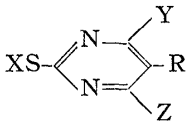
UDC 615.778[547.854.83]

89. Sumiko Watanabe, Tadakazu Tsuji, and Shigeshi Toyoshima : Syntheses and Antimicrobial Activity of 5-Alkyl-2-thiouracil Derivatives.

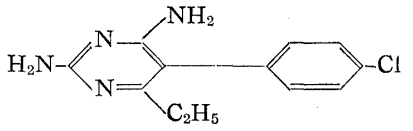
(Pharmaceutical Institute, Keio-Gijuku University[*1])

As described in the previous papers[1], compounds related to pyrimidine were synthesized and examined as to their antiviral activity by our research group in view of searching inhibitor to viral synthesis of nucleic acid. In continution of those studies, the authors took up 2-thio-5-alkyluracil derivatives having the following general formula and their antiviral activity was examined in these studies.



Wherein, X stands for hydrogen atom or alkyl group, Y for hydroxyl or amino group and Z for hydrogen atom, methyl group, hydroxyl group or chlorine atom. Among these compounds, any remarkably effective one was not found on viruses, but some of these compounds was found active on toxoplasma Gondii, one kind of intracellular parasites in mice.[2] This finding prompted the authors to examine antitoxoplasmosal activity of these compounds.

Any therapeutically effective agent has not been found on human toxoplasmosis yet. Sulfanilamide derivatives having substituted pyrimidine rings at N^1-position, such as sulfadiazine, sulfamethazine and sulfamerazine, were claimed to be effective on toxoplasma in mice, if the administration for the treatment on this parasite was initiated within the third day after the inoculation and continued for 14 days. On the other hand, pyrimethazine, 5-(*p*-chlorophenyl)-6-ethyl-2,4-diaminopyrimidine (I) jointly with sulfadiazine was reported to be curatively effective on the parasite in mice.[3]



I

Some of cured mice, however, was found to retain the virulent toxoplasma and remain carriers.[3] Therefore, it may be said that any highly chemotherapeutic agent on toxoplasmosis has not been revealed yet. Sabin and Olitsky[4] succeeded in the cultivation of toxoplasma in tissue culture and concluded that the development of this parasite was within host cells and any growth could not be observed in any cell-free medium. These findings suggested that toxoplasma bore a resemblance to virus in point of intracellular parasite. This suggestion led the authors to set about the solution of the problems on antitoxoplasmosal drugs. This paper describes the syntheses and antitoxoplasmosal activity of 2-thio-5-alkyluracil derivatives.

Synthesis of 2-Thio-5-alkyluracil and 2-Thio-5-alkyl-6-methyluracil——Anderson *et al.*[5] and Russell,[6] synthesized several compounds of 2-thio-5-alkyluracil, and Falco *et al.*[7] synthesized 2-thio-5-alkyl-6-methyluracil. According to these reports, compounds

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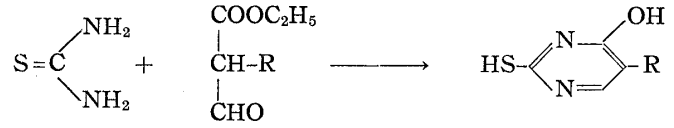
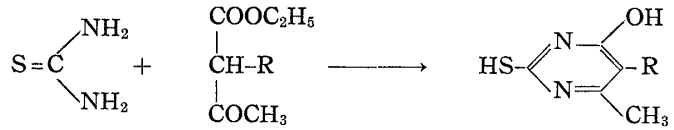
1) T. Tsuji, S. Watanabe, Y. Nakadai, S. Toyoshima : This Bulletin, **10**, 9 (1962); M. Muraoka, A. Takada : Keio J. Medicine, **11**, 95 (1962); I. Nakata, T. Ueda : Yakugaku Zasshi, **80**, 1065 (1960).
2) P.H. Van Thiel : Animal Parasites in Man (D. Van Nostrand Company INC) p. 137 (1961).
3) D.E. Eyles, E. Coleman : Antibiotics and Chemotherapy, **5**, 525 (1955).
4) A.B. Sabin, P.K. Olitsky : Science, **85**, 336 (1937).
5) G. Anderson, I.F. Halverstadt, W.H. Miller, R.O. Roblin : J. Am. Chem. Soc., **67**, 2199 (1945).
6) P. Russell : *Ibid.*, **73**, 3767 (1951).
7) E. Falco, S. DuBreuil, C.H. Hitchings : *Ibid.*, **73**, 3760 (1951).

of this series were prepared by the condensation of ethyl α-alkyl-formylacetate or ethyl 2-alkylacetoacetate with thiourea, as shown in equation (1) and (2).



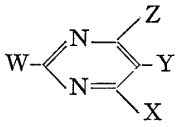
(1)



(2)

R=alkyl group

The unknown compounds among this siries are listed in Table I.

TABLE I. 

W	X	Y	Z	m.p. (°C)	Appearance	Molecular formula	Analyses (%) Calcd. C	H	N	Found C	H	N
SH	H	C_4H_9	OH	150~151	colorless prisms	$C_8H_{12}ON_2S$	—	—	15.12	—	—	14.97
〃	〃	C_6H_{13}	〃	171~172	〃	$C_{10}H_{16}ON_2S$	—	—	13.20	—	—	12.80
〃	CH_3	〃	〃	181~183	〃	$C_{11}H_{18}ON_2S$	58.39	8.02	12.38	58.15	8.08	12.31
〃	〃	C_8H_{17}	〃	178~180	〃	$C_{13}H_{22}ON_2S$	61.39	8.72	11.02	61.61	8.92	11.02
〃	〃	$C_{10}H_{21}$	〃	175~176	colorless fine needles	$C_{15}H_{26}ON_2S$	63.80	9.28	9.92	63.35	9.42	10.12
〃	〃	$C_{12}H_{25}$	〃	170~172	〃	$C_{17}H_{30}ON_2S$	—	—	9.03	—	—	8.97
〃	NH_2	C_2H_5	〃	283~284	colorless prisms	$C_6H_9ON_3S$	42.10	5.03	24.55	41.98	4.89	24.25
〃	〃	C_3H_7	〃	247~248	〃	$C_7H_{11}ON_3S$	—	—	22.69	—	—	22.41
〃	〃	C_4H_9	〃	251~252	〃	$C_8H_{13}ON_3S$	—	—	21.10	—	—	21.00
〃	〃	C_5H_{11}	〃	257	colorless fine needles	$C_9H_{15}ON_3S$	—	—	19.71	—	—	19.52
〃	〃	C_6H_{13}	〃	245~246	needles	$C_{10}H_{17}ON_3S$	—	—	18.49	—	—	18.22
CH_3S	〃	C_2H_5	〃	215~217	fine needles	$C_7H_{11}ON_3S$	—	—	22.69	—	—	22.73
〃	〃	C_3H_7	〃	193~194	〃	$C_8H_{13}ON_3S$	—	—	21.10	—	—	20.96
〃	〃	C_4H_9	〃	170~171	〃	$C_9H_{15}ON_3S$	—	—	19.71	—	—	19.85
〃	〃	C_5H_{11}	〃	167~168	colorless plates	$C_{10}H_{17}ON_3S$	52.85	7.54	18.49	52.80	7.49	18.30
〃	〃	C_6H_{13}	〃	165~166	〃	$C_{11}H_{19}ON_3S$	—	—	17.42	—	—	17.58
〃	〃	C_8H_{17}	〃	160~161	〃	$C_{13}H_{23}ON_3S$	—	—	15.60	—	—	15.88
〃	〃	$C_{10}H_{21}$	〃	134~135	〃	$C_{15}H_{29}ON_3S$	—	—	14.13	—	—	14.32
CH_3S[a]	〃	CH_3	Cl	242	colorless prisms	$C_6H_9N_3SCl_2$	—	—	18.58	—	—	18.81
〃	〃	C_2H_5	〃	215~216	〃	$C_7H_{11}N_3SCl_2$	—	—	17.51	—	—	17.31
〃	〃	C_3H_7	〃	204~205	〃	$C_8H_{13}N_3SCl_2$	—	—	16.53	—	—	16.26
〃	〃	C_4H_9	〃	215~216	colorless needles	$C_9H_{15}N_3SCl_2$	—	—	15.66	—	—	15.61
〃	〃	C_5H_{11}	〃	214	〃	$C_{10}H_{17}N_3SCl_2$	—	—	14.89	—	—	14.78
〃	〃	C_6H_{13}	〃	194~195	colorless prisms	$C_{11}H_{19}N_3SCl_2$	—	—	14.18	—	—	13.93
〃	〃	$C_{10}H_{21}$	〃	173	〃	$C_{15}H_{27}N_3SCl_2$	—	—	12.27	—	—	12.01

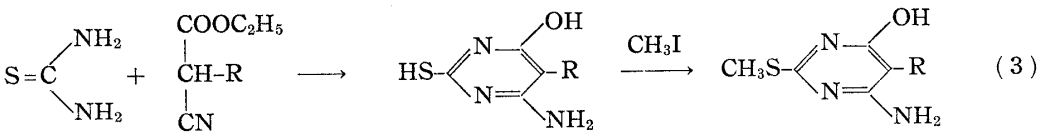
a) HCl salt.

Synthesis of 2-Thio-5-alkyl-6-aminouracil and 2-Methylthio-5-alkyl-6-amino-4-pyrimidinol——According to the synthetic method of Nakata *et al.*,[1] Nishikawa[8] and

8) T. Nishikawa : Bull. Chem. Soc. Japan, **56**, 936, 1487 (1953).

Weygand *et al.*,[9] 2-thio-5-methyl-6-aminouracil was obtained by the condensation of thiourea with ethyl alkylcyanoacetate, and this compound was converted to its 2-methylthio derivative by the reaction with methyl iodide.

Applying this method to the syntheses of the objective compounds, the compounds of this series were synthesized, as indicated in equation (3). The new compounds among the series are illustrated in Table I.



(3)

R=alkyl group.

Synthesis of 2-methylthio-4-chloro-5-alkyl-6-aminopyrimidine——Any compounds of 2-methylthio-4-chloro-5-alkyl-6-aminopyrimidine were unknown. These compounds were synthesized by the chlorination of 2-methylthio-5-alkyl-6-amino-4-pyrimidinol with phosphoryl oxychloride as shown in equation (4).

Compounds thus obtained are listed in Table I.

(4)

R=alkyl group.

Screening tests with Compounds Synthesized——The compounds were examined as to their activity on toxoplasma Gondii in mice. The experimental procedures were as follows: Mice of ca. 15 g. in body weight were inoculated intraperitoneally with 2×10^5 of toxoplasma Gondii. The administration of a tested compound was initiated on one day prior to the inoculation and continued once daily for five days.

All tested compounds were injected intraperitoneally into mice, and a single dose of each of the compounds was equivalent to 1/15 of its LD_{50}. After that, these mice were observed for 30 days, and the number of the died mice and the date of death were recorded.*[2] the results are shown in Table II.

As shown in the Table, some of the compounds were found fairly effective on the toxoplasma in mice, that is, the time of the death of the infected mice were observed to be delayed by the administration of these compounds.

The relationship between the chemical structures and antitoxoplasmosal activity of the above 2-thio-5-alkyluracil derivatives may be reviewed as follows.

Among alkylated thiouracil, the death-delaying effect was found in the compounds which possessed higher alkyl groups than ethyl group. The effectiveness of the alkylated thiouracil is in the following order, as shown in Table II.

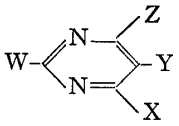
$$C_6>C_8>C_{12}>C_3=C_4=C_5$$

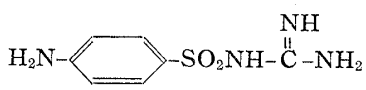
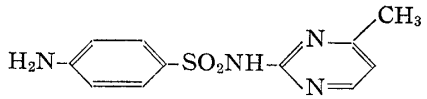
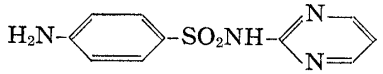
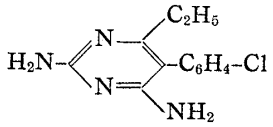
Table II shows that several compounds were found active against the toxoplasma in concentration of 2×10^5 among the series of 2-thio-5-alkyl-6-amino-4-pyrimidinol, 2-methylthio-5-alkyl-6-amino-4-pyrimidinol, and 2-methylthio-4-chloro-5-alkyl-6-aminopyrimidine.

*[2] This screening test is considered too drastic to search primarily an active agent. However, this test was inevitably employed, since there was not found any other appropriate screening method at present.

9) F. Weygand, A. Wacker, F. Wirth: Z. Naturforsch., **6b**, 25 (1951).

TABLE II.



Y	Z	W	X	Single dose (mg./kg.)	Average value of survival days	Difference between survival days of the treated and the control group of mice
H	OH	H	CH_3	14.2	5.8	+0.6
OC_2H_5	〃	〃	H	17.2	5.6	0.4
C_4H_9	〃	〃	〃	18.4	5.6	0.4
C_6H_{13}	〃	〃	〃	21.2	5.8	0.6
C_6H_5	〃	〃	〃	20.4	5.4	0.2
C_2H_5	〃	〃	CH_3	17.0	5.6	0.4
C_3H_7	〃	〃	〃	18.4	6.2	1.0
C_4H_9	〃	〃	〃	19.8	6.2	1.0
C_5H_{11}	〃	〃	〃	21.2	6.2	1.0
C_6H_{13}	〃	〃	〃	22.6	7.0	1.8
C_8H_{17}	〃	〃	〃	25.4	6.6	1.4
$C_{12}H_{25}$	〃	〃	〃	31.0	6.4	1.2
CH_3	〃	〃	H	14.2	5.6	0.4
$C_{10}H_{21}$	〃	CH_3	CH_3	28.2	5.6	0.4
OC_2H_5	〃	C_2H_5	H	20.0	5.8	0.6
	Control			—	5.2	—
C_2H_5	OH	H	NH_2	17.1	8.8	+1.0
C_3H_7	〃	〃	〃	18.5	8.6	0.8
C_4H_9	〃	〃	〃	19.9	8.2	0.4
C_5H_{11}	〃	〃	〃	21.3	8.6	0.8
C_8H_{17}	〃	〃	〃	25.5	8.8	1.0
$C_{10}H_{21}$	〃	〃	〃	28.3	8.2	0.4
	Control			—	7.8	—
CH_3	OH	CH_3	NH_2	17.1	8.2	+0.6
C_2H_5	〃	〃	〃	18.5	7.8	0.2
C_3H_7	〃	〃	〃	19.9	8.6	1.0
C_4H_9	〃	〃	〃	21.3	8.2	0.6
C_5H_{11}	〃	〃	〃	22.7	9.0	1.4
C_8H_{17}	〃	〃	〃	26.9	8.2	0.6
	Control			—	7.6	—
CH_3	Cl	CH_3	NH_2	22.6	6.6	+1.4
C_3H_7	〃	〃	〃	25.4	6.2	1.0
C_4H_9	〃	〃	〃	26.8	6.2	1.0
C_5H_{11}	〃	〃	〃	28.2	6.4	1.2
C_6H_{13}	〃	〃	〃	29.4	6.2	1.0
C_8H_{17}	〃	〃	〃	31.5	5.8	0.6
	Control			—	5.2	—
				23.2	7.8	+0.6
				26.4	8.6	1.6
				25.0	8.4	1.4
				20.0	13.4	6.4
Control				—	7.0	—

From the comparison of Table II, 2-methylthio-4-chloro-5-alkyl-6-aminopyrimidine was more effective than 2-thio-5-alkyl-6-aminouracil and 2-methylthio-5-alkyl-6-amino-4-pyrimidinol against the infection of toxoplasma in mice.

As inferred from Table II, it may be said that the activity of 2-thio-5-hexyl-6-methyluracil, 2-methylthio-5-pentyl-6-amino-4-pyrimidinol and 2-methylthio-4-chloro-5-methyl-6-aminopyrimidine might be considered as nearly the same to that of sulfadiazine or sulfamethazine, though their effect was weaker than that of pyrimethazine.

At any rate, it is of interest that some of alkylated thiouracil derivatives exerted the delaying effect of the death caused by the infection of toxoplasma in mice. These findings will be a milestone to find more promising antitoxoplasmosal agents. On the basis of these studies, the syntheses and screening tests of compounds of seversl types are now on progress. The results along this line will be reported in the near future.

Experimental

General Procedure of Synthesis of Pyrimidine Derivatives : 2-Thio-5-alkyluracil——The procedure was modified from that of Anderson[5] for the synthesis of 2-thio-5-propyluracil.

A solution of ethyl ester of fatty acid (0.1 mol.) and ethyl formate (0.2 mol.) was added to a stirred mixture of powdered sodium (0.13 mol.) in dry ether during 4 hr. The mixture was allowed to stand overnight. The solvent and the unreacted EtOH were removed by evaporation at a room temperature. Powdered thiourea (0.08 mole) and abs. EtOH were added to the residue and the mixture was refluxed for 7 hr. on a water bath. EtOH was removed *in vacuo* and the dried residue was dissolved in H_2O. After the solution was acidified with HCl, the precipitate was filtered off, washed with H_2O and purified by recrystallization from dil. EtOH.

2-Thio-5-alkyl-6-methyluracil——The series of these compounds were prepared by condensation of ethyl α-alkylacetoacetate with thiourea in EtONa in EtOH, according to Russell's method.[5] The crude product was recrystallized from dil. EtOH.

2-Thio-5-alkyl-6-aminouracil and 2-Methylthio-5-alkyl-6-amino-4-pyrimidinol——These compounds were prepared by the condensation of ethyl alkylcyanoacetate with thiourea.[1] A mixture of 2-mercapto compound (0.1 mole), EtONa (0.1 mole) in EtOH and CH_3I (0.13 mole) was refluxed for 3~4 hr. After removal of the solvent by distillation, the residue was recrystallized from EtOH.

2-Methylthio-4-chloro-5-alkyl-6-aminopyrimidine——A mixture of 0.01 mole of 2-alkylthiopyrimidine and 30 cc. of $POCl_3$ was refluxed in an oil bath for 5~6 hr. After distillation of excess $POCl_3$ *in vacuo*, the residue was poured into ice water and made alkaline with *N*NaOH. The separated crude product was collected and dissolved in MeOH. Dry HCl gas was introduced into the solution. After removal of MeOH, the residue was recrystallized from dil. EtOH.

For the screening tests of compounds on toxoplasma, the great help was given to the authors from Prof. H. Matsubayashi of Department of Parasitology, Keio university. and Dr. S. Saito, Instructor of the same Department. The authors wish to express their thanks to them.

Summary

To find chemotherapeutic agents for polio, adenoviruses and toxoplasma Gondii, 2-thio-5-alkyl-6-aminouracil, 2-methylthio-5-alkyl-6-amino-4-pyrimidinol, 2-methylthio-4-chloro-5-alkyl-6-aminopyrimidine, 2-thio-5-alkyluracil and 2-thio-5-alkyl-6-methyluracil were synthesized.

All of the compounds did not show any inhibitory effect on the viruses, but some of the compounds were found fairly effective against the infection of toxoplasma in mice. Death-delaying effect of 2-thio-5-hexyl-6-methyluracil, 2-methylthio-5-pentyl-6-amino-4-pyrimidinol and 2-methylthio-4-chloro-5-alkyl-6-aminopyrimidine which possess lower alkyl groups than hexyl group was found effective nearly equal to that of sulfadiazine or sulfamethazine.

(Received July 9, 1962)