UDC 615.778.25

2. Tadakazu Tsuji, Sumiko Watanabe, Yasuko Nakadai, and Shigeshi Toyoshima : Synthesis and Antimicrobial Activity of N^1-(2-Alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide.

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

Many new drugs have been prepared by replacing oxygen atom in the structure of existing drugs with sulfur atom. However, such idea has hardly been put to practical use in the field of antimicrobial agents.

This idea was used for a new agent by the substitution of alkoxyl group in N^1-(2,6-dimethoxy-4-pyrimidinyl)sulfanilamide, known as one of long-acting sulfanilamide drugs, with alkylthio group and N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide was synthesized to test their antimicrobial activity.

N^1-(2-Alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide

Compounds of the type of N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide have not been reported up till now and the synthesis of N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl)-sulfanilamide was undertaken by condensation of 2-alkylthio-4-alkoxy-6-aminopyrimidine with *p*-acetamidobenzenesulfonyl chloride and hydrolysis of the resulting condensate with sodium hydroxide solution, according to the general method of synthesis of sulfanilamide drugs, as shown in Chart 1.

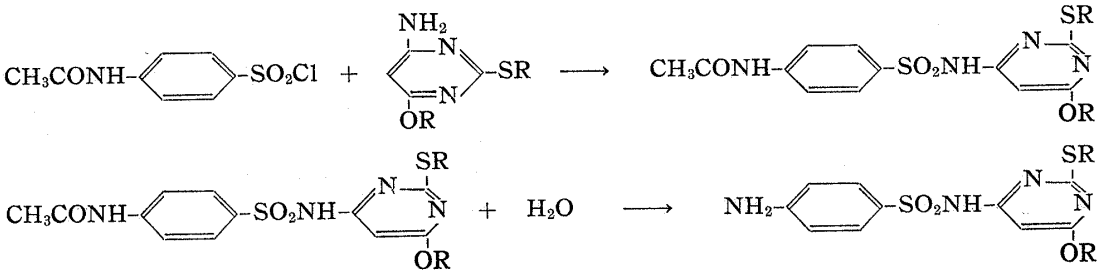

Chart 1. R=alkyl group

For the synthesis of the objective sulfanilamides, it was necessary to prepare N^1-component, i.e. 2-alkylthio-4-amino-6-alkoxypyrimidine. Among compounds of this series, only 2-methylthio-4-amino-6-methoxypyrimidine was reported by Jones.[1]

According to the method of Jones, 2-mercapto-6-amino-4-pyrimidinol was prepared by reacting thiourea with ethyl cyanoacetate in the presence of sodium ethoxide and the resulting product was converted into 2-alkylthio-6-amino-4-pyrimidinol with alkyl iodide or alkyl bromide as shown in Chart 2.

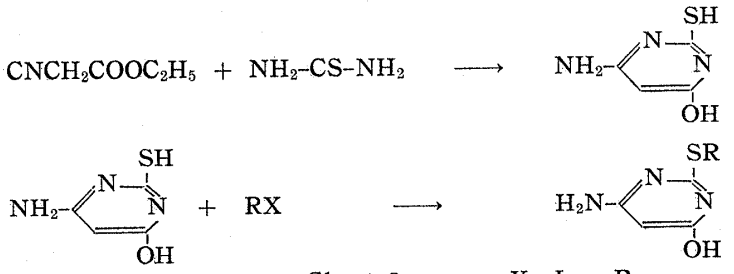

Chart 2. X=I or Br

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1) C.O. Jones : J. Biol. Chem., **20**, 157 (1915).

2-Alkylthio-4-amino-6-alkoxypyrimidine was obtained by chlorination of 2-alkylthio-6-amino-4-pyrimidinol with phosphorus oxychloride and alkylation of the resulting product, 2-alkylthio-4-amino-6-chloropyrimidine, with sodium alkoxide as shown in Chart 3. The

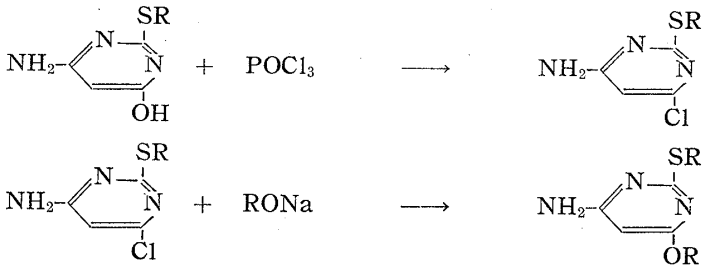


Chart 3.

objective sulfonamides were obtained in a good yield by reaction of *p*-acetamidobenzenesulfonyl chloride with 2-alkylthio-4-amino-6-alkoxypyrimidine in dehyd. pyridine and hydrolysis of the resulting acetylated product with sodium hydroxide solution.

N^1-(6-Ethylthio-2-methylthio-4-pyrimidinyl)sulfanilamide

This compound was synthesized by a similar procedure as for N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide. The preceding intermediate, 2-methylthio-4-amino-6-ethylthiopyrimidine was prepared by reaction of 2-methylthio-4-amino-6-chloropyrimidine with ethylthio sodium in excess ethanol. N^1-(6-Ethylthio-2-methylthio-4-pyrimidinyl)-sulfanilamide was obtained in a good yield by reaction of 2-methylthio-4-amino-6-ethylthiopyrimidine with *p*-acetamidobenzenesulfonyl chloride in dehyd. pyridine and hydrolysis of the resulting product with sodium hydroxide solution.

Screening for Antibacterial Activity

The compounds of 2-alkylthio-4-amino-6-alkoxypyrimidine, N^4-acetyl-N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide, and N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl)-sulfanilamide series were screened for their antibacterial activity, using *Escherichia coli* C_{14} and K_{12}, *Aerobactor aerogenes* 1033, *Salmonella enteritidis* No. 11, and *Staphylococcus aureus* Terashima, as described in the Experimental part. It was noted that all compounds of 2-alkylthio-4-amino-6-alkoxypyrimidine and N^4-acetyl-N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide series were ineffective on these bacteria, except N^1-(2-methylthio-6-methoxy (and ethoxy)-4-pyrimidinyl) sulfanilamides, which were effective on some of these bacteria. It is noteworthy that the latter compound has an effect comparable to that of N^1-(2,6-dimethoxy-4-pyrimidinyl)sulfanilamide as shown in Table VI. It is interesting that the lower alkyl members of this series seemed to be more effective than the higher compounds.

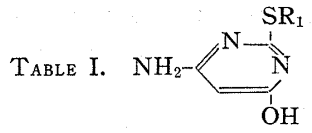
Similar tendency had been noticed among compounds of N^1-(6-alkoxy-3-pyridazinyl)-sulfanilamide,[2] N^1-(6-alkylthio-3-pyridazinyl)sulfanilamide,[2] and N^1-(2,6-dialkoxy-4-pyrimidinyl)benzenesulfonamide.

It may be considered that the replacement of oxygen with sulfur atom might influence antibacterial spectrum of sulfanilamide drugs and that the compounds of this type might be effective selectively on a specific bacterium. Work on this problem will be reported in the near future.

Experimental

General Method for Synthesis of 2-Alkylthio-6-amino-4-pyrimidinol——A solution of EtONa, 0.1 mole of 2-mercapto-6-amino-4-pyrimidinol, and 0.13 mole of alkyl halide was refluxed for 2~3 hr. After removal of the solvent by distillation, the residue was recrystallized from EtOH. Analytical data for the compounds synthesized are summarized in Table I.

2) T. Ishida, *et al.*: Paper read at the 12th Annual Meeting of the Pharmaceutical Society of Japan (1959).

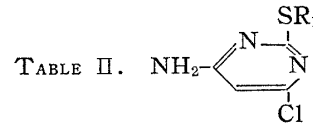
Table I. 

No.	R_1	m.p. (°C)	Mol. formula	N (%) Calcd.	N (%) Found
1	CH_3*	267	$C_5H_7ON_3S$	26.74	26.70
2	C_2H_5*	219	$C_6H_9ON_3S$	24.55	24.31
3	C_3H_7	210～211	$C_7H_{11}ON_3S$	22.69	22.46
4	iso-C_3H_7	300	$C_7H_{11}ON_3S$	22.69	22.43
5	C_4H_9	190～191	$C_8H_{13}ON_3S$	21.10	20.92

* C. O. Jones : Am. Chem. J., **34**, 181 (1905).

2-Methylthio-4-amino-6-ethylthiopyrimidine——To a solution of 3.5 g. of 2-methylthio-4-amino-6-chloropyrimidine dissolved in 10 cc. of dehyd. EtOH, a solution of EtSNa prepared from 0.05 g. of Na in 25 cc. of dehyd. EtOH and 1.3 g. of EtSH was added. The reaction mixture was refluxed for 3 hr. After removal of the solvent, the residual oily product solidified upon addition of water. After drying, the crude product was used for the next reaction.

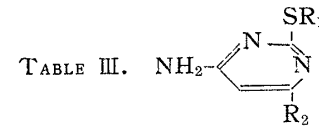
General Method for Synthesis of 2-Alkylthio-4-amino-6-chloropyrimidine——A mixture of 0.09 mole of 2-alkylthio-6-amino-4-pyrimidinol and 30 cc. of $POCl_3$ was refluxed in an oil bath for 5～6 hr. After distillation of excess $POCl_3$ *in vacuo*, the residue was poured into ice water and made alkaline with *N* NaOH. The crude product that separated was collected, washed with a small amount of ice water, and recrystallized from EtOH. Analytical data for the compounds synthesized are listed in Table II.

Table II. 

No.	R_1	m.p. (°C)	Mol. formula	N (%) Calcd.	N (%) Found
1	CH_3*	127	$C_5H_6N_3SCl$	23.92	23.89
2	C_2H_5	115	$C_6H_8N_3SCl$	22.21	22.41
3	C_3H_7	101～101.5	$C_7H_{10}N_3SCl$	20.63	20.71
4	C_4H_9	81～82	$C_8H_{12}N_3SCl$	19.53	19.22

* C. O. Jones : Am. Chem. J., **34**, 183 (1905).

General Method for Synthesis of 2-Alkylthio-4-alkoxy-6-aminopyrimidine——A mixture of 0.05 mole of 2-alkylthio-4-amino-6-chloropyrimidine and a solution of EtONa prepared from 1.25 g. of metallic Na and 30 cc. of EtOH, was refluxed for 3 hr. After removal of the solvent, the precipitate was collected, washed with water, and recrystallized from EtOH. Analytical data for the compounds obtained are listed in Table III.

Table III. 

No.	R_1	R_2	m.p. (°C)	Mol. formula	N (%) Calcd.	N (%) Found
1	CH_3	OCH_3	144	$C_6H_9ON_3S$	24.55	24.49
2	C_2H_5	OCH_3	106～107	$C_7H_{11}ON_3S$	22.69	22.78
3	C_3H_7	OCH_3	90～91	$C_8H_{13}ON_3S$	21.10	21.02
4	C_4H_9	OCH_3	63～65	$C_9H_{15}ON_3S$	19.71	19.75
5	CH_3	OC_2H_5	93～95	$C_7H_{11}ON_3S$	22.69	22.41
6*	CH_3	OC_3H_7		$C_8H_{11}ON_3S$		
7*	CH_3	iso-OC_3H_7		$C_8H_{11}ON_3S$		
8*	CH_3	OC_4H_9		$C_9H_{13}ON_3S$		
9*	CH_3	SC_2H_5		$C_7H_{11}N_3S_2$		

* The product did not solidify, but was used for the synthesis of the corresponding sulfonamide without further purification.

General Method for Synthesis of N^1-(2-Alkylthio-6-alkoxy-4-pyrimidinyl) sulfanilamide—To a solution of 0.02 mole of 2-alkylthio-4-alkoxy-6-aminopyrimidine in 35 cc. of dehyd. pyridine, 4.4 g. of *p*-acetamidobenzenesulfonyl chloride was added. After allowing to stand overnight, the solvent was evaporated *in vacuo*, the residue was poured into ice water, and the precipitate produced was recrystallized from EtOH. N^4-Acetyl-N^1-(2-alkylthio-6-alkoxy-4-pyrimidinyl) sulfanilamide thus obtained was dissolved in 5 cc. of 10% NaOH and the solution was heated on a water bath for 5~6 hr. After cool, the reaction mixture was acidified with AcOH and the product was recrystallized from EtOH. The compounds of the above two series are listed in Tables IV and V.

Table IV. $CH_3CONH-C_6H_4-SO_2NH-C_4H_2N_2(SR_1)R_2$

No.	R_1	R_2	m.p. (°C)	Mol. formula	N (%) Calcd.	N (%) Found
1	CH_3	OCH_3	228	$C_{14}H_{16}O_4N_4S_2$	15.21	15.20
2	C_2H_5	OCH_3	201~202	$C_{15}H_{18}O_4N_4S_2$	14.66	14.57
3	C_3H_7	OCH_3	228	$C_{16}H_{20}O_4N_4S_2$	14.14	14.52
4	C_4H_9	OCH_3	228~229	$C_{17}H_{22}O_4N_4S_2$	13.65	13.50
5	CH_3	OC_2H_5	245	$C_{15}H_{18}O_4N_4S_2$	14.66	14.48
6	CH_3	OC_3H_7	186~187	$C_{16}H_{20}O_4N_4S_2$	14.14	14.40
7	CH_3	iso-OC_3H_7	239~240	$C_{16}H_{20}O_4N_4S_2$	14.14	13.90
8	CH_3	OC_4H_9	164	$C_{17}H_{22}O_4N_4S_2$	13.65	13.76
9	CH_3	SC_2H_5	233~234	$C_{15}H_{18}O_3N_4S_3$	14.07	13.81

Table V. $NH_2-C_6H_4-SO_2NH-C_4H_2N_2(SR_1)R_2$

No.	R_1	R_2	m.p. (°C)	Mol. formula	N (%) Calcd.	N (%) Found
1	CH_3	OCH_3	179~180	$C_{12}H_{14}O_3N_4S$	17.17	17.08
2	C_2H_5	OCH_3	170~171	$C_{12}H_{16}O_3N_4S$	16.47	16.55
3	C_3H_7	OCH_3	167~168	$C_{14}H_{18}O_3N_4S$	15.81	15.79
4	C_4H_9	OCH_3	143	$C_{15}H_{20}O_3N_4S$	15.21	15.48
5	CH_3	OC_2H_5	180~181	$C_{13}H_{16}O_3N_4S$	16.47	16.64
6	CH_3	OC_3H_7	133~134	$C_{14}H_{18}O_3N_4S$	15.81	15.52
7	CH_3	iso-OC_3H_7	134~135	$C_{14}H_{18}O_3N_4S$	15.81	15.57
8	CH_3	SC_2H_5	134~135	$C_{15}H_{16}O_2N_4S_2$	15.72	15.81

Screening Test for Antibacterial Activity—*Escherichia coli* K_{12}, and C_{14}, *Aerobactor aerogenes* 1033, *Salmonella enteritidis* No. 11, and *Staphylococcus aureus* Terashima were used in this test. The organism was cultured overnight on nutrient agar slant at 37° and was suspended in saline solution.

Table VI. $NH_2-C_6H_4-SO_2NH-C_4H_2N_2(SR_1)R_2$

R_1	R_2	*E. coli.* C_{14}	*E. coli.* K_{12}	*Sal. enter.* No. 11	*A. aerog.* 1033	*Staph. aureus* Terashima
CH_3	OCH_3	4×10^{-4}	4×10^{-4}	$>4\times10^{-4}$	$>4\times10^{-4}$	2×10^{-5}
CH_3	OC_2H_5	4×10^{-4}	4×10^{-4}	4×10^{-4}	4×10^{-4}	2×10^{-5}
CH_3	OC_3H_7	$>2\times10^{-4}$	$>2\times10^{-4}$	$>2\times10^{-4}$	$>2\times10^{-4}$	2×10^{-4}
CH_3	iso-OC_3H_7	$>2\times10^{-4}$	$>2\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$
C_2H_5	OCH_3	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	5×10^{-5}
C_3H_7	OCH_3	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	2×10^{-4}
C_4H_9	OCH_3	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	2×10^{-4}
CH_3	SC_2H_5	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	$>4\times10^{-4}$	10^{-4}
Sulfadimethoxine		10^{-4}	10^{-4}	4×10^{-4}	2×10^{-4}	2×10^{-5}
Sulfamethoxypyridazine		10^{-4}	2×10^{-5}	10^{-4}	10^{-4}	10^{-4}

The inoculum size was about 2000. The culture medium used contained : K_2HPO_4 0.7%, KH_2PO_4 0.3%, $(NH_4)_2SO_4$ 0.2%, $MgSO_4$ 0.01%, casamino acid 1.5%, nicotinic acid 0.01%, thiamine 0.01%, and glucose 0.2%, pH 7.0. The compound was taken as effective when complete supression of colony-formation is observed after incubation for 48 hr. and considered as ineffective when one or more colonies were observed.

Summary

N^1-(2-Alkylthio-6-alkoxy-4-pyrimidinyl)sulfanilamide and N^1-[2,6-bis(alkylthio)-4-pyrimidinyl]sulfanilamide were synthesized and were screened for their antibacterial activity using *Escherichia coli* C_{14} and K_{12}, *Aerobactor aerogenes* 1033, *Salmonella enteritidis* No. 11, *Staphylococcus aureus* Terashima.

N^1-(2-Methylthio-6-methoxy(and ethoxy)-4-pyrimidinyl)sulfanilamides were effective on some of these bacteria. The latter compound had an effect comparable to that of sulfadimethoxine.

(Received December 13, 1960)

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3. Hisashi Tanaka and Akira Yokoyama : Studies on Sulfur-containing Chelating Agents. VII.*1 Syntheses of β-Mercaptothiolic Acid Esters and their Metal Chelates.

(*Faculty of Pharmacy, Kyoto University**2)

In the previous papers,[1],*1 synthesis of β-mercapto-acid esters and their metal chelates was reported. The present paper deals with the synthesis of β-mercaptothiolic acid esters and their metal chelates.

As in the case of β-mercapto-acid esters, the chelate-formation of β-mercaptothiolic acid esters would occur through the combination of carbonyl group of the ester and mercapto group. Considering the difference between the character of sulfur in thiolic acid ester and that of oxygen in the ester, that is, sulfur is more polarizable and less electronegative than oxygen, β-mercaptothiolic acid ester would be expected to show different attitude towards chelate-formation from that of β-mercapto-acid ester. The synthesis of β-mercaptothiolic acid esters was therefore planned to compare their chelating ability with that of β-mercapto-acid esters.

S-Alkyl or S-aryl esters of β-mercaptothiohydrocinnamic acid were prepared as β-mercaptothiolic acid esters. Addition of hydrogen sulfide to S-esters of thiocinnamic acid was found to be satisfactory for the preparation of the S-esters of β-mercaptothiohydrocinnamic acid. As shown in Chart 1, hydrogen sulfide was reacted with S-alkyl or S-aryl thiocinnamate in a manner which was found to be the best in the case of β-mercapto-acid esters. S-Alkyl or S-aryl thiocinnamates were prepared by the method described by Bestram.[2] Separation of β-mercaptothiolic acid esters from the reaction mixture

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*1 Part VI. H. Tanaka, A. Yokoyama : This Bulletin, **9**, 110 (1961).
*2 Yoshida, Sakyo-ku, Kyoto (田中 久, 横山 陽).
1) Part V. H. Tanaka, A. Yokoyama : This Bulletin, **9**, 66 (1961).
2) H. J. Bestram : Chem. Ber., **92**, 530 (1959).