UDC 547.859.3〔547.792.9〕

169. Yasuo Makisumi and Hideo Kanō : Synthesis of Potential Anticancer Agents. III.[1] 7-Substituted *s*-Triazolo〔2,3-*a*〕-pyrimidines and their Halogenated Compounds.

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In the preceding of this series,[1] the syntheses of a number of 7-substituted 5-methyl-*s*-triazolo〔2,3-*a*〕pyrimidines and their 6-halogeno derivatives for the purpose of biological evaluation were described. Some of them inhibited the growth of microorganisms and were effective against transplantable tumor in animals. It seemed of interest to prepare 7-substituted *s*-triazolo〔2,3-*a*〕pyrimidines, which involves some isomeric compounds of natural purines and their antagonists.

The starting compound, 7-hydroxy-*s*-triazolo〔2,3-*a*〕pyrimidine (I), was prepared by the following two methods. Condensation of malic acid and 3-amino-*s*-triazole in fuming sulfuric acid at low temperature gave (I) as white needles, m.p. 286~287°, in ca. 45% yield. It seemed that in this reaction malic acid was transferred to 2-formylacetic acid by decarboxylation and dehydrogenation by the action of fuming sulfuric acid, and 2-formylacetic acid was condensed with 3-amino-*s*-triazole.

On the other hand, the same compound (I) was obtained by heating 2-hydrazino-4-hydroxypyrimidine and formic acid for six hours. During the course of this study, Shirakawa[2] reported the same preparation and disclosed that 2-hydrazino-4-hydroxypyrimidine was formylated to 2-formylhydrazino-4-hydroxypyrimidine which condensed intramolecularly to 5-hydroxy-*s*-triazolo〔4,3-*a*〕pyrimidine (I′). This compound (I′) could be readily rearranged to (I) on boiling with formic acid.

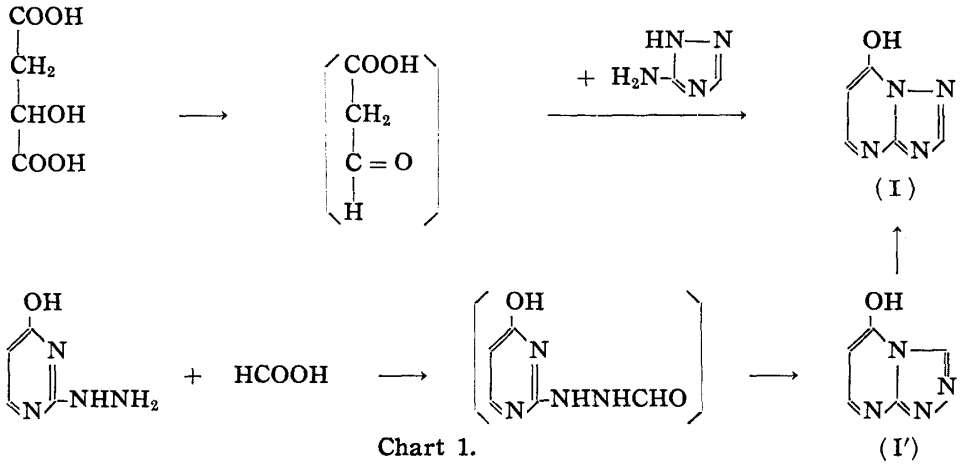

Chart 1.

(I) was converted into 7-chloro-*s*-triazolo〔2,3-*a*〕pyrimidine (II) by the action of phosphoryl chloride, which was readily hydrolysed with dilute acid or alkali to the original material (I). This fact showed that chlorine atom at 7-position in *s*-triazolo〔2,3-*a*〕-pyrimidine is reactive. Therefore, the following reactions were carried out under relatively mild conditions.

Dehalogenation of (II) using palladium-charcoal as a catalyst in water gave

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1) Part II : This Bulletin, **7**, 903(1959).

2) Paper read at the Kinki Local Meeting of the Pharmaceutical Society of Japan, December 20, 1958.

s-triazolo[2,3-*a*]pyrimidine (III), and the reaction of (II) with equimolar amount of thiourea produced 7-mercapto derivative (IV) in a quantitative yield. Treatment of (II) with ethanolic ammonia solution in a sealed tube at 120~130° gave 7-amino derivative (V), and that with furfurylamine in ethanol gave 7-furfurylamino derivative (VI).

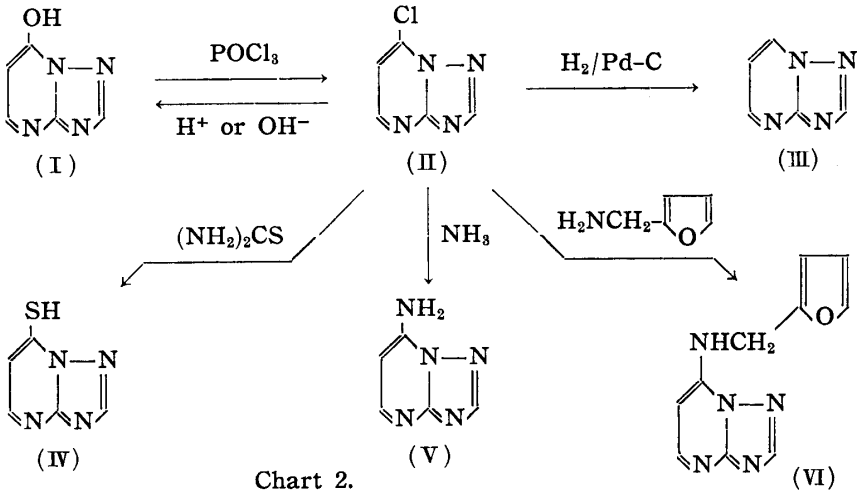

Chart 2.

In the foregoing paper,[1] it was shown that the chlorination or bromination of 7-hydroxy- and 7-amino-5-methyl-*s*-triazolo[2,3-*a*]pyrimidine with chlorine or bromine in acetic acid or water gave the corresponding 6-halogenated derivatives. This halogenation seemed to occur by the -M effect of the hydroxy or amino group in the 7-position of *s*-triazolo[2,3-*a*]pyrimidine.

By the same idea, halogenation of 7-hydroxy and 7-amino compounds (I and V) was attempted. The reaction of (I) and (V) with chlorine or bromine in glacial acetic acid gave their monohalogenated products. These products would be 6-halo-7-hydroxy derivatives (VII and VIII) and 6-halo-7-amino derivatives (XI and XII).

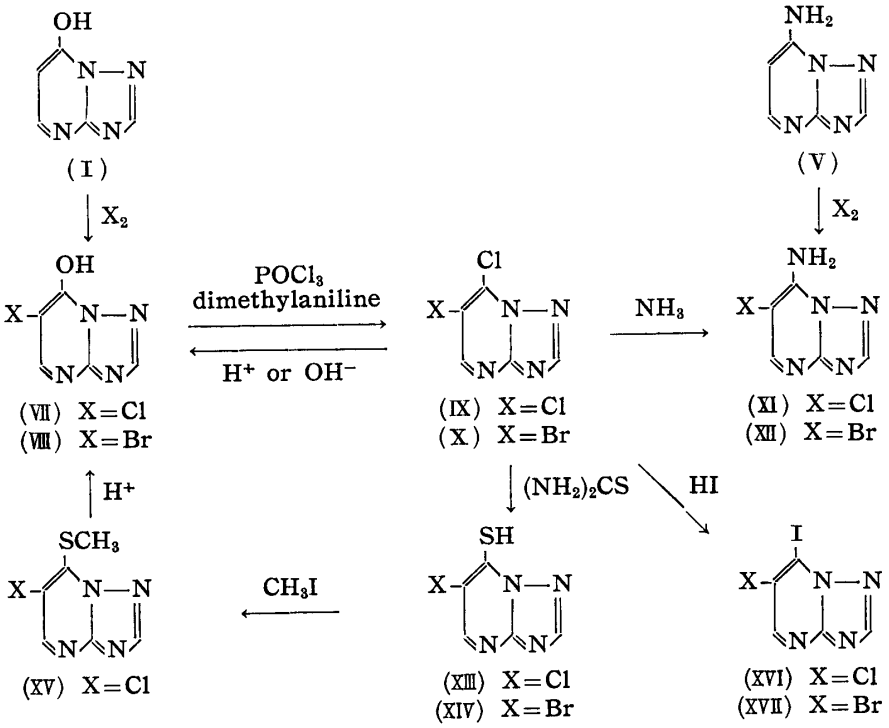

Chart 3.

These 6-halo-7-hydroxy derivatives (VII and VIII) were converted into 6-halo-7-chloro-*s*-triazolo[2,3-*a*]pyrimidines (IX and X) by the action of phosphoryl chloride in the presence of dimethylaniline. As the chlorine atom at 7-position of (IX) and (X) is very reactive, these compounds were readily hydrolysed to the corresponding 7-hydroxy-6-halogeno derivatives (VII and VIII) on warming with dilute acid or alkali solution, and also converted into (XI and XII) by conc. ammonia at room temperature. These were identical with (XI) and (XII) obtained by halogenation of 7-amino-*s*-triazolo[2,3-*a*]pyrimidine. Furthermore, (IX) and (X) were converted into 6-halo-7-mercapto derivatives (XIII and XIV) on refluxing with thiourea in ethanol for 20~30 minutes and also into 6-halo-7-iodo derivatives (XVI and XVII) by the action of conc. hydrogen iodide solution at room temperature. Methylation of (XIII) with methyl iodide in dil. sodium hydroxide solution afforded 7-methylthio compound (XV), which was hydrolysed to (VII) with 25% sulfuric acid. (XVI) and (XVII) were also hydrolysed to the corresponding 6-halo-7-hydroxy derivatives (VII and VIII) on heating with dilute alkaline solution.

By these reactions it became evident that the halogen atom at 7-position of *s*-triazolo[2,3-*a*]pyrimidine reacted readily with nucleophilic reagents, but halogen atom at 6-position was very resistant to nucleophilic reagents.

In preliminary biological tests, (II), (IV), (XIII), and (XIV) were found to inhibit the growth of *Lactobacillus casei* and *Streptococcus faecalis*, and (IX) and (X) inhibited the growth of *Escherichia coli*. Some of these derivatives were effective against transplantable tumor in animals. Biological details will be published elsewhere.

The authors are deeply grateful to Dr. K. Takeda, Director of this Laboratory, for his kind advice and encouragement. Their thanks are also due to the members of analysis room of this Laboratory for elementary analysis.

Experimental*2

7-Hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (I)—a) 160 cc. of 10% fuming H_2SO_4 was cooled and 90 g. of malic acid was added with good agitation at such a rate that the temperature did not rise above 0°. After the addition of malic acid was completed, 50.4 g. of 3-amino-*s*-triazole was added to the resulting solution under the same condition. The mixture was allowed to warm spontaneously to room temperature and stood overnight. It was then heated cautiously on a water bath with vigorous stirring for about 1 hr. The reaction mixture was cooled and poured on ice. The resulting solution was adjusted to pH 6 with NH_4OH, the precipitated crystals were collected by filtration, washed with water, and recrystallized from water to 36 g. of white needles, m.p. 286~287°. *Anal.* Calcd. for $C_5H_4ON_4$: C, 44.12; H, 2.96; N, 41.17. Found: C, 44.29; H, 2.81; N, 41.25.

b) A mixture of 1.2 g. of 2-hydrazino-4-hydroxypyrimidine and 7.2 cc. of formic acid was refluxed for 6 hr. The excess of formic acid was removed under reduced pressure and the residue was diluted with EtOH. The resulting crystals were collected, washed with water, and recrystallized from water to 1 g. of white needles, m.p. 287°. This sample showed no depression on admixture with the sample prepared by the method a) described above. *Anal.* Calcd. for $C_5H_4ON_4$: C, 44.12; H, 2.96; N, 41.17. Found: C, 44.14; H, 3.36; N, 41.44.

7-Chloro-*s*-triazolo[2,3-*a*]pyrimidine (II)—A mixture of 13.6 g. of (I) and 68 cc. of $POCl_3$ was refluxed under anhydrous conditions for 1~1.5 hr. The solution was concentrated *in vacuo* to a viscous syrup, which was poured with stirring on crushed ice. The solution was then thoroughly agitated and carefully made alkaline with NH_4OH. The precipitate was collected, washed with water, and dried (9.8 g.). The filtrate was extracted three times with $CHCl_3$, which was dried over Na_2SO_4, and evaporation of $CHCl_3$ gave 1.7 g. of a crude product. Both the products were combined and recrystallized from water to 10 g. of white needles, m.p. 175~176°. *Anal.* Calcd. for $C_5H_3N_4Cl$: C, 38.87; H, 1.97; N, 36.27. Found: C, 38.90; H, 2.23; N, 36.60.

***s*-Triazolo[2,3-*a*]pyrimidine (III)**—3.0 g. of (II) was shaken at room temperature with 1 g. of 10% Pd-C in 30 cc. of water at 1 atm. of hydrogen. A theoretical uptake of 1 mole of H_2 occurred during about 2 hr. After removal of the catalyst, the solution was adjusted to pH 7 with NH_4OH and evaporated to dryness *in vacuo*. The residue was extracted with $CHCl_3$ and evaporation of the

*2 All m.p.s are uncorrected.

solvent gave 1.1 g. of pale yellow solid which was recrystallized from benzene to white prisms, m.p. 151°. *Anal.* Calcd. for $C_5H_4N_4$: C, 50.00; H, 3.36; N, 46.66. Found: C, 50.22; H, 3.63; N, 46.37.

7-Mercapto-*s*-triazolo[2,3-*a*]pyrimidine (IV)—A mixture of 1.5 g. of (II) and an equimolar quantity of thiourea in 30 cc. of dehyd. EtOH was refluxed, by which the solid dissolved and soon a yellow crystalline product deposited. After refluxing for 20 min., the mixture was chilled, the product was collected, and recrystallized from EtOH to 1.2 g. of pale yellow needles, m.p. 294~295° (decomp.). *Anal.* Calcd. for $C_5H_4N_4S$: C, 39.46; H, 2.65; N, 36.82. Found: C, 39.44; H, 2.68; N, 37.03.

7-Amino-*s*-triazolo[2,3-*a*]pyrimidine (V)—In a bomb were placed 2 g. of (II) and 25 cc. of dehyd. EtOH saturated with dry NH_3 at 0°, and the bomb was heated at 120~130° for 5 hr. After cool, the resulting product was collected and recrystallized from water to 1.2 g. of white needles, m.p. 279~280°. *Anal.* Calcd. for $C_5H_5N_5$: C, 44.44; H, 3.70; N, 51.85. Found: C, 44.41; H, 3.70; N, 51.99.

7-Furfurylamino-*s*-triazolo[2,3-*a*]pyrimidine (VI)—To a solution of 1 g. of (II) in 25 cc. of EtOH, a solution of 0.7 g. of furfurylamine in 2 cc. of EtOH was added slowly. After refluxing for about 20 min., evaporation of the solvent gave 1.2 g. of crude product. Recrystallization from water gave 1.0 g. of white needles, m.p. 199~200°. *Anal.* Calcd. for $C_{10}H_9ON_5$: C, 55.81; H, 4.22; N, 32.54. Found: C, 55.63; H, 4.68; N, 32.82.

6-Chloro-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (VII)—To a solution of 1.4 g. of (I) in 25 cc. of glacial AcOH, 0.7 g. of Cl_2 gas was absorbed. After stirring for 3 hr., the precipitated product was collected and recrystallized from 50% EtOH to 1.0 g. of white needles, m.p. over 300°. *Anal.* Calcd. for $C_5H_3ON_4Cl$: C, 35.19; H, 1.76; N, 32.85. Found: C, 35.48; H, 2.03; N, 32.98.

6-Bromo-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (VIII)—To a solution of 0.5 g. of (I) dissolved in 20 cc. of glacial AcOH, a solution of 0.6 g of Br_2 in 10 cc. of glacial AcOH was added slowly in drops and the reaction mixture was decolorized, when the product deposited. After stirring at room temperature for 3 hr., the precipitate was collected, washed with EtOH, and recrystallized from water to 0.6 g. of white scales, m.p. over 300°. *Anal.* Calcd. for $C_5H_3ON_4Br$: C, 27.91; H, 1.40; N, 26.05. Found: C, 28.17; H, 1.66; N, 26.16.

6,7-Dichloro-*s*-triazolo[2,3-*a*]pyrimidine (IX)—To a mixture of 1.2 g. of (VII) in 20 cc. of $POCl_3$, 4 cc. of dimethylaniline was added and the mixture was refluxed for 2 hr. The excess $POCl_3$ was removed under reduced pressure on a water bath, the syrupy residue was poured with vigorous stirring into an ice water, and the aqueous solution was extracted with three 20-cc. portions of $CHCl_3$. The $CHCl_3$ extract was washed with two 30-cc. portions of cold water and dried over $CaCl_2$. Evaporation of $CHCl_3$ left 1.2 g. of a crude product, which was recrystallized from EtOH to 1.0 g. of pale yellow prisms, m.p. 139~140°. *Anal.* Calcd. for $C_5H_2N_4Cl_2$: C, 31.74; H, 1.06; N, 29.63. Found: C, 32.11; H, 1.28; N, 29.94.

6-Bromo-7-chloro-*s*-triazolo[2,3-*a*]pyrimidine (X)—A mixture of 3 g. of (VIII), 30 cc. of $POCl_3$, and 6 cc. of dimethylaniline was refluxed for 2 hr. The reaction mixture was treated by the same procedure as for (IX). Pale yellow needles (3 g.), m.p. 183°. *Anal.* Calcd. for $C_5H_2N_4BrCl$: C, 25.70; H, 0.86; N, 23.98. Found: C, 25.98; H, 1.09; N, 23.96.

6-Chloro-7-amino-*s*-triazolo[2,3-*a*]pyrimidine (XI)—a) To a solution of 0.5 g. of (V) in 10 cc. of glacial AcOH, 0.27 g. of Cl_2 gas was absorbed and after stirring this mixture at room temperature for 2 hr., the precipitated crystals were collected, dissolved in water, and neutralized with Na_2CO_3 solution. The resulting product was collected by filtration and recrystallized from EtOH to 0.25 g. of colorless needles, m.p. 268~268.5°. *Anal.* Calcd. for $C_5H_4N_5Cl$: C, 35.40; H, 2.36; N, 41.30. Found: C. 35.32; H, 2.51; N, 41.52.

b) To a solution of 0.5 g. of (IX) dissolved in 10 cc. of EtOH, 5 cc. of conc. NH_4OH was added and the mixture was allowed to stand at room temperature for 8 hr. The separated crystals (0.45 g.) were recrystallized fron dil. EtOH to colorless needles, m.p. 268°. This sample showed no depression on admixture with the sample prepared from (V) described above.

6-Bromo-7-amino-*s*-triazolo[2,3-*a*]pyrimidine (XII)—a) To a solution of 0.5 g. of (V) in 10 cc. of glacial AcOH, a solution of 0.6 g. of Br_2 in 5 cc. of glacial AcOH was slowly added dropwise, by which the reaction mixture was decolorized and crystals deposited. After stirring at room temperature for 3 hr., the separated crystals were collected, washed with EtOH, and recrystallized from dil. EtOH to 0.4 g. of white needles, m.p. 278° (decomp.). *Anal.* Calcd. for $C_5H_4N_5Br$: C, 28.04; H, 1.84; N, 32.71. Found: C, 28.32; H, 2.00; N, 33.01.

b) (XII) was also obtained from 0.5 g. of (X) by the same procedure as for (XI). Colorless needles (0.45 g.), m.p. 278° (decomp.) (from dil. EtOH). *Anal.* Calcd. for $C_5H_4N_5Br$: C, 28.04; H, 1.84; N, 32.71. Found: C, 28.15; H, 1.99; N, 32.93.

6-Chloro-7-mercapto-*s*-triazolo[2,3-*a*]pyrimidine (XIII)—A mixture of 0.5 g. of (IX) and 0.2 g. of thiourea in 50 cc. of dehyd. EtOH was refluxed, by which the solid dissolved and soon a yellow crystalline product deposited. After refluxing for 30 min., the mixture was chilled and the product was collected by filtration to 0.45 g. of almost pure crystals. The crystals were dissolved in 5%

NaOH, filtered with charcoal, and precipitated with AcOH to yellow-white needles, m.p. 312° (decomp.). *Anal.* Calcd. for $C_5H_3N_4ClS$: C, 32.17; H, 1.61; N, 30.03. Found: C, 32.55; H, 1.82; N, 30.17.

6-Bromo-7-mercapto-*s*-triazolo[2,3-*a*]pyrimidine (XIV)—A mixture of 0.5 g. of (X) and 0.2 g. of thiourea in 30 cc. of dehyd. EtOH was refluxed for 20 min. After cool, the separated yellow crystals were collected and washed with EtOH to 0.48 g. of yellow needles, m.p. 243° (decomp.). *Anal.* Calcd. for $C_5H_3N_4BrS$: C, 25.97; H, 1.30; N, 24.24. Found: C, 25.94; H, 1.61; N, 24.54.

6-Chloro-7-methylthio-*s*-triazolo[2,3-*a*]pyrimidine (XV)—A solution of 0.6 g. of (XIII) dissolved in 14 cc. of 1% NaOH was shaken at room temperature with 0.5 g. of MeI for 1 hr. The resulting crystals were collected and recrystallized from water to 0.3 g. colorless prisms, m.p. 85~86°. *Anal.* Calcd. for $C_6H_5N_4ClS$: C, 35.91; H, 2.49; N, 27.93. Found: C, 36.08; H, 2.40; N, 28.21.

Hydrolysis of 6-Chloro-7-methylthio-*s*-triazolo[2,3-*a*]pyrimidine (XV)—A mixture of 0.3 g. of (XV) and 5 cc. of 25% H_2SO_4 was heated on a water bath for 1 hr. The white crystalline product was recrystallized from 50% EtOH to 0.2 g. of white needles, m.p. over 300°. This substance is 6-chloro-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (VII). *Anal.* Calcd. for $C_5H_3ON_4Cl$: C, 35.19; H, 1.76; N, 32.85. Found: C, 35.21; H, 1.98; N, 32.95.

6-Chloro-7-iodo-*s*-triazolo[2,3-*a*]pyrimidine (XVI)—To 10 cc. of conc. HI (d=1.7) in an ice-bath, 0.5 g. of (IX) was added slowly with stirring. The mixture was allowed to stand overnight at room temperature, the resulting crystals were collected, and recrystallized from water to 0.3 g. of colorless prisms, m.p. 233° (decomp.). *Anal.* Calcd. for $C_5H_2N_4ClI$: C, 21.39; H, 0.71; N, 19.96. Found: C, 21.50; H, 0.81; N, 20.24.

6-Bromo-7-iodo-*s*-triazolo[2,3-*a*]pyrimidine (XVII)—This compound was obtained from 0.5 g. of (X) by the same procedure as for (XVI). Colorless prisms (0.32 g.), m.p. 233~233.5° (decomp.). *Anal.* Calcd. for $C_5H_2N_4BrI$: C, 18.46; H, 0.62; N, 17.23. Found: C, 18.89; H, 0.65; N, 17.56.

Summary

Six kinds of 7-substituted *s*-triazolo[2,3-*a*]pyrimidines, which are related to some isomeric compounds of natural purines and their antagonists, were prepared. Halogenation of 7-hydroxy- and 7-amino-*s*-triazolo[2,3-*a*]pyrimidines with chlorine or bromine gave the corresponding 6-halogeno derivatives (VII), (VIII), (XI), and (XII). Some other 7-substituted 6-halogeno derivatives (XI), (XII), (XIII), and (XIV) were also derived from (IX) and (X) by the usual substitution reactions. Some of these compounds showed biological activity.

(Received May 20, 1959)