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103. Yoshihisa Mizuno, Morio Ikehara, and Kyoichi A. Watanabe: Potential Antimetabolites. I. Selective Thiation of Uracil and 1,2,4-Triazine-3,5(2*H*, 4*H*)-dione (6-Azauracil).*1

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Derivatives of 1,2,4-triazine have recently attracted interest because of their biological activity as the analogs of naturally occurring pyrimidines. For example, Prusoff and Holmes[1] were the first to investigate one of such compounds, 6-methyl-1,2,4-triazine-3,5(2*H*, 4*H*)-dione (6-azathymine) (I; R=CH_3) as an antagonist of thymine and demonstrated its ability to inhibit the growth of various species of bacteria.[2] Later, it was found by Handschumacher and Welch[3] and also independently by Sorm, Jakubovic and Slechta[4] that 1,2,4-triazine-3,5(2*H*, 4*H*)-dione (6-azauracil) (I; R=H) has a marked inhibitory effect on a number of microorganisms. Quite recently, Elion, *et al.* reported[5] the biological behavior of 5-amino-1,2,4-trtazin-3(2*H*)-one (6-azacytosine) (III, R=H). The accumulation of its nucleoside in the medium of Escherichia coli B grown in the presence of 6-azacytosine was observed by Hitchings and his coworkers.[6] All these derivatives were tested in the chemotherapy of experimental neoplastic diseases and among them 6-azacytosine was found to be most effective against Adenocarcinoma 755 despite the low antibacterial activity. Work aiming at the clarification of the mechanism of their antimetabolic actions of 6-azathymine[7] and 6-azauracil[8] has been also carried out intensively, but the mechanism has been clarified only to a certain extent.

One of troubles to investigators working in this field is that 1,2,4-triazine derivatives, especially 6-azauracil and 6-azacytosine are not always easily available. It seems, therefore, of value to devise an improved synthesis of these biologically interesting derivatives.

The present paper deals with a selective thiation of 1,2,4-triazine-3,5(2*H*, 4*H*)-dione (6-azauracil) leading to an improved method of preparation of 6-azacytosine.

Survey of the literature showed that 5-chloro derivatives of 1,2,4-triazine series were

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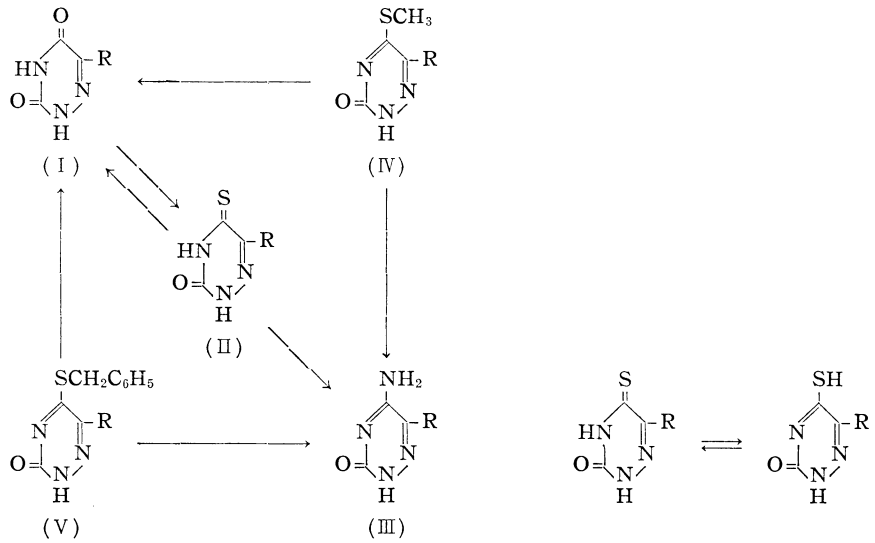
1) W.H. Prusoff, W.L. Holmes: Federation Proc., **11**, 271 (1952).
2) W.H. Prusoff, W.L. Holmes, A.D. Welch: Cancer Research, **14**, 570 (1954).
3) R.E. Handschumacher, A.D. Welch: Federation Proc., **15**, 267 (1956).
4) F. Sorm, A. Jakubovic, L. Slechta: Experientia, **12**, 271 (1956).
5) G.B. Elion, S. Bieber, H. Nathan, G.H. Hitchings: Cancer Research, **18**, 802 (1958).
6) S. Bresnik, S. Singer, G.H. Hitchings: Biochem. et Biophys. Acta, **37**, 251 (1960).
7) W.H. Prusoff, A.D. Welch: *Ibid.*, **20**, 209 (1956).
8) R.E. Handschumacher: *Ibid.*, **23**, 428 (1957); J. Skoda, F. Sorm: *Ibid.*, **28**, 659 (1958); R.E. Handschumacher: J. Biol. Chem., **234**, 2992 (1959); *Idem*: *Ibid.*, **235**, 764 (1960).

extremely unstable[9] and these could hardly serve as an intermediate for the synthesis of 6-azacytosine, while this could be prepared by amination of 5-mercapto-1,2,4-triazin-3(2*H*)-one (II; R=H). Shortcoming of the latter method[10] is the poor yield of the key intermediate, the 5-mercapto-1,2,4-triazine derivative (II; R=H) from the corresponding oxy-derivative (I; R=H). Therefore, the synthesis of 6-azacytosine by way of 5-mercapto-1,2,4-triazine derivative will be much improved, provided that a convenient method of synthesis of the key intermediate could be devised. Again extensive survey of the literature dealing with thiation of oxy-derivatives of pyrimidine series revealed[10~13] that there was a certain difference of reactivity toward phosphorus pentasulfide among hydroxyl groups according to positions, at least in the case of N-substituted ones, which suggested that even in the case of N-unsubstituted dioxypyrimidines, monothiation might be effected by treatment with an appropriate amount of phosphorus pentasulfide.

In order to find a suitable amount of phosphorus pentasulfide required for monothiation, the effect of the amount of phosphorus pentasulfide upon the yield of 5-mercapto-1,2,4-triazin-3(2*H*)-one obtained was examined, keeping the amount of the oxy-triazine base constant, and the results obtained are summarized in Table I. As

Table I.

6-Azauracil		Phosphorus Sulfide (P_4S_{10})				Yield of 5-Mercapto-derivative	
(g.)	(mole)	(g.)	P→S	P-S-P	Total S	(g.)	(%)
2.5	.022	0.9	0.008	0.012	0.020	0.8	28.6
2.5	.022	1.4	0.013	0.020	0.033	1.0	35.7
2.5	.022	2.2	0.020	0.030	0.050	2.0	71.4
2.5	.022	2.3	0.021	0.031	0.052	2.1	75.0
2.5	.022	2.4	0.022	0.033	0.054	2.8	100.0
2.5	.022	2.5	0.023	0.034	0.057	2.2	78.6
2.5	.022	2.6	0.024	0.035	0.059	1.8	64.3
2.5	.022	7.5	0.068	0.101	0.171	0.2	7.1



9) P.K. Chang, T.L.V. Ulbricht: J. Am. Chem. Soc., **80**, 976 (1958).
10) E.A. Falco, E. Pappas, C.H. Hitchings: *Ibid.*, **78**, 1938 (1956).
11) J.J. Fox, D. Van Praag, I. Wempen, I.L. Doerr, L. Cheong, J.E. Knoll, A. Bendich, G.B. Brown: *Ibid.*, **81**, 178 (1959).
12) G.B. Elion, G.H. Hitchings: *Ibid.*, **69**, 2138 (1947).

shown in the table the 5-monomercapto product was obtained in almost quantitative yield when molar ratio of phosphorus pentasulfide to the base was 1:4 (on the basis of the chemical formula of P_4S_{10}) or 1:2 (on the basis of the chemical formula of P_2S_5).

The use of larger amount of phosphorus pentasulfide resulted in decrease of the yield of the monomercapto compound, and correspondingly an increase in yield of dimer capto derivative was observed.

In the case of monothiation of uracil, the use of the same ratio of phosphorus pentasulfide to the base again gave a satisfactory result.

These results might be explained on the assumption that only the coordinately linked four sulfur atoms in the phosphorus sulfide molecule take part in the thiation reaction and six sulfurs linked covalently to phosphorus are ineffective in the reaction (See Fig. 1).

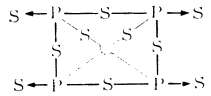


Fig. 1.
Molecular Structure of P_4S_{10}[14]

Falco and Hitchings' method[10] of preparation of 6-azacytosine was further improved by using 5-alkylthio-1,2,4-triazine derivatives instead of 5-mercapto-1,2,4-triazine (II). The improvement of the present method is based on the fact that alkylthio groups are more easily replaced by nucleophilic reagents than the corresponding unsubstituted sulfur. Introduction of methyl group on sulfur will have another advantage in the case of glycosidation of the base. Discussion and experiments along this line will be described in the following paper.

Experimental

1, 2, 4-Triazine-3, 5(2*H*,4*H*)-dione (6-Azauracil) (I; R=H)[*3]——This compound was prepared by Welch's procedure improved as follows: Crude 3-mercapto-1,2,4-triazin-5(4*H*)-one-6-carboxylic acid[15] (20 g.) was poured into 4*N* HNO_3 (400 cc.) and refluxed mildly for. 1 hr. A trace of impurity was filtered off. White prisms of 1,2,4-triazine-3,5(2*H*,4*H*)-dione-6-carboxylic acid gradually separated from the filtrate. The yield was almost quantitative. When the carboxylic acid was heated at 230~250° for 30 min., decarboxylation was complete, and 6-azauracil (I; R=H) was produced almost theoretically. Recrystallization from H_2O gave white, fine crystals, m.p. 275°.

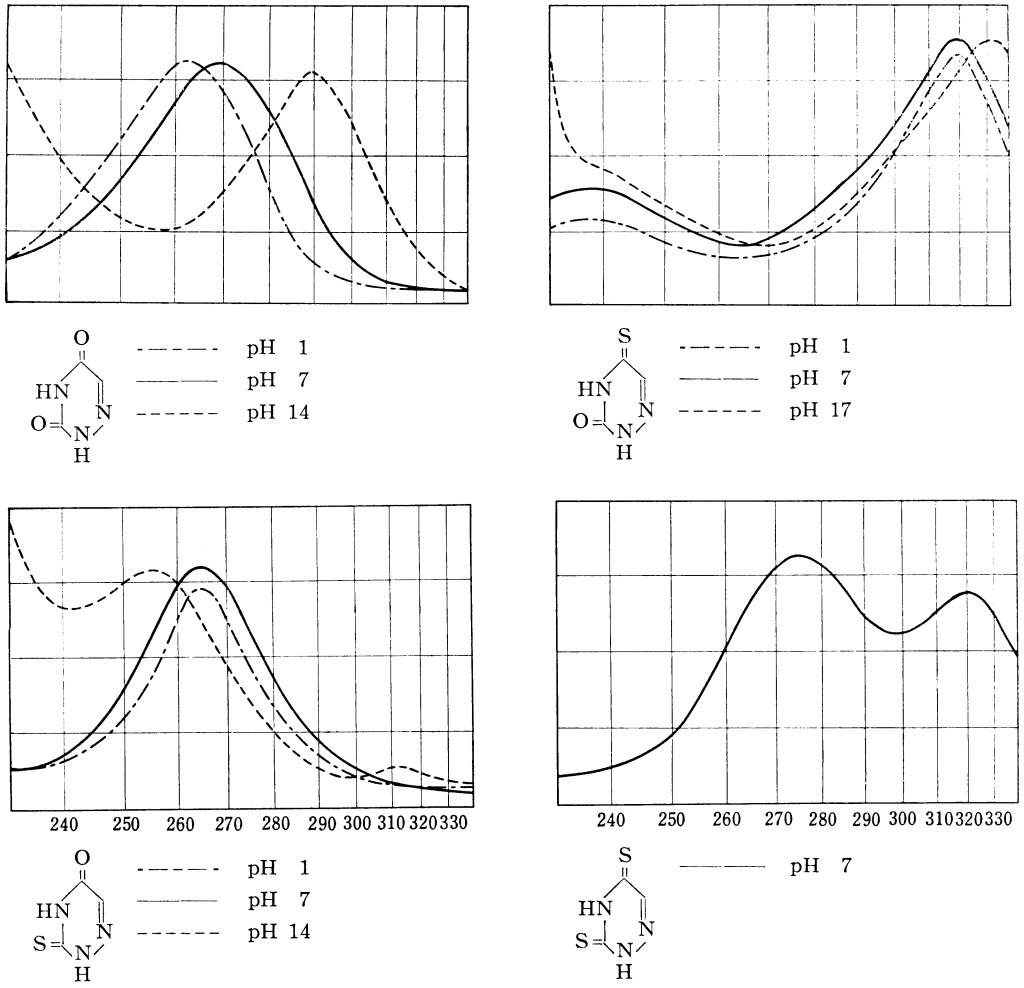
5-Mercapto-1,2,4-triazine-3(2*H*)-one (II; R=H)——A suspension of freshly ground P_4S_{10} (7.5 g.) and 6-azauracil (8.0 g., 0.071 mole) in pyridine (250 cc.)[*4] was heated at reflux temperature for 3 hr., and the solvent was evaporated in a reduced pressure. The residue was diluted with H_2O (150 cc.), adjusted to pH 9 with 2*N* NaOH, and a trace of insoluble material was filtered off. The filtrate was washed with Et_2O (100 cc. × 2), brought to pH 3 with 2*N* HCl, and extracted with Et_2O (100 cc. × 5). Et_2O extracts were dried over Na_2SO_4 and evaporated to dryness on a water bath to give 5-mercapto-1,2,4-triazin-3(2*H*)-one (II; R=H) (9.0 g., 98.5%). Bright orange prisms, m.p. 222° (reported m.p. 213~214°[10]). The product thus obtained was sufficiently pure for synthetic use.

5-(Benzylthio)-1,2,4-triazine-3(2*H*)-one (V; R=H)——The above crude mercapto derivative (II; R=H) (4.9 g., 0.038 mole) was dissolved in 0.3*N* NaOH (350 cc.) with mechanical stirring and a trace of insoluble substance was filtered off. To the filtrate BzCl (5.0 g., 0.038 mole) was added and the

*3 One of the starting materials of 6-azauracil, disodium mesoxalate was kindly supplied by Shionogi & Co., Ltd., to which the authors' thanks are due.

*4 The property and the amount of the solvent affected greatly to the yield. When dehydrated pyridine was used as a solvent, the reaction mixture darkened considerably. Tarry residue was produced and isolation of the desired product from it was extremely laborious. Almost theoretical yield was obtained if an appropriate volume of low-boiling pyridine (b.p. 113°), which may contain an appreciable amount of H_2O, was used.

13) J. J. Fox, D. Van Praag *Ibid.*, **82**, 486 (1960).
14) J. R. Van Wazer: "Phosphorous and its Compounds", **1**, 291, 270 (1958). Interscience Publ. Inc., New York.
15) A. D. Welch: J. Am. Chem. Soc.' **78**, 1258 (1956).

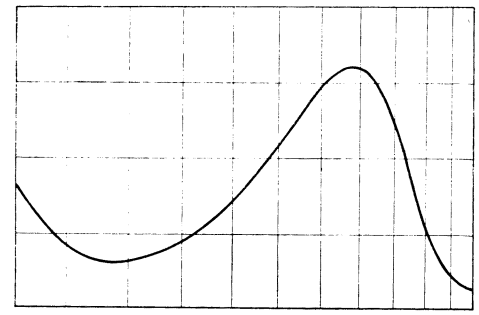


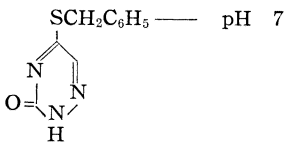
stirring was continued for about 3 hr. at 12°. The solution was acidified with AcOH and yellowish feathers of (V; R=H) precipitated, which, after recrystallization from EtOH, formed yellow needles, m.p. 201° (7.2 g., 86.7%). *Anal.* Calcd. for $C_{10}H_9ON_3S$: C, 54.8; 4, 4.1; N, 19.2. Found: C, 54.6; H, 4.5; N, 19.9.

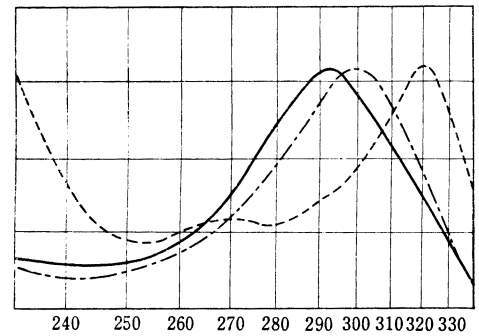
5-(Methylthio)-1, 2, 4-triazine-3(2*H*)-one (IV; R=H)——The mercapto triazine (II; R=H) (6.5 g., 0.05 mole) was methylated by dissolving in 0.2*N* NaOH (250 cc.), MeI (7.8 g., 0.055 mole) was added and the mixture was stirred mechanically for 1 hr. The solution was adjusted to pH 6.2 and concentrated to about 80 cc. in a diminished pressure. The separated yellow crystals were filtered off and (IV; R=H) was extracted from the filtrate with $CHCl_3$ (80 cc. × 3). $CHCl_3$ layer was dried over anhyd. Na_2SO_4, then the solvent was evaporated to dryness. The residue and the crude (IV; R=H) separated from aqueous layer were crystallized from EtOH and hexane to yellow needles, m.p. 177~179°. Yield, 6.2 g. (86.7%). *Anal.* Calcd. for $C_4H_5ON_3S$: C, 33.6; H, 3.5; N, 29.4. Found: C, 33.9; H, 3.3; N, 29.6.

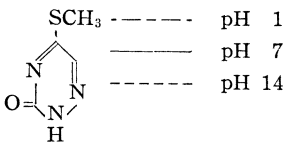
5-Amino-1,2,4-triazine-3(2*H*)-one (6-Azacytosine)(III; R=H)——(a) To the thoroughly dried mercapto triazine (II; R=H) (6.0 g., 0.0465 mole), 600 cc. of NH_3-MeOH (satd. at 0) wasad ded. After the mixture was heated in an autoclave at 100~120° for 20 hr., the solvent was evaporated on a water bath in a reduced pressure. The residue was recrystallized from H_2O to 6-azacytosine as white prisms, which did not melt below 320°. Yield, 3.3 g. (63.3%). *Anal.* Calcd. for $C_3H_4ON_4$: C, 32.1; H, 3.6; N, 50.0. Found: C, 32.5; H, 3.3; N, 49.7.

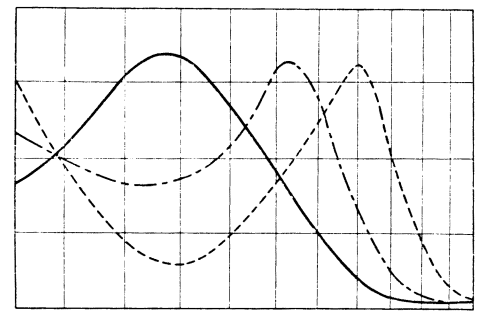
(b) Treatment of the mercapto derivative (II; R=H) (1.3 g., 0.01 mole) with NH_3-MeOH (satd. at 0°) (60 cc.) in a sealed tube at room temperature for 1 week afforded 6-azacytosine. Recrystallization from H_2O gave 0.7 g. (62.5%) of pure material.

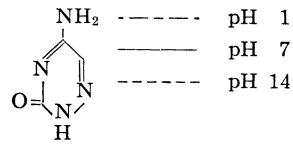












(c) Analogous treatment of methylthio (IV; R=H) or benzylthio derivative (V; R=H) for 2 days with NH_3-MeOH as in method separated 6-azacytosine as white prisms in an almost quantitative yield.

5-(Benzylamino)-1,2,4-triazine-3(2*H*)-one——A mixture of 5.0 g. of 5-mercapto-1,2,4-triazin-3(2*H*)-one and 40 cc. of benzylamine was mildly heated. The triazine soon dissolved with effervescence and the mixture darkened considerably, but the solution soon became green, then turned yellow. After 8 min., excess of benzylamine was removed *in vacuo*. The residue, when crystallized from pyridine afforded slightly yellow needles (7.0 g., or 89.7%), m.p. 211°. *Anal.* Calcd. for $C_{10}H_{10}ON_4$: C, 59.41; H, 4.95; N, 27.27. Found: C, 59.78; H, 5.22; N, 27.21.

Conversion of Mercapto- or Alkylthiotriazine into 6-Azauracil——(a) Hydrolysis of alkylthiotriazine: Methylthio- or benzylthiotriazine (0.01 mole) was heated with conc. HCl (120 cc.) at 80° for 12 hr., boiled for 30 min., and filtered while hot to remove a small amount of insoluble matter. After the filtrate was concentrated to about 40 cc. in a diminished pressure, the residue was allowed to stand in an ice box overnight, 6-azauracil separated quantitatively.

(b) Oxidation with Nitric Acid: To 0.01 mole of mercapto-(II; R=H) or alkylthio-(IV; R=H or V, R=H) derivative 80 cc. of 3*N* HNO_3 was added, the mixture was heated under reflux for 1 hr., filtered and the filtrate was concentrated to about 40 cc. A quantitative amount of 6-azauracil gradually separated, m.p. 272°. Admixture with authentic sample melted 273°.

Above evidences led to the identification of the structures of alkylated mercaptotriazines as S-alkyl derivatives (IV; R=H) and (V; R=H).

Thiation of Uracil——A mixture of uracil (2.5 g., 0.02 mole) and P_4S_{10} (2.2 g.) in pyridine*5 (80 cc.) was refluxed with stirring for 3 hr. The bright orange supernatant was separated by decantation and evaporated to dryness in a reduced pressure. The bright orange needles, m.p. 320°,[17] of 4-mercapto-2(1*H*)-pyrimidinone were obtained when the residue was crystallized from H_2O. Yield, 2.2 g., or 76.9%. The structure was determined by comparing the ultraviolet absorption spectrum of the thiation product with that reported for 4-mercapto-2(1*H*)-pyrimidinone.[18]

Treatment of 4-Mercapto-2(1*H*)-pyrimidinone with Ammonia——(a) A sealed tube containing 500 mg. of the above mercaptopyrimidine in 30 cc. of NH_3-MeOH (satd. at 0°) was heated for 40 hr. at 100°. After the solvent was evaporated to dryness, the residue was crystallized from H_2O to white prisms, m.p. 300° (decomp.). Comparison of the ultraviolet absorption spectrum of this compound with that of cytosine[19] led to the identification of this compound as cytosine. *Anal.* Calcd. for $C_4H_5ON_3$: C, 43.2; H, 4.5; N, 37.8. Found: C, 43.4; H, 4.5; N, 37.6.

(b) 4-(Methylthio)-2(1*H*)-pyrimidinone[20] (300 mg.) was treated with NH_3-MeOH (satd. at 0°) for 1 week, at room temperature. A theoretical yield of cytosine was obtained on evaporation of the solvent to dryness.

Under the similar condition cytosine was not obtained from 4-mercapto-2(1*H*)-pyrimidinone.

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Summary

1,2,4-Triazine-3,5(2*H*, 4*H*)-dione (6-azauracil) (I; R=H) and uracil were partially thiated to afford 5-mercapto-1,2,4-triazin-3(2*H*)-one (II; R=H) and 4-mercapto-2(1*H*)-pyrimidinone, respectively, in good yields by the use of limited amount of phosphorus pentasulfide. These are the first examples of the success of monothiation in triazine and pyrimidine series. The alkylation of 5-mercapto-1,2,4-triazin-3(2*H*)-one gave the corresponding alkylthio derivatives (IV and V; R=H) from which 5-amino-1,2,4-triazin-3(2*H*)-one (6-azacytosine) (III; R=H) was synthesized in an excellent yield.

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*5 Only suitably hydr. pyridine (b.p. 113°) was applicable. Otherwise it was fairly difficult to isolate the desired product in a pure state.

17) H. L. Wheeler, L. M. Liddle: Am. Chem. J., **40**, 547 (1908).
18) G. B. Elion, W. S. Ide, C. H. Hitchings: J. Am. Chem. Soc., **68**, 2137 (1946).
19) D. Shugar, J. J. Fox: Biochim. et Biophys. Acta, **9**, 199 (1952).
20) H. L. Wheeler, T. B. Johnson: Am, Chem. J., **42**, 30 (1909).