UDC 547.854.1.07 : 547.945

158. Takeo Ueda, Tadakazu Tsuji, and Hiroko Momona (née Koibuchi) : The Direct Thiation of Pyrimidinol Derivatives.

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Hurst[1] reported that 2,6-diaminopurine and 7-methyl-2,6-dichloropurine showed an inhibitory effect on vaccina virus in chick embryo and murine encephalitis virus in mouse brain culture, but not on the viruses in mice at all. After that, Hollinshead[2] claimed that 2,6-diaminopurine inhibited the multiplication of the type-1, WS strain of poliomyelitis virus in tissue culture of monkey testicle, monkey kidney and HeLa cells. These findings suggested that those inhibitory effects might originate from the hindrance to nucleic acid biosynthesis of viruses by the antimetabolic action of those deformed purine derivatives. Taking this assumption into consideration, the authors synthesized several compounds, particularly thiated, related to metabolic purine bases forming viral nucleic acid, and examined as to their activity on various viruses.

The present report describes a synthetic method which allows the replacement of 4-hydroxyl group of amino-4-pyrimidinol with sulfhydryl in one step, through the action of phosphorus pentasulfide in a tertiary amine. In connection with this method, 5-acylamino-6-amino-4-pyrimidinol or purinol were thiated, which are also reported herein.

Direct Thiation of Amino-4-pyrimidinol

Elion and Hitchings[3] was able to thiate some uracils, but not aminopyrimidinol such as isocytosine, using phosphorus pentasulfide and tetralin. Recently, Mizuno and Ikehara[4] succeeded in the monothiation of uracil in pyridine by using a half mole of phosphorus pentasulfide for one mole of uracil, and they assumed that only the coordinately linked four sulfur atoms in phosphorus sulfide (P_4S_{10}) might take part in the monothiation of uracil.

Although the thiation of amino-4-pyrimidinol with phosphorus pentasulfide was unsuccessful by using either tetralin or pyridine, the authors found that this thiation was furnished successfully, when triethylamine or 3-picoline was employed as a solvent. The experimental results are shown in Table I. Moreover, 2,6-lutidine and 4-picoline

Table I.

Exp.	Starting material	Solvent	Time of reflux (hr.)	Product	Yield (%)
1	2-methyl-6-amino-4-pyrimidinol	triethylamine	10	2-methyl-6-amino-4-pyrimidinethiol	50.2
2	〃	3-picoline	1	〃	50.1
3	〃	2,6-lutidine	15	〃	26.6
4	6-amino-2,4-pyrimidinediol	3-picoline	2	6-amino-2,4-pyrimidinedithiol	42.7
5	2-mercapto-6-amino-4-pyrimidinol	3-picoline	1.5	〃	22.7
6	2-methyl-5,6-diamino-4-pyrimidinol	triethylamine	15	2-methyl-5,6-diamino-4-pyrimidinethiol	50.7
7	〃	3-picoline	1	〃	48.7
8	〃	2,6-lutidine	16	〃	28.6

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1) E.W. Hurst, R. Hull : Pharmacol. Rev., 8, 199 (1956).
2) A. Hollinshead, P.K. Smith : J. Pharmacol. Exptl. Therap., **123**, 54 (1958).
3) G.B. Elion, G.H. Hitchings : J. Am. Chem. Soc., **69**, 2138 (1947).
4) Y. Mizuno, M. Ikehara, K.A. Watanabe : This Bulletin, **10**, 647 (1962).

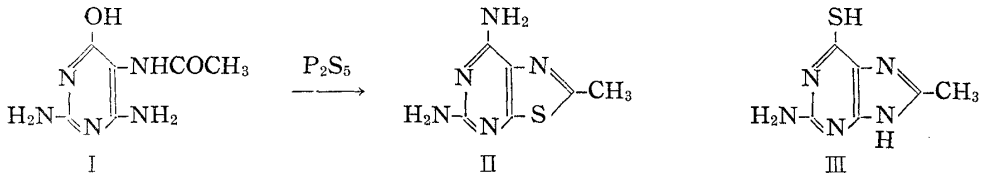
were employed for the reaction. The yield of thiol compounds was observed to fall fairly down by using 2,6-lutidine as a solvent, and an employment of 4-picoline did not afford any amount of thiol compound. From these results, the suitability of solvents for this reaction might concern with their basicity and solubility to a reaction mixture.

Isocytosine and 2,6-diamino-4-pyrimidinol were not thiated by using any of these solvents. From this finding, it may be said that 4-pyrimidinol possessing an amino group at 2-position was unable to be thiated by this method.

Thiation of 5-Acylamino-6-amino-4-pyrimidinol

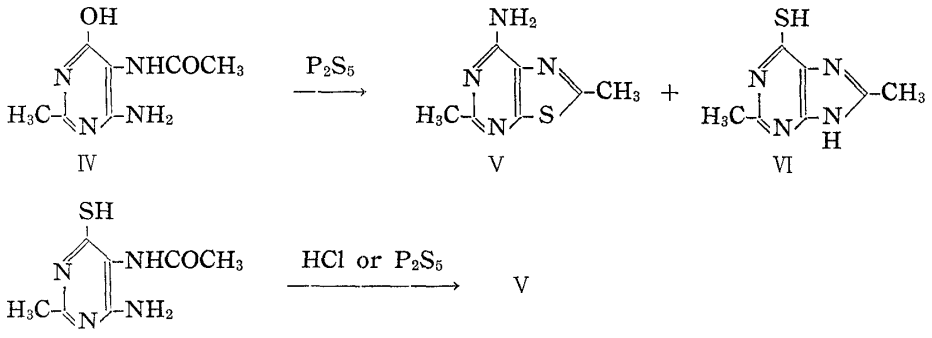
Falco[5] prepared 2-methyl-4,6-diaminothiazolo[5,4-*d*]pyrimidine (II) by the treatment of 5-acetamido-2,6-diamino-4-pyrimidinol (I) with phosphorus pentasulfide in tetralin. On the contrary, Daves[6] obtained 2-amino-8-methyl-6-purinethiol (III) from the same reactant (II), using phosphorus pentasulfide in pyridine.

The authors reexamined Daves' experiments under the same condition to that described by Daves. The ultraviolet spectra (pH 1) of the crude product exhibited a weak absorption at 350 mμ besides the extensive one at 265 mμ. This index might suggest an existence of II and the contamination with III. After a purification of the crude product has been completed, II was exclusively fastened.



I II III

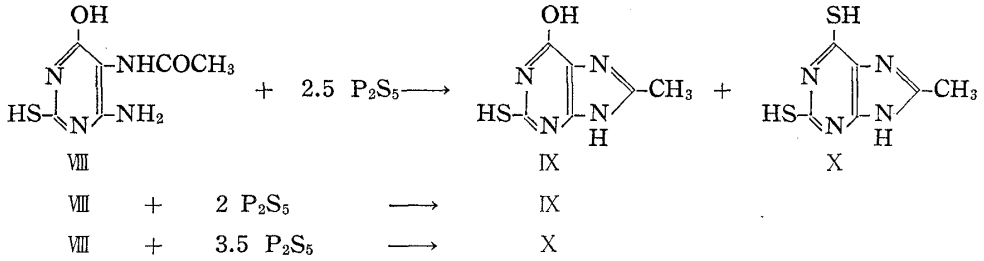
Attention was then turned to the thiation of some compounds of 2-substituted-5-acylamino-6-amino-4-pyrimidinol, which might involve cyclization to either thiazolo[5,4-*d*]pyrimidine or purinethiol derivative. The treatment of 2-methyl-5-acetamido-6-amino-4-pyrimidinol (IV) with phosphorus pentasulfide in pyridine gave a considerable yield of thiazolopyrimidine (V) and a small amount of purinethiol (VI). For the identification of product (V), which was prepared by the treatment of 2-methyl-5-acetamido-6-amino-4-pyrimidinol (VII) with diluted hydrochloric acid according to the method reported by Ishidate[7] or with phosphorus pentasulfide in pyridine.



IV V VI

Next, 2-mercapto-5-acetamido-6-amino-4-pyrimidinol (VIII) was refluxed with phosphorus pentasulfide (2.5 moles per mole of VIII) to give a mixture of 2-mercapto-8-methyl-6-purinol (IX) and 8-methyl-2,6-purinedithiol (X). When a molar ratio of phosphorus pentasulfide to pyrimidine (VIII) was 2:1 or 3.5:1, the product was proved as IX

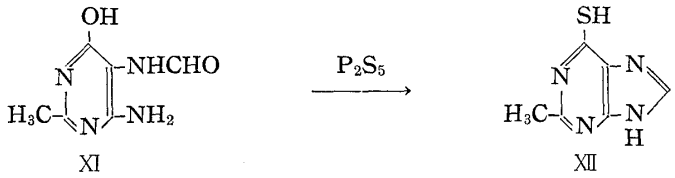
5) E. A. Falco : J. Am. Chem. Soc., **72**, 3203 (1950).
6) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel, A. G. Beaman : *Ibid.*, 82, 2633 (1960).
7) M. Ishidate, H. Yuki : This Bulletin, 8, 131 (1960).



or X, respectively. The present results indicate that a cyclization to purine ring precedes to a thiation of 5-hydroxyl group in the reaction of VIII with phosphorus pentasulfide in pyridine.

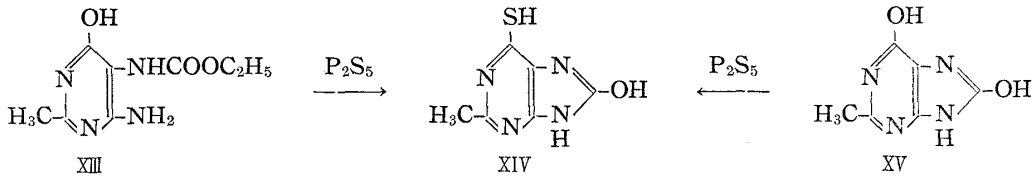
When one of 6-purinethiol or thiazolopyrimidine derivatives which were obtained above was refluxed with phosphorus pentasulfide in pyridine, neither any change nor reverse rearrangement between the both ring structures was observed. Therefore, it appears that in these thiations, the ratio of yield between the two different type ring products might change according to a sort of group at 2-position in 2-substituted-5-acetamido-6-amino-4-pyrimidinol.

As for 2-methyl-5-acylamino-6-amino-4-pyrimidinol, 5-formamido-derivative (XI) was cyclized to purine (XII), while 5-acetamido-derivative (IV) to a mixture of thiazolopyrimidine (V) and purine (VI). From these facts, the carbon number of 5-acyl group might affect to the ease of purine ring formation.



The analogous tendency was observed about the preparation of 2-amino-8-alkyl-6-purinol from 5-acylamino-2,6-diamino-4-pyrimidinol. 8-Methyl- or 8-ethyl-purine derivatives were obtainable, but 8-propyl derivative was not obtained, when Traube's method[8] was employed.

Ethyl 2-methyl-4-amino-6-hydroxy-5-pyrimidinecarbamate (XIII) was thiated to yield 2-methyl-6-mercapto-8-purinol (XIV), which was identified by infrared spectra with a sample prepared from the thiation of 2-methyl-6,8-purinediol (XV).



Thiation of 6-Purinols

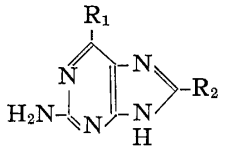
According to the usual method, several 6-purinol derivatives were thiated with phosphorus pentasulfide in pyridine. The compounds thereby obtained and related compounds were shown in Table II and III.

Screening Test on Poliomyelitis Virus

All products obtained above were screened as to their antiviral activities on the type-1, Mahoney strain of poliomyelitis virus in Hep. No. 2 cells. Only 2-methyl-5-(*p*-tolylsulfonamido)-6-amino-4-pyrimidinol exerted a slight activity on poliomyelitis

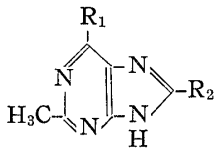
8) W. Traube : Ann., **432**, 283 (1923).

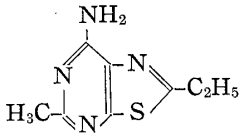
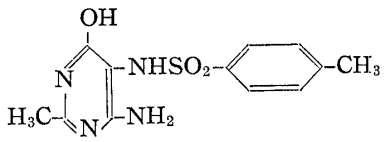
Table II.



R_1	R_2	Formula	N (%) Calcd.	N (%) Found
OH[8]	CH_3	$C_6H_7ON_5$	43.41	—
OH[8]	C_2H_5	$C_7H_9ON_5$	39.09	—
OH[10]	OH	$C_5H_5O_2N_5$	41.91	—
OH	SH	$C_5H_5ON_5S$	38.25	38.02
OH[10]	SCH_3	$C_6H_7ON_5S\cdot H_2O$	32.55	—
OH	SC_2H_5	$C_7H_9ON_5S\cdot H_2O$	30.56	30.53
SH[6]	CH_3	$C_6H_7N_5S\cdot \frac{1}{2}H_2O$	36.83	—
SH	C_2H_5	$C_7H_9N_5S$	35.82	35.54
NH_2[12]	H	$C_5H_6N_6$	55.98	—

Table III.



R_1	R_2	Formula	N (%) Calcd.	N (%) Found
OH	OH	$C_6H_6O_2N_4\cdot H_2O$	30.43	30.36
OH	SH	$C_6H_6ON_4S$	30.76	30.50
SH	SH	$C_6H_6N_4S_2$	28.28	28.33
		$C_8H_{10}N_4S$	28.85	28.95
		$C_{12}H_{14}O_3N_4S$	19.04	19.23

virus, but any of other compounds did not show any activity. Hollinshead[2] reported that 2,6-diaminopurine exerted the antiviral activity on the type-1 strain of poliomyelitis virus, while Syverton[12] has denied its activity. The present result also denies its activity.

Experimental

A) General Procedure for the Thiation of Amino-4-pyrimidinols——Fine powdered amino-4-pyrimidinol (0.005 mole) was suspended in 20 cc. of the solvent, which was indicated in Table I. To this suspension, 2.78 g.of P_2S_5 (0.0125 mole) was added portionwise, and the mixture was refluxed on an oil bath. After the solvent was removed off in reduced pressure, the residue was poured into H_2O and the whole was refluxed for 1 hr. Then, the mixture was made basic with NaOH, and filtered. The hot filtrate was neutralized with AcOH, and cooled. Reprecipitation was carried out twice more from dil. NaOH with AcOH. For further purification, the product was recrystallized from H_2O.

9) G.E. Gifford, H.E. Robertson, J.T. Syverton : Proc. Soc. Exp. Biol. Med., **86**, 515 (1954).
10) L.F. Cavalieri, A. Bendich : J. Am. Chem. Soc., **72**, 2587 (1950).
11) R.K. Robins : *Ibid.*, **80**, 6671 (1958).
12) R.K. Robins, K.J. Dille, C.H. Willit, B.E. Christensen : *Ibid.*, **75**, 263 (1953).

2-Methyl-6-amino-4-pyrimidinethiol——Pale yellow needles, m.p. 298° (decomp.). SH, positive. *Anal.* Calcd. for $C_5H_7N_3S$: N, 29.78. Found : N, 30.03.

6-Amino-2,4-pyrimidinedithiol——Yellow prisms, m.p. 309° (decomp.). SH, positive. *Anal.* Calcd. for $C_4H_5N_3S_2$: N, 26.41. Found : N, 26.48.

2-Methyl-5,6-diamino-4-pyrimidinethiol——Brownish yellow prisms, m.p. 285° (decomp.). SH, positive. *Anal.* Calcd. for $C_5H_8N_4S$: N, 35.88. Found : N, 35.93.

B) General Procedure for the Thiation of 5-Acylamino-6-amino-4-pyrimidinols——A mixture of 5-acylamino-6-amino-4-pyrimidinol (0.005 mole) and 2.78 g. of P_2S_5 (0.0125 mole) was suspended in 30 cc. of pyridine, and refluxed for 8 hr. The excess pyridine was distilled under reduced pressure on a water bath. To the residue was added 30 cc. of H_2O, and the whole was allowed to stand for 12 hr. After warmed on a water bath for 1 hr., the mixture was made basic with NaOH, and chilled overnight. The insoluble fraction, thiazolopyrimidine derivative, was collected by filtration. The filtrate was adjusted to pH 5.6 with HCl, and the precipitates, purine derivative, were collected.

2-Methyl-4,6-diaminothiazolo[5,4-*d*]pyrimidine (II)——Recrystallized from H_2O to give 730 mg. (81.1%) of colorless prisms, m.p. 248~250° (lit., m.p. 255~257°,[13] 246~250°[5]). *Anal.* Calcd. for $C_6H_7N_5S$: N, 38.66. Found : N, 39.00. The UV spectra of the product was identical to that reported by Hitchings.[5]

4-Amino-2,6-dimethylthiazolo[5,4-*d*]pyrimidine (V)——a) Prepared by the general method. Recrystallized from H_2O to colorless prisms, m.p. 192~194° (740 mg., 81.5%). *Anal.* Calcd. for $C_7H_8N_4S$: N, 31.10. Found : N, 30.97.

b) Prepared from 2-methyl-5,6-diamino-4-pyrimidinethiol *via* 5-acetyl derivative (VII).

i) 2-Methyl-5-acetamido-6-amino-4-pyrimidinethiol (VII) : To 500 mg. of finely powdered 2-methyl-5,6-diamino-4-pyrimidinethiol, 2.5 cc. of Ac_2O was added, and the whole was warmed on a water bath for 10 min. After cooling, the precipitates were filtered, and dissolved in 10% NaOH. The solution was filtered, and adjusted to pH 5.6. The precipitates were recrystallized from H_2O to give 450 mg. of colorless plates, m.p. 305° (decomp.). *Anal.* Calcd. for $C_7H_{10}ON_4S$: N, 28.27. Found : N, 28.35.

ii) 4-Amino-2,6-dimethylthiazolo[5,4-*d*]pyrimidine (V) : A mixture of 400 mg. of VII and 4 cc. of 10% HCl was refluxed for 10 min. Then, the solution was made basic with NaOH. The resulted precipitates were collected, suspended in H_2O and adjusted to pH 6 with AcOH. The product was recrystallized from dil. EtOH to colorless plates, m.p. 195~196°. Yield, 320 mg. *Anal.* Calcd. for $C_7H_8N_4S$: N, 31.10. Found : N, 30.98. No depression of melting point was observed on admixture with a sample prepared by method a).

c) Prepared from VII (200 mg.) with P_2S_5 in pyridine by the general method. Colorless plates, m.p. 192~194°.

2,8-Dimethyl-6-purinethiol (VI)——Recrystallized from H_2O to colorless powder, m.p. >300° (110 mg., 12.2%). *Anal.* Calcd. for $C_7H_8N_4S$: N, 31.10. Found : N, 31.29. UV $\lambda_{max}^{0.1N\,HCl}$ mμ (log ε) : 227 (4.02), 328 (4.25).

2-Mercapto-8-methyl-6-purinol (IX)——Used 2.22 g. of P_2S_5 (0.01 mole). Colorless crystals, m.p. >300° (810 mg., 89.0%). *Anal.* Calcd. for $C_6H_6ON_4S$: N, 30.76; S, 17.60. Found : N, 30.48; S, 17.85. UV $\lambda_{max}^{H_2O}$ mμ (log ε) : 276 (4.25) at pH 10.4 (NaOH-glycine-NaCl buffer).

8-Methyl-2,6-purinedithiol (X)——Used 3.89 g. of P_2S_5 (0.0175 mole). Yellow crystals, m.p. >300° (860 mg., 86.9%). *Anal.* Calcd. for $C_6H_6N_4S$: N, 28.28. Found : N, 28.39. UV $\lambda_{max}^{H_2O}$ mμ (log ε) : 250 (4.30), 280 (shoulder, 4.20), 347 (4.12) at pH 10.4 (NaOH-glycine-NaCl buffer).

2-Methyl-6-purinethiol (XII)——Recrystallized from H_2O to pale yellow crystals, m.p. >300°. *Anal.* Calcd. for $C_6H_6N_4S$: N, 33.73. Found : N, 33.77. The UV spectra of this compound was identical to that described by Robins.[14]

Ethyl 2-Methyl-4-amino-6-hydroxy-5-pyrimidinecarbamate (XIII)——To a cooled solution of 1.4 g. of 5,6-diamino-2-methyl-4-pyrimidinol in 12 cc. of 10% NaOH, 1.3 g. of ethyl chloroformate was added. After stirred for 1 hr., the solution was acidified with AcOH. The precipitates were dissolved in 15% NaOH, and acidified with AcOH to yield 1.3 g. of yellow crystals, m.p. >300°. *Anal.* Calcd. for $C_8H_{12}O_3N_4$: N, 26.40. Found : N, 26.26.

2-Methyl-6-mercapto-8-purinol (XIV)——a) Prepared from XIII by the general method (reflux time, 32 hr.). Yellow crystals, m.p. >300°. *Anal.* Calcd. for $C_6H_6ON_4S \cdot \frac{1}{2}H_2O$: C, 37.69; H, 3.61; N, 29.31. Found : C, 37.36; H, 3.25; N, 29.15.

b) Applying to Robins' report,[11] this compound was prepared by refluxing a mixture of 2-methyl-6,8-purinediol (XV) and P_2S_5 in pyridine for 24 hr. *Anal.* Calcd. for $C_6H_6ON_4S \cdot \frac{1}{2}H_2O$: N, 29.31. Found : N, 29.24. Comparison of the IR spectra showed this compound to be identical to that prepared by method a).

C) Thiation of 6-Purinols

2-Amino-8-alkyl-6-purinol——i) Ethyl (acylamino)cyanoacetate : Prepared by the acylation of ethyl aminocyanoacetate according to the usual method.

13) S. J. Childress, R. L. McKee : J. Am. Chem. Soc., **73**, 3862 (1951).
14) R. K. Robins, J. Liu : J. Org. Chem., **21**, 695 (1956).

Ethyl (propionamido)cyanoacetate : Colorless needles, m.p. 109~110°. *Anal.* Calcd. for $C_8H_{12}O_3N_2$: N, 15.21. Found : N, 15.44.

Ethyl (butyramido)cyanoacetate : Colorless plates, m.p. 104~105°. *Anal.* Calcd. for $C_9H_{14}O_3N_2$: N, 14.13. Found : N, 14.08.

ii) 5-Acylamino-2,6-diamino-4-pyrimidinol : Prepared by the condensation of ethyl (acylamino)cyanoacetate with guanidine, according to Acker's paper.[15]

5-Propionamido-2,6-diamino-4-pyrimidinol : Colorless prisms, m.p. 267~268° (decomp.). *Anal.* Calcd. for $C_7H_{11}O_2N_5 \cdot H_2O$: N, 32.54. Found : N, 32.51.

5-Butyramido-2,6-diamino-4-pyrimidinol : Colorless prisms, m.p. 291~292°. *Anal.* Calcd. for $C_8H_{13}O_2N_5 \cdot H_2O$: N, 30.55. Found : N, 30.33.

iii) 2-Amino-8-alkyl-6-purinol : Prepared by the treatment of 5-acylamino-2,6-diamino-4-pyrimidinol with NaOH, according to Traube's method.[8] 5-Butyramido-2,6-diamino-4-pyrimidinol and other higher 5-acyl derivatives were failed to cyclize by this method.

2-Amino-8-mercapto-6-purinol——Prepared from 2,5,6-triamino-4-pyrimidinol and thiourea applying the method described by Robins[11] and Cavalieri.[10] Yellow crystals, m.p. >300°.

2-Amino-8-ethylthio-6-purinol——Prepared by treating 2-amino-6-hydroxy-8-purinethiol with ethyl iodide, referring Beaman's paper.[6] Brown crystals, m.p. >300°.

2-Amino-8-ethyl-6-purinethiol——Prepared by the thiation of 2-amino-8-ethyl-6-purinol with P_2S_5 and pyridine. Orange yellow prisms, m.p. >300°.

2-Methyl-6,8-purinediol——Prepared by the condensation of 5,6-diamino-2-methyl-4-pyrimidinol with urea, applying the method reported by Beaman.[6] Colorless crystals, m.p. >300°.

2-Methyl-8-mercapto-6-purinol——Prepared by the condensation of 2-methyl-5,6-diamino-4-pyrimidinol with thiourea. Yellow powder, m.p. >300°.

2-Methyl-6,8-purinedithiol——Prepared by the thiation of 2-methyl-8-mercapto-6-purinol with P_2S_5 and pyridine referring the Robins' report.[11] Yellow crystals, m.p. >300°.

2-Ethyl-4-amino-6-methylthiazolo[5,4-*d*]pyrimidine——Prepared from 2-methyl-5,6-diamino-4-pyrimidinethiol *via* 5-propionyl derivative, according to the preparation of which reported by Ishidate.[7] Intermediate, 2-methyl-5-propionamido-6-amino-4-pyrimidinol was used for further synthesis without purification. Colorless needles, m.p. 169.5~170.5°.

2-Methyl-5-(*p*-tolylsulfonamido)-6-amino-4-pyrimidinol——To a solution of 2-methyl-5,6-diamino-4-pyrimidinol in 9 cc. of 10% NaOH, 1.9 g. of *p*-tolylsulfonyl chloride was added portionwise by shaking. After continued the shaking for 2 hr., the resulted solution was neutralized with AcOH. The product was recrystallized from H_2O to pale yellow prisms, m.p. 291°(decomp.). *Anal.* Calcd. for $C_{12}H_{14}O_3N_4S$: N, 19.04. Found : N, 19.23.

Summary

The replacement of 4-hydroxyl group of aminopyrimidinols by sulfhydryl in one step, through the action of phosphorus pentasulfide in triethylamine or 3-picoline. 5-Acylamino-6-amino-4-pyrimidinols were treated with phosphorus pentasulfide in pyridine, to yield thiazolo[5,4-*d*]pyrimidine and 6-purinethiol compounds. The yield of both two type compounds were changed according to a functional group in the starting substance. These products, in addition, other 6-purinethiol and related compounds were screened as to their antiviral activities on poliomyelitis virus. Any of the compounds, however, did not exert a significant activity on the virus.

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15) D.S. Acker, J.E. Castle : J. Org. Chem., **23**, 2010 (1958).