ω-dialkylaminoalkyl)-phenothiazine and N-substituted 10-glycylphenothiazine were newly synthesized and their activity on adenovirus was examined, using the tissue culture method.

Synthesis of Alkylphenothiazine

To synthesize the compounds of above two series, alkylphenothiazine was employed as a starting material, which have not been synthesized, except 3-methyl,[2] 3-ethyl,[3] and 2-ethyl[4] derivatives.

The derivatives substituted with alkyl group in 1- or 3-position of phenothiazine ring were prepared according to the general method illustrated in Chart 1.

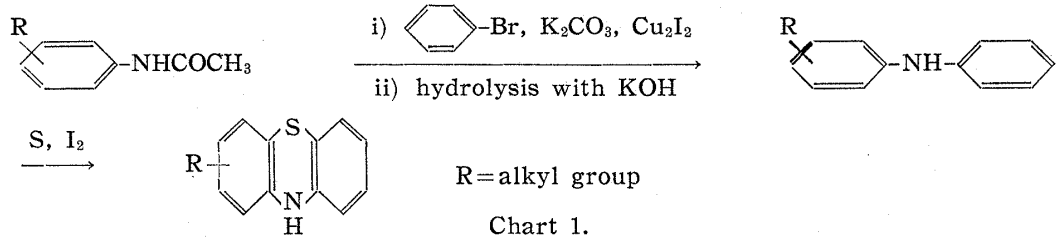

Chart 1.

First, alkyldiphenylamine was prepared by the condensation of alkylacetanilide and bromobenzene in the presence of potassium carbonate and cuprous iodide, followed by hydrolysis with ethanolic potassium hydroxide solution. Alkyldiphenylamine was converted

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*1 This paper constitutes part of a series entitled "Researches on Chemotherapeutic Drugs against Viruses" by Takeo Ueda. Part XXXIII : This Bulletin, **9**, 908 (1961).

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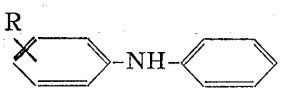
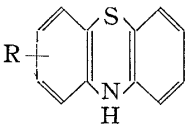
1) K. Takahashi, K. Ohki, T. Mizuma, S. Toyoshima : This Bulletin, 8, 758 (1960); T. Tsuji, T. Mizuma, S. Toyoshima : *Ibid.*, 8, 763 (1960); A. Takada, S. Toyoshima : *Ibid.*, 8, 767 (1960).
2) H. Gilman, D. A. Shirley : J. Am. Chem. Soc., **66**, 888 (1944).
3) G. Cauquil, A. Casadevall, E. Casadevall : Compt. rend., **236**, 1363 (1953).
4) A. Burger, J. B. Clements : J. Org. Chem., **19**, 1113 (1954).

to alkylphenothiazine by thermal reaction with sulfur in the presence of iodine as the catalyst. The data for the compounds of alkyldiphenylamines and alkylphenothiazines prepared as above are listed in Tables I and II.

TABLE I. 

R	b.p. (°C/mm.Hg)	m.p. (°C)	Yield (%)	Appearance	Formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
2-CH_3	131/3	—	56	Colorless oil	$C_{13}H_{13}N$	——		——		7.64	7.58
4-CH_3	138~141/4	88~89	74	Colorless plates	$C_{13}H_{13}N$	——		——		7.64	7.59
2-C_2H_5	131~132/3	—	50	Colorless oil	$C_{14}H_{15}N$	85.23	85.22	7.66	7.94	7.10	7.29
4-C_2H_5	148~153/3	85~86	45	Colorless plates	$C_{14}H_{15}N$	85.23	84.85	7.66	7.64	7.10	7.12
4-C_3H_7	163~165/3	65~66	87	〃	$C_{15}H_{17}N$	——		——		6.63	6.45
4-C_4H_9	163~168/3	—	45	Colorless oil	$C_{16}H_{19}N$	——		——		6.22	6.35
4-C_5H_{11}	178~185/3	—	32	〃	$C_{17}H_{21}N$	——		——		5.86	5.93

TABLE II. 

R	b.p. (°C/mm.Hg)	m.p. (°C)	Yield (%)	Appearance	Formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
1-CH_3	—	136	16	Yellow prisms	$C_{13}H_{11}NS$	73.22	73.31	5.20	5.20	6.57	6.73
3-CH_3	192~193/2.5	171~172	33	Yellow plates	$C_{13}H_{11}NS$	73.22	73.18	5.20	5.31	6.57	6.61
1-C_2H_5	180~183/2.5	126	20	Yellow prisms	$C_{14}H_{13}NS$	73.99	74.20	5.77	5.87	6.16	6.34
3-C_2H_5	187~190/2	132	36	Yellow plates	$C_{14}H_{13}NS$	73.99	73.55	5.77	5.92	6.16	5.92
3-C_3H_7	179~185/0.7	136~137	22	〃	$C_{15}H_{15}NS$	74.66	74.36	6.27	6.21	5.81	5.88
3-C_4H_9	220/3	125~126	42	〃	$C_{16}H_{17}NS$	75.24	75.08	6.71	6.70	5.49	5.62
3-C_5H_{11}	210~218/1	112	14	〃	$C_{17}H_{19}NS$	75.81	75.92	7.11	7.03	5.20	5.31

The method described above was, however, found unsuitable for the preparation of 2-alkyl derivatives, because two kinds of isomers substituted at 2- or 4-position of phenothiazine ring were produced. Therefore, the method for obtaining 2-ethylphenothiazine by the reduction of 2-acetylphenothiazine was considered for 2-alkylphenothiazines. By employing the modification of this method, 2-alkylphenothiazine was prepared through the reduction of 2-alkanoylphenothiazine, according to the scheme shown in Chart 2.

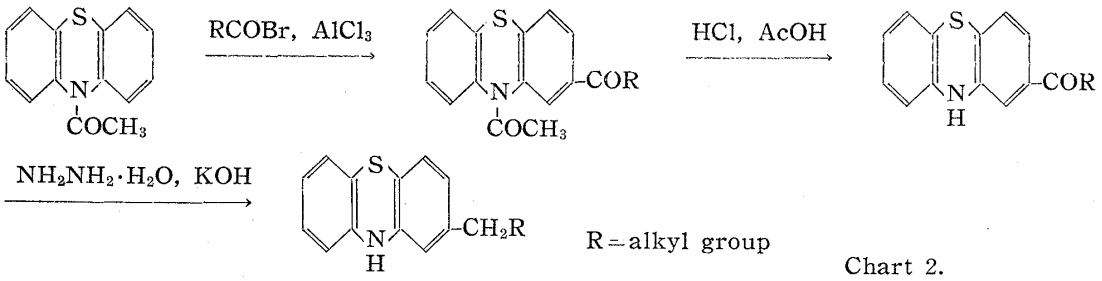
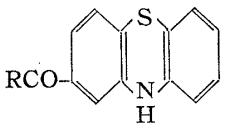


Chart 2.

The intermediate, 10-acetyl-2-alkanoylphenothiazine was obtained in nearly quantitative yield by the Friedel-Crafts condensation of 10-acetylphenothiazine with alkanoyl bromide. After deacetylation of the product with acetic acid and hydrochloric acid, the resulting 2-alkanoylphenothiazine was submitted to reduction by the Wolff-Kischner method. 2-Alkanoylphenothiazine and 2-alkylphenothiazine thus obtained are listed in Tables III and IV.

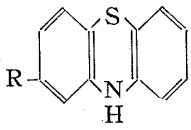
TABLE III.



RCO	m.p. (°C)	Yield (%)	Formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
CH_3CO	189~195	42	$C_{14}H_{11}ONS$	—		—		5.80	5.73
C_2H_5CO	170	38	$C_{15}H_{13}ONS$	70.58	70.24	5.13	5.09	5.49	5.58
C_3H_7CO	197	49	$C_{16}H_{15}ONS$	71.36	71.20	5.61	5.67	5.20	5.04
C_4H_9CO	197~198	53	$C_{17}H_{17}ONS$	—		—		4.94	4.87
$C_5H_{11}CO$	191~192	60	$C_{18}H_{19}ONS$	72.70	72.79	6.44	6.55	4.71	4.71
$C_7H_{15}CO$	189~190	48	$C_{20}H_{23}ONS$	—		—		4.30	4.39

All compounds are orange needles.

TABLE IV.



R	m.p. (°C)	Yield (%)	Formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
C_2H_5	135	50	$C_{14}H_{13}NS$	—		—		6.16	6.05
C_3H_7	143	66	$C_{15}H_{15}NS$	74.66	74.80	6.27	6.28	5.81	5.79
C_4H_9	133~134	56	$C_{16}H_{17}NS$	75.24	75.23	6.71	6.75	5.49	5.68
C_5H_{11}	136	43	$C_{17}H_{19}NS$	—		—		5.20	5.35
C_6H_{13}	124~125	83	$C_{18}H_{21}NS$	—		—		4.94	5.05
C_8H_{17}	116	50	$C_{20}H_{25}NS$	—		—		4.50	4.53

All compounds are colorless needles.

Synthesis of 10-(ω-Dialkylaminoalkyl)-alkylphenothiazines

Compounds of 10-(ω-dialkylaminoalkyl)-alkylphenothiazine series have not been reported yet, but its parent compound, 10-(2-dialkylaminoethyl)-phenothiazine and 10-(3-dialkylaminopropyl)-phenothiazine have already been synthesized by various workers.[2,5] Taking this into consideration, 10-(ω-dialkylaminoalkyl)-alkylphenothiazine was synthesized by the condensation of alkylphenothiazine with ω-dialkylaminoalkyl chloride in the presence of sodium amide in xylene. The reaction route is shown in Chart 3.

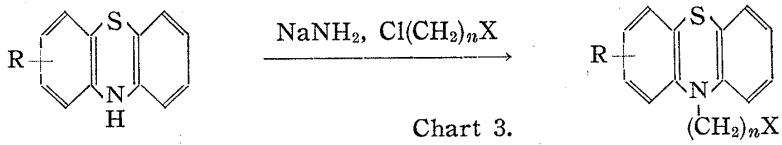


Chart 3.

R = alkyl group
X = dialkylamino group
n = 2 or 3

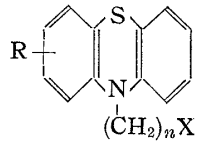
Thus, 15 compounds of 10-(ω-dialkylaminoalkyl)-alkylphenothiazine were obtained. These compounds in the form of free base are listed in Table V, and Table VI shows their citrate which were submitted to screening test for antiviral activity.

Synthesis of N-Substituted 10-Glycyl-alkylphenothiazine

Any compound of 10-glycylalkyl-phenothiazine derivatives have not been synthesized, but their parent compound, N-substituted glycylphenothiazine, was already synthesized from 10-(2-chloroacetyl)phenothiazine and amines by Dahlbom, *et al.*[6] Taking this

5) P. Viaud: J. Pharm. Pharmacol., **6**, 361 (1954); H.L. Yale, F. Sowinski, J. Bernstein: J. Am. Chem. Soc., **79**, 4375 (1957); P. Charpentier: U.S. Pat. 2,519,886.
6) R. Dahlbom, T. Ekstrand: Acta Chem. Scand., **5**, 102 (1951).

TABLE V.



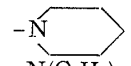
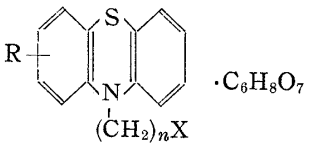
R	n	X	b.p. (°C/mm. Hg)	Yield (%)	Formula	Analysis (%) C Calcd.	C Found	H Calcd.	H Found	N Calcd.	N Found
1-CH_3	2	$-N(C_2H_5)_2$	187/2	40	$C_{19}H_{24}N_2S$	73.04	72.74	7.74	7.69	8.97	8.67
1-C_2H_5	2	〃	187～189/2	38	$C_{20}H_{26}N_2S$	73.59	73.33	8.03	7.87	8.58	8.37
2-C_2H_5	2	〃	207～209/2	44	$C_{20}H_{26}N_2S$	73.59	73.39	8.03	7.94	8.58	8,45
2-C_3H_7	2	〃	209/2	33	$C_{21}H_{28}N_2S$	74.08	74.02	8.29	8.38	8.23	7.80
2-C_4H_9	2	〃	223～225/3	31	$C_{22}H_{30}N_2S$	74.54	74.63	8.53	8.48	7.90	7.98
〃	2		225～228/1	23	$C_{23}H_{30}N_2S$	—		—		7.64	7.62
2-C_5H_{11}	2	$-N(C_2H_5)_2$	228/2	21	$C_{23}H_{32}N_2S$	—		—		7.60	7.82
2-C_6H_{13}	2	〃	235/3	18	$C_{24}H_{34}N_2S$	75.35	75.38	8.96	8.95	7.32	7.02
3-CH_3	2	〃	201～203/2	42	$C_{19}H_{24}N_2S$	73.04	73.17	7.74	7.89	8.97	8.84
〃	2	$-N(CH_3)_2$	187/0.6	15	$C_{17}H_{20}N_2S$	—		—		9.85	9.73
〃	3	$-N(C_2H_5)_2$	205～207/2	20	$C_{20}H_{26}N_2S$	73.59	73.15	8.03	7.90	8.58	8.40
3-C_2H_5	2	〃	202～204/2	41	$C_{20}H_{26}N_2S$	73.59	73.30	8.03	7.74	8.58	8.47
〃	3	〃	212～215/3	30	$C_{21}H_{28}N_2S$	74.08	74.17	8.29	8.26	8.23	8.23
3-C_3H_7	2	〃	211～215/3	23	$C_{21}H_{28}N_2S$	74.08	73.87	8.29	8.22	8.23	8.27

TABLE VI.



R	n	X	m.p. (°C)	Recrystn. solvent	Formula	Analysis (%) N Calcd.	N Found
1-CH_3	2	$-N(C_2H_5)_2$	132 (decomp.)	EtOH-Me_2CO	$C_{26}H_{34}O_7N_2S$	5.55	5.42
2-C_2H_5	2	〃	48～50	Me_2CO-Et_2O	$C_{26}H_{34}O_7N_2S$	5.40	5.41
2-C_3H_7	2	〃	58～60	〃	$C_{27}H_{36}O_7N_2S$	5.26	5.14
2-C_4H_9	2	〃	64～65	〃	$C_{28}H_{38}O_7N_2S$	5.13	4.91
2-C_5H_{11}	2	〃	53～54	〃	$C_{29}H_{40}O_7N_2S$	5.00	4.98
2-C_6H_{13}	2	〃	47～48	〃	$C_{30}H_{42}O_7N_2S$	4.88	4.96
3-CH_3	2	〃	153 (decomp.)	EtOH-Me_2CO	$C_{25}H_{32}O_7N_2S$	5.55	5.66
〃	3	〃	86～87	Me_2CO	$C_{26}H_{34}O_7N_2S$	5.40	5.49
3-C_2H_5	2	〃	138～139 (decomp.)	〃	$C_{26}H_{34}O_7N_2S$	5.40	5.43
〃	3	〃	78～79	〃	$C_{27}H_{36}O_7N_2S$	5.26	5.46
3-C_3H_7	2	〃	105～107	〃	$C_{27}H_{36}O_7N_2S$	5.26	5.28
3-C_4H_9[a]	2	〃	97～98	〃	$C_{28}H_{38}O_7N_2S$	5.13	5.23

a) Prepared by treating the reaction mixture with citric acid without purification by distillation.

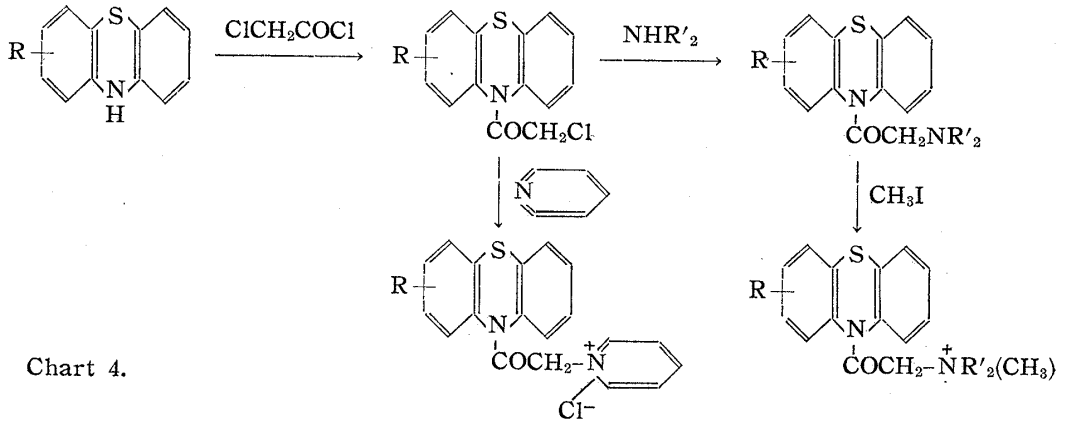


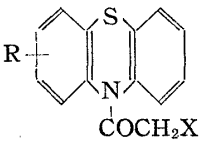
Chart 4.

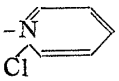
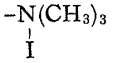
R=alkyl group R'2N=1-piperidyl or dimethylamino group

method of Dahlbom into consideration, 10-glycyl-alkylphenothiazine derivatives were synthesized, as shown in Chart 4.

As shown in Chart 4, 10-(2-chloroacetyl)-alkylphenothiazine employed as the intermediate was prepared by reacting alkylphenothiazine with chloroacetyl chloride. By the reaction of dialkylamine with 10-(2-chloroacetyl) derivative, 10-(N,N-dialkylglycyl)-alkylphenothiazine was obtained. 10-(N,N-Dimethylglycyl)-alkylphenothiazine was converted to its quarternary ammonium salt through reaction with methyl iodide, while 10-(2-pyridiniumacetyl)-alkylphenothiazine was obtained by reaction of 10-(2-chloroacetyl)-alkylphenothiazine with pyridine.

Thus, nine compounds of 10-(2-pyridiniumacetyl)-alkylphenothiazine chloride series, three compounds of 10-(2-trimethylammoniumacetyl)-alkylphenothiazine iodide series, and three compounds of 10-[2-(1-piperidyl)-acetyl]-alkylphenothiazine series were prepared with and these compounds are listed in Table Ⅶ.

Table Ⅶ. 

R	X	m.p. (℃)	Yield (%)	Formula	Analysis (%) N	
					Calcd.	Found
2-C_2H_5		227～228 (decomp.)	48.2	$C_{21}H_{19}ON_2ClS$	7.32	7.38
2-C_2H_5	〃	192～193(〃)	46.8	$C_{22}H_{21}ON_2ClS$	7.06	7.16
2-C_4H_9	〃	207(〃)	51.3	$C_{23}H_{23}ON_2ClS$	6.82	6.77
2-C_6H_{13}	〃	205(〃)	47.1	$C_{25}H_{27}ON_2ClS$	6.38	6.46
2-C_8H_{17}	〃	187(〃)	40.6	$C_{27}H_{31}ON_2ClS$	5.98	6.01
3-CH_3	〃	229～230(〃)	36.6	$C_{20}H_{17}ON_2ClS$	7.60	7.65
3-C_3H_7	〃	217～218(〃)	48.9	$C_{22}H_{21}ON_2ClS$	7.06	6.87
3-C_4H_9	〃	216～217(〃)	13.0	$C_{23}H_{23}ON_2ClS$	6.82	6.98
3-C_5H_{11}	〃	216(〃)	42.7	$C_{24}H_{25}ON_2ClS$	6.62	6.59
2-C_2H_5		119～120	34.5	$C_{21}H_{24}ON_2S$	7.95	7.92
2-C_3H_7	〃	124～125	38.7	$C_{22}H_{26}ON_2S$	7.36	7.45
2-C_4H_9	〃	72～73	32.4	$C_{23}H_{28}ON_2S$	7.65	7.72
8-CH_3		214～216	47.8	$C_{18}H_{21}ON_2IS$	6.09	6.18
3-C_3H_7	〃	207～208	26.0	$C_{20}H_{25}ON_2IS$	5.74	5.46
3-C_4H_9	〃	212	23.0	$C_{21}H_{27}ON_2IS$	5.58	5.41

All compounds of 10-(2-pyridiniumacetyl) derivatives are colorless plates, all of 10-[2-(1-piperidyl)-acetyl] derivatives are colorless prisms, and all of 10-(2-trimethylammoniumacetyl) derivatives are yellow needles.

Screening Test with 10-(ω-Dialkylaminoalkyl)-alkylphenothiazine and N-Substituted Glycylalkylphenothiazine

Screening tests of the compounds synthesized were carried out by using the adenovirus Types Ⅰ, Ⅱ, Ⅲ, and Ⅵ in the tissue culture system, as described in the experimental part. The results obtained are shown in Tables Ⅷ, Ⅸ, and Ⅹ.

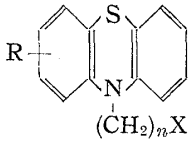
As can be seen in Table Ⅷ, 10-(3-diethylaminopropyl)-3-methyl- and 10-(3-diethylaminopropyl)-3-ethyl-phenothiazine were found to possess direct inactivating action on the adenovirus, and these two effective compounds were also inhibitory against cytopathogenic effect induced by $5\times TCD_{50}$ of adenovirus, as shown in Table Ⅸ. By the test with $50\times TCD_{50}$, however, none of the compounds were found to exert any effect on the virus.

As shown in Table Ⅹ, all compounds of 10-(2-pyridiniumacetyl)-alkylphenothiazine chloride series were found to have direct inactivating action on the adenovirus

Type I, showing more than 0.5 difference from the control, but other series of 10-glycyl-alkylphenothiazine were not effective. On the other hand, none of the compounds were inhibitory against cytopathogenic effect produced by $10\times TCD_{50}$ of adenovirus.

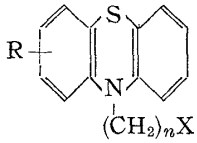
From the relationship between the antiviral activity and chemical structure of compounds of these series, the length or position of alkyl group substituted in the phenothi

Table VIII. Direct Inactivating Action of 10-(ω-Dialkylaminoalkyl)-alkylphenothiazine against Adenovirus



R	n	X	TCD_{50} Type I 10^{-x}	Type II 10^{-x}	Type III 10^{-x}	Type VI 10^{-x}
1-CH_3	2	-$N(C_2H_5)_2$	3.5	3.5	4.0	4.0
1-C_2H_5	2	〃	3.5	3.5	4.0	3.8
2-C_2H_5	2	〃	3.5	3.5	3.5	3.5
2-C_3H_7	2	〃	4.0	3.5	3.5	3.5
2-C_4H_9	2	〃	3.5	3.5	3.5	3.5
〃	2	-N⟨ ⟩	3.0	3.5	4.5	3.5
2-C_5H_{11}	2	-$N(C_2H_5)_2$	4.0	4.0	4.25	3.5
2-C_6H_{13}	2	〃	3.5	3.5	3.5	3.5
3-CH_3	2	〃	3.5	4.0	3.75	3.5
〃	2	-$N(CH_3)_2$	3.5	4.0	3.5	3.5
〃	3	-$N(C_2H_5)_2$	2.5	2.5	3.0	2.5
3-C_2H_5	2	〃	4.0	3.5	4.0	3.5
〃	3	〃	2.5	2.5	2.0	2.0
3-C_3H_7	2	〃	3.75	3.5	4.0	3.5
3-C_4H_9	2	〃	3.5	3.5	3.5	3.5
Untreated			3.5	3.5	4.5	3.5

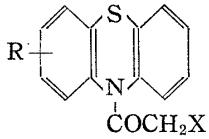
Table IX. Inhibitory Effect on Cytopathogenic Effect produced by $5\times TCD_{50}$ of Adenovirus

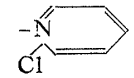
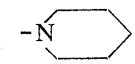
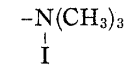


R	n	X	Max. nontoxic dose (10^{-x} M)	Type I	Type II	Type III	Type VI
1-CH_3	2	-$N(C_2H_5)_2$	6	0/4[a]	0/4	0/4	0/4
1-C_2H_5	2	〃	5	0/4	0/4	0/4	0/4
2-C_2H_5	2	〃	5	0/4	0/4	0/4	0/4
2-C_3H_7	2	〃	5	0/4	0/4	0/4	0/4
2-C_4H_9	2	〃	5	0/4	0/4	0/4	0/4
〃	2	-N⟨ ⟩	5	0/4	0/4	0/4	0/4
2-C_5H_{11}	2	-$N(C_2H_5)_2$	5	0/4	0/4	0/4	0/4
2-C_6H_{13}	2	〃	5	0/4	0/4	0/4	0/4
3-CH_3	2	〃	5	0/4	0/4	0/4	0/4
〃	2	-$N(CH_3)_2$	5	0/4	0/4	0/4	0/4
〃	3	-$N(C_2H_5)_2$	5	2/4	3/4	2/4	4/4
3-C_2H_5	2	〃	5	0/4	0/4	0/4	0/4
〃	3	〃	5	3/4	3/4	2/4	4/4
3-C_3H_7	2	〃	5	0/4	0/4	0/4	0/4
3-C_4H_9	2	〃	5	0/4	0/4	0/4	0/4
Untreated				0/4	0/4	0/4	0/4

a) The numerator represents the number of tubes without cytopathogenic effect and the denominator, total number of tubes.

Table X. Direct Inactivating Action of N-Substituted 10-Glycylalkylphenothiazine against Adenovirus Type I



R	X	Compd. concn. (10^{-x} M)	TCD_{50} Untreated group 10^{-x}	TCD_{50} Treated group 10^{-x}
2-C_2H_5		5	3.5	2.5
2-C_3H_7	〃	5	3.5	2.5
2-C_4H_9	〃	6	3.5	2.5
2-C_6H_{13}	〃	6	3.5	2.5
2-C_8H_{17}	〃	6	3.5	3.0
3-CH_3	〃	5	3.5	2.5
3-C_3H_7	〃	5	3.5	2.5
3-C_3H_7	〃	5	3.5	2.5
3-C_4H_9	〃	6	3.5	2.5
3-C_5H_{11}	〃	6	3.5	3.0
2-C_2H_5		5	3.5	3.5
2-C_3H_7	〃	5	3.5	3.5
2-C_4H_9	〃	3	3.5	3.5
3-CH_3		5	3.5	3.25
3-C_3H_7	〃	6	3.5	3.25

azine ring was considered not to influence the effect, but the substituent attached to nitrogen atom of phenothiazine ring seemed important in producing marked activity on the virus. Therefore, it seems that a more effective compound might be anticipated by introduction of a longer alkyl chain or other substituent into 10-position of phenothiazine. The work on this problem will be reported in the near future.

Experimental

General Procedure for Synthesis of 1- or 3-Alkylphenothiazine

Alkyldiphenylamine——A mixture of 0.027 mole of alkylacetanilide, 0.027 mole of PhBr, 2.5 g. of K_2CO_3, and 1 g. of Cu_2I_2 was heated under reflux for 25 hr. After cool, the reaction mixture was extracted with Et_2O. The Et_2O residue was refluxed with 2.5 g. of KOH in 35 cc. of EtOH for 5 hr. The reaction mixture was poured into 1 L. of water and extracted with Et_2O. After removal of Et_2O, the residue was distilled *in vacuo*.

Alkylphenothiazine——A mixture of 0.039 mole of alkyldiphenylamine, 2.5 g. of S, and 0.1 g. of I_2 was heated at 195° for 10~15 min. After cool, the reaction mixture was extracted with benzene. The benzene extract was chromatographed through a column of Al_2O_3, eluted with benzene, and the crude material was submitted to vacuum distillation. The distillate was purified by recrystallization from hexane.

General Procedure for Synthesis of 2-Alkylphenothiazines

10-Acetyl-2-alkanoylphenothiazine——To a mixture of 0.2 mole of 10-acetylphenothiazine and 0.21 mole of alkanoyl bromide in 2 L. of CS_2, 54 g. of anhyd. $AlCl_3$ was added with vigorous stirring. After stirring under reflux on a water bath for 48 hr., the supernatant CS_2 was discarded. The residue was poured into ice and HCl. The crude 10-acetyl-2-alkanoylphenothiazine was collected by filtration and submitted to subsequent reaction without further purification.

2-Alkanoylphenothiazine——A solution of 0.031 mole of 10-acetyl-2-alkanoylphenothiazine in 100 cc. of 20% HCl was heated under reflux for 10 min. After cool, the precipitate was collected by filtration, washed with water, and recrystallized from benzene.

2-Alkylphenothiazine——A mixture of 0.025 mole of 2-alkanoylphenothiazine, 4.1 g. of KOH, and 4.2 cc. of 80% $NH_2NH_2 \cdot H_2O$ in 33 cc. of ethylene glycol was refluxed for 2 hr. After distillation of

the mixture up to b.p. 190~195°, the residual solution was heated under reflux for 6 hr. The precipitate was collected by filtration, washed with water, and recrystallized from hexane.

General Procedure for Synthesis of 10-(ω-Dialkylaminoalkyl)alkylphenothiazine——To a solution of 0.014 mole of alkylphenothiazine in 20 cc. of dehyd. xylene, 0.7 g. of $NaNH_2$ was added gradually. After stirring for 2 hr. under reflux, a solution of 0.017 mole of ω-dialkylaminoalkyl chloride was added at 15~20° and the mixture was refluxed for 3 hr. in an oil bath. The reaction mixture was extracted with 5% HCl, the HCl extract was neutralized with 5% NaOH, and taken up in Et_2O. After removal of Et_2O by evaporation, the residual oil was purified by vacuum distillation. The citrate was prepared by treatment with citric acid in Et_2O.

General Procedure for Synthesis of N-Substituted 10-Glycylalkylphenothiazine

10-(2-Chloroacetyl)-alkylphenothiazine——To a solution of 0.01 mole of alkylphenothiazine in 20 cc. of xylene, 0.011 mole of chloroacetyl chloride was added. After refluxing for 8 hr., xylene was distilled off and residual syrup was submitted to next reaction without further purification.

10-(2-Pyridiniumacetyl)-alkylphenothiazine Chloride——A solution of 0.003 mole of 10-(2-chloroacetyl)alkylphenothiazine and 1 cc. of pyridine in 10 cc. of benzene was heated on a water bath for 8 hr. After cool, the precipitate was collected by suction and recrystallized from a mixture of EtOH-AmOH-hexane.

10-[2-(1-Piperidyl)acetyl]-alkylphenothiazine——A solution of 0.005 mole of 10-(2-chloroacetyl)-alkylphenothiazine and 1.0 g. of piperidine in 10 cc. of benzene was refluxed on a water bath for 8 hr. The reaction mixture was washed with water and extracted with 5% HCl. The HCl extract was neutralized with 5% NaOH and extracted with Et_2O. After removal of Et_2O, the residue was recrystallized from hexane.

10-(2-Trimethylammoniumacetyl)-alkylphenothiazine Iodide——A solution of 0.003 mole of 10-(2-chloroacetyl)alkylphenothiazine and 0.4 g. of Me_2NH in 10 cc. of benzene was warmed at 50° for 16 hr. The reaction mixture was treated by the same procedure as for piperidyl derivatives. After removal of Et_2O, the residual syrup was refluxed with 0.7 g. of MeI in 5 cc. of MeOH for 10 hr., MeOH was evaporated, and the residue was recrystallized from a mixture of EtOH-AmOH-hexane.

Screening Test for Antiviral Activity

1) Virus Inactivating Action : Adenovirus Types I, II, III, and VI were employed. Each 0.1 cc. of various viral dilution of adenovirus and 10× maximum nontoxic dose of the test compound were mixed in a test tube and 0.8 cc. of maintenance medium was added. For the maintenance medium, the YLA medium added with 5% horse serum was employed. After incubation at 37° for 24 hr., 1.0 cc. of this mixture was added into the tube in which the monolayer sheet of the HeLa cells had been established and further incubated at 37° for 7 days. The efficacy was determined according to the calculation of TCD_{50} of both the control and the treated, by using Reed and Muench's method[7] from the microscopic examination of cytopathogenic effect.

2) Inhibition of Cytopathogenic Effect induced by Adenovirus : To each of the tubes with established monolayer sheet of HeLa cells, 0.1 cc. of maximum nontoxic concentration of the test compound, and 0.8 cc. of the maintenance medium were added. Then, 0.1 cc. of the viral dilution was inoculated. The viral dilution employed was $5\times TCD_{50}$, $10\times TCD_{50}$, and $50\times TCD_{50}$ ($TCD_{50}=10^{-3.5}$/cc.). After incubation at 37° for 7 days, the efficacy was determined from microscopic examination of cytopathogenic effect.

Summary

Fifteen compounds of 10-(ω-dialkylaminoalkyl)-alkylphenothiazine series, nine compounds of 10-(2-pyridiniumacetyl)-alkylphenothiazine series, three compounds of 10-[2-(1-piperidyl)-acetyl]-alkylphenothiazine series, and three compounds of 10-(2-trimethylammoniumacetyl)alkylphenothiazine iodide series, which were substituted with alkyl group in 1-, 2-, or 3-position of the phenothiazine ring, were synthesized and their antiviral activity on adenovirus was tested by the tissue culture method. Among these compounds, 10-(3-diethylaminopropyl)-3-methyl- and 10-(3-diethylaminopropyl)-3-ethylphenothiazine, and all compounds of 10-(2-pyridiniumacetyl)-alkylphenothiazine chloride series were found to possess direct inactivating effect on the adenovirus. On cytopathogenic effect induced by adenovirus, 10-(3-diethylaminopropyl)-3-methyl and 10-(3-diethylaminopropyl)-3-ethyl derivatives also inhibited in $5\times TCD_{50}$ of virus.

(Received September 14, 1960)

7) L. J. Reed, H. Muench : Am. J. Hyg., **27**, 493 (1938).