UDC 615.771.7 : 547.859.3

135, Yasuo Makisumi : Synthesis of Potential Anticancer Agents. VII.*2 6-Nitro- and 6-Amino-*s*-triazolo[2,3-*a*]pyrimidines.

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It has been reported by Hitchings and co-workers[1] that 5-nitrouracil has anti-folic acid activity and that 5-aminouracil is antagonistic to uracil.

Various derivatives of *s*-triazolo[2,3-*a*]pyrimidine which were prepared by the authors in the previous work of this series, were found to be more antagonistic to pyrimidine and folic acid than to purine.[2] In connection with these facts, an attempt was made to synthesize 6-nitro- and 6-amino-*s*-triazolo[2,3-*a*]pyrimidines.

In the previous work,[3] halogenation of *s*-triazolo[2,3-*a*]pyrimidine derivatives was examined and it was assumed that *s*-triazolo[2,3-*a*]pyrimidines, especially their derivatives containing a hydroxyl or amino group at 5- or 7-position would be nitrated at 6-position. Therefore, nitration of *s*-triazolo[2,3-*a*]pyrimidine derivatives was carried out.

s-Triazolo[2,3-*a*]pyrimidine (I), its 5,7-dimethyl (II), 7-amino (III), 5-methyl-7-amino (IV), 7-hydroxy (V), 5-methyl-7-hydroxy (VI), 5-hydroxy-7-amino (VII), and 5,7-dihydroxy (VIII) derivatives were treated with a mixture of fuming nitric acid and concentrated sulfuric acid or glacial acetic acid at a low temperature. In the case of (V) to (VIII), mononitro derivatives (Va～VIIIa) were obtained, but in the case of (I) to (IV), the original materials were recovered even at a high reaction temperature. These nitro derivatives were slightly yellow or yellow crystalline substance and formed ammonium salts with ammonium hydroxide.

On the other hand, condensation of ethyl nitromalonate with 5-amino-*s*-triazole (IX) in the presence of sodium ethoxide produced a compound of $C_7H_7O_4N_9$ instead of (VIIIa). This compound (X) was found to have the groups NH_2, NO_2, and a lactam C=O by infrared spectrum and an aromatic primary amine by means of the diazo-coupling method. Treatment of (X) with dilute hydrochloric acid separated it into (VIIIa) and (IX). (X) was also prepared by treatment of a mixture of (VIIIa) and (IX) in water. Thus, it was proved that (X) is a salt of 5,7-dihydroxy-6-nitro-*s*-triazolo[2,3-*a*]pyrimidine and 5-amino-*s*-triazole, and nitro group of (VIIIa) is attached to 6-position of (VIII).

As these four nitro derivatives (Va～VIIIa) were insoluble in most of the common organic solvents, they were dissolved in sodium hydrogencarbonate solution and converted by catalytic reduction into the corresponding 6-amino derivatives (Vb～VIIIb).

It was assumed that the amino group at 6-position of *s*-triazolo[2,3-*a*]pyrimidine ring would show general characteristics of an aromatic amino group, compared to those at 5- or 7-position which lacked these characters.

Diazo-coupling test was positive in the case of (Vb) and (VIb), white crystals (XI) were obtained by the action of nitrous acid in the case of (VIIb). This product was considered to be 1*H*,6*H*-*s*-triazolo[2,3-*a*]-*v*-triazolo[4,5-*e*]pyrimidin-4-ol by the results of elemental analysis and infrared spectrum. It was deduced that (XI) was produced by the *v*-triazole ring closure between the amino group at 7-position and the diazonium group formed by reaction of nitrous acid and the amino group at 6-position. Diazo-coupling test of (VIIIb)

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*2 Part VI : This Bulletin, **9**, 814 (1961).

1) G.H. Hitchings, E.A. Falco, P.B. Ressell, H. Vanderwerf : Ann. N.Y. Acad. Sci., **52**, 1318 (1950).
2) T. Okabayashi, *et al.* : This Bulletin, **8**, 158, 162 (1960).
3) Part V. Y. Makisumi : *Ibid.*, **9**, 808 (1961).

(I) $R_1=R_2=H$
(II) $R_1=R_2=CH_3$
(III) $R_1=H$, $R_2=NH_2$
(IV) $R_1=CH_3$, $R_2=NH_2$

(V) $R_1=H$, $R_2=OH$
(VI) $R_1=CH_3$, $R_2=OH$
(VII) $R_1=OH$, $R_2=NH_2$
(VIII) $R_1=R_2=OH$

(Va) $R_1=H$, $R_2=OH$
(VIa) $R_1=CH_3$, $R_2=OH$
(VIIa) $R_1=OH$, $R_2=NH_2$
(VIIIa) $R_1=R_2=OH$

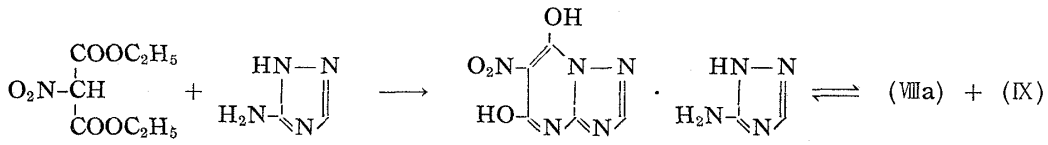
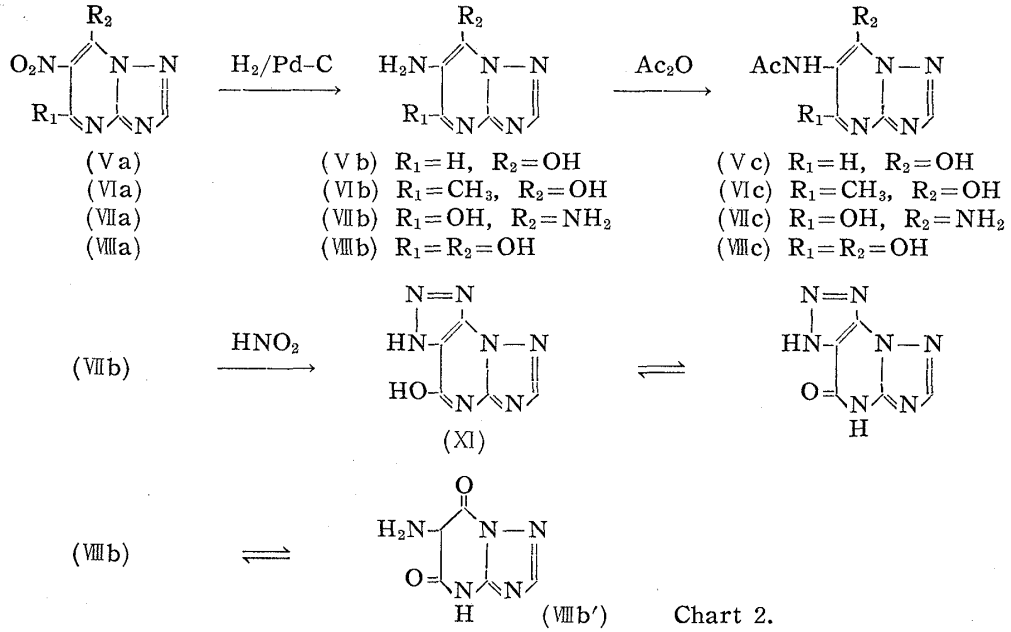


Chart 1.

was negative, probably because the amino group at 6-position of (VIIIb) lacks aromatic characters by formation of the keto form (VIIIb').

In acetylation using acetic anhydride, (Vb), (VIb), and (VIIb) were smoothly converted into 6-acetamido derivatives (Vc, VIc, and VIIc). Acetylation of (VIIIb) was somewhat difficult and produced the 6-acetamido derivative (VIIIc) after heating for 3 hours.



(Vb) $R_1=H$, $R_2=OH$
(VIb) $R_1=CH_3$, $R_2=OH$
(VIIb) $R_1=OH$, $R_2=NH_2$
(VIIIb) $R_1=R_2=OH$

(Vc) $R_1=H$, $R_2=OH$
(VIc) $R_1=CH_3$, $R_2=OH$
(VIIc) $R_1=OH$, $R_2=NH_2$
(VIIIc) $R_1=R_2=OH$

Chart 2.

Treatment of ethyl 2-acetamidoacetoacetate with (IX) in glacial acetic acid gave 5-methyl-6-acetamido-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine. Similarly, reaction of ethyl acetamidocyanoacetate or ethyl acetamidomalonate with (IX) in the presence of sodium ethoxide in boiling ethanol gave 5-hydroxy-6-acetamido-7-amino or 5,7-dihydroxy-6-acetamido

derivatives, respectively. These 6-acetamido derivatives were converted into 6-amino derivatives by hydrolysis with 10% hydrochloric acid.

6-Acetamido and 6-amino derivatives obtained here were identified by mixed melting point and comparison of infrared spectra with the samples (VIb～VIIIb and VIc～VIIIc) derived from the reduction of nitro derivatives (VIa～VIIIa).

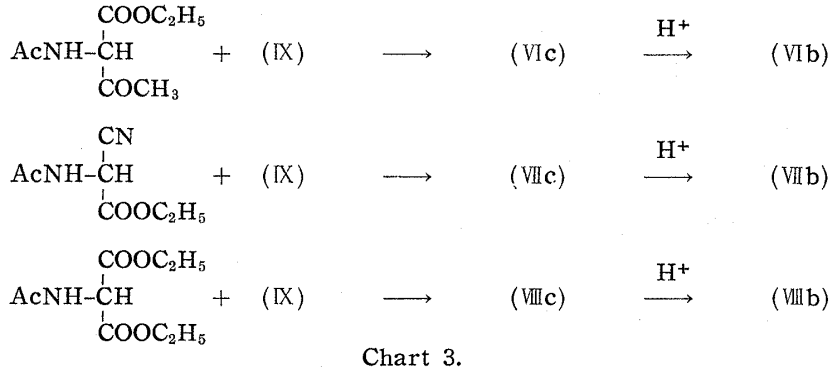


Chart 3.

This evidence indicated that the nitration of *s*-triazolo[2,3-*a*]pyrimidine derivatives always takes place at 6-position when at least one of their 5- and 7-positions is substituted with a hydroxyl group.

Biological activity of these compounds will be reported elsewhere.

Experimental*3

Nitration of *s*-triazolo[2,3-*a*]pyrimidines

6-Nitro-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (Va)——In small portions, 2.0 g. of (V) was added to a mixture of 2.8 cc. of fuming nitric acid (sp. gr. 1.50) and 2.8 cc. of conc. H_2SO_4 at 10～15° with stirring. After stirring for 2 hr. at the same temperature, the reaction solution was poured into ice-water with stirring and the resulting yellowish white product was collected by filtration, washed with H_2O, and dried. This product (2.0 g.) was recrystallized from H_2O to slightly yellow needles, m.p. 284°(decomp.). *Anal.* Calcd. for $C_5H_3O_3N_5$: C, 33.16; H, 1.67; N, 38.67. Found : C, 33.36; H, 2.04; N, 38.89.

5-Methyl-6-nitro-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (VIa)——In small portions, 2.0 g. of (VI) was added to a mixture of 3.5 cc. of fuming nitric acid (sp. gr. 1.50) and 3.5 cc. of conc. H_2SO_4 and the mixture was treated as described above to give 1.6 g. of (VIa). The filtrate was basified with conc. NH_4OH and the separated NH_4 salt was collected, dissolved in hot H_2O, acidified with HCl, and gave 0.9 g. of (VIa). These products were combined and recrystallized from H_2O to yellow pillars, m.p. 232°(decomp.). *Anal.* Calcd. for $C_6H_5O_3N_5$: C, 36.93; H, 2.58; N, 35.89. Found : C, 37.05; H, 2.94; N, 36.11.

5-Hydroxy-6-nitro-7-amino-*s*-triazolo[2,3-*a*]pyrimidine (VIIa)——A portion of 2.0 g. of (VII) was reacted with 7 cc. of the mixed acid and treated by the same method as described above. The resulting crystals were recrystallized from H_2O to 1.0 g. of slightly yellow prisms, m.p. 303°(decomp.). *Anal.* Calcd. for $C_5H_4O_3N_6$: C, 30.62; H, 2.06; N, 42.85. Found : C, 30.60; H, 2.54; N, 42.99.

5,7-Dihydroxy-6-nitro-*s*-triazolo[2,3-*a*]pyrimidine (VIIIa)——A portion of 3.0 g. of (VIII) was reacted with a mixture of 4.0 g. of fuming nitric acid (sp. gr. 1.50) and 7.2 g. of glacial AcOH, and treated in the same way as above. The resulting crystals were recrystallized from H_2O to 3.1 g. of slightly yellow prisms, m.p. 241°(decomp.). *Anal.* Calcd. for $C_5H_3O_4N_5 \cdot H_2O$: C, 27.91; H, 2.34; N, 32.56. Found: C, 27.90; H, 2.44; N, 32.77.

Reaction of Ethyl Nitromalonate with (IX)——To a solution of 1.2 g. of Na in 30 cc. of dehyd. EtOH, 5.0 g. of ethyl nitromalonate and 2.05 g. of (IX) were added and the mixture was refluxed for 8 hr. After cool, the resulting precipitate was collected, dissolved in H_2O, and acidified with dil. HCl. The separated crystals were collected, washed with H_2O, and recrystallized from H_2O to slightly yellow crystalline powder (0.4 g.), m.p. 275°(decomp.). *Anal.* Calcd. for $C_7H_7O_4N_9$(X) : C, 29.89; H, 2.49; N, 44.94. Found : C, 30.17; H, 2.81; N, 45.11.

*3 All melting points are uncorrected.

This compound (0.2 g.) was warmed with 5 cc. of 10% HCl for 10 min. on a water bath, the insoluble substance was collected by filtration while hot, and recrystallized from H_2O to 0.15 g. of slightly yellow prisms (VIIIa), m.p. 241°(decomp.). *Anal.* Calcd. for $C_5H_3O_4N_5 \cdot H_2O$: C, 27.91; H, 2.34; N, 32.56. Found : C, 27.75; H, 2.76; N, 32.73.

The portion soluble in 10% HCl was neutralized with dil. Na_2CO_3 and evaporated to dryness *in vacuo*. The residue was dissolved in a small volume of H_2O and a saturated aqueous solution of picric acid was added to this solution. The resulting picrate was collected and recrystallized from H_2O to yellow needles, m.p. 229~230°(decomp.), which was identified with authentic specimen of picrate of (IX).

Formation of Salt of (VIIa) and (IX)——A portion of 0.5 g. of (VIIIa) and 0.22 g. of (IX) were dissolved in a large amount of hot H_2O and the hot solution was filtered. After the filtrate cooled, the precipitated slightly yellow crystalline powder was collected, washed with H_2O, and dried to 0.6 g. of (X), m.p. 275°(decomp.). *Anal.* Calcd. for $C_7H_7O_4N_9$: C, 29.89; H, 2.49; N, 44.84. Found : C, 29.90; H, 2.86; N, 44.73.

Reduction of 6-Nitro-*s*-triazolo[2,3-*a*]pyrimidines

6-Amino-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (Vb)——A solution of 2.8 g. of (Va) in 140 cc. of 1% $NaHCO_3$ solution was shaken in H_2 atmosphere with 0.5 g. of 10% Pd-C, and 3 equiv. moles of H_2 was absorbed. The catalyst was filtered off and the filtrate was acidified with AcOH. The white precipitate that deposited was recrystallized from H_2O to white needles, m.p. 278°(decomp.). *Anal.* Calcd. for $C_5H_5ON_5$: C, 39.78; H, 3.33; N, 46.34. Found : C, 40.14; H, 3.79; N, 46.22.

This compound was warmed with Ac_2O on a water bath for 15 min. and the resulting crystals were recrystallized from H_2O to white needles, m.p. 325°(decomp.). *Anal.* Calcd. for $C_7H_7O_2N_5$ (6-acetamido-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (Vc)) : C, 43.52; H, 3.65; N, 36.26. Found : C, 43.83; H, 3.88; N, 36.09.

5-Methyl-6-amino-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (VIb)——A solution of 4 g. of (VIa) in 160 cc. of 1% $NaHCO_3$ solution was hydrogenated over 0.8 g. of 10% Pd-C by the same method as above. The resulting product was recrystallized from EtOH to 3.1 g. of colorless needles, m.p. 281°(decomp.). *Anal.* Calcd. for $C_6H_7ON_5$: C, 43.63; H, 4.27; N, 42.41. Found : C, 43.71; H, 4.68; N, 42.42.

This compound was warmed with Ac_2O for 15 min. and the resulting crystals were recrystallized from EtOH to white needles (VIc), m.p. 292°(decomp.). *Anal.* Calcd. for $C_8H_9O_2N_5$: C, 46.37; H, 4.38 N, 33.80. Found : C, 46.56; H, 4.45; N, 33.68.

5-Hydroxy-6,7-diamino-*s*-triazolo[2,3-*a*]pyrimidine (VIIb)——A solution of 0.5 g. of (VIIa) dissolved in a solution of 0.2 g. of $NaHCO_3$ in 140 cc. of water was hydrogenated over 0.2 g. of 10% Pd-C by the same method as above. The resulting crystals were recrystallized from hydr. EtOH to 0.3 g. of slightly yellow needles, m.p. above 320°. *Anal.* Calcd. for $C_5H_6ON_6$: C, 36.14; H, 3.64; N, 50.59. Found : 36.31; H, 3.99; N, 50.23.

This compound was warmed with Ac_2O on a water bath for 30 min. and the resulting crystals were recrystallized from hydr. EtOH to white scales (VIIc), m.p. above 320°. *Anal.* Calcd. for $C_7H_8O_2N_6 \cdot H_2O$: C, 37.17; H, 4.46; N, 37.16. Found : C, 37.31; H, 4.59; N, 37.41.

5,7-Dihydroxy-6-amino-*s*-triazolo[2,3-*a*]pyrimidine (VIIIb)——A solution of 4.0 g. of (VIIIa) dissolved in a solution of 2.0 g. of $NaHCO_3$ in 120 cc. of H_2O was hydrogenated over 1.0 g. of 10% Pd-C by the same method as above. The resulting crystals were recrystallized from H_2O to 3.0 g. of colorless needles, m.p. 282°(decomp.). *Anal.* Calcd. for $C_5H_5O_2N_5 \cdot H_2O$: C, 32.43; H, 3.81; N, 37.83. Found: C, 32.17; H, 4.12; N, 37.42.

This compound was heated with Ac_2O on a water bath for 3 hr. and the resulting crystals were recrystallized from hydr. EtOH to colorless scales (VIIIc), m.p. 277°(decomp.). *Anal.* Calcd. for $C_7H_7O_3N_5$: C, 40.19; H, 3.37; N, 33.48. Found : C, 40.51; H, 3.70; N, 33.09.

Reaction of (VIIb) with Nitrous Acid——To a solution of 0.1 g. of (VIIb) in 1 cc. of 10% HCl, 0.5 cc. of 10% $NaNO_2$ was added dropwise. The white crystals that deposited were collected, washed with H_2O, and recrystallized from EtOH to 0.09 g. of colorless pillars, m.p. 313~314°(decomp.). *Anal.* Calcd. for $C_5H_3ON_7 \cdot H_2O$ (XI) : C, 30.77; H, 2.58; N, 50.25; H_2O, 9.23. Found : C, 30.80; H, 2.85; N, 50.45; H_2O, 8.96. IR (in Nujol) : 1710 cm^{-1} (lactam C=O).

Direct Synthesis of 6-Acetamido-*s*-triazolo[2,3-*a*]pyrimidines

5-Methyl-6-acetamido-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (VIc)——A mixture of 3.3 g. of ethyl 2-acetamidoacetoacetate and 1.5 g. of (IX) in 20 cc. of glacial AcOH was refluxed for 3 hr. The reaction mixture was concentrated *in vacuo* on a water bath and the residue was diluted with EtOH. The separated crystals were collected and recrystallized from EtOH to 1.6 g. of (VIc), m.p. 292° (decomp.). *Anal.* Calcd. for $C_8H_7O_2N_5$: C, 46.37; H, 4.38; N, 33.80. Found : C, 46.76; H, 4.81; N, 33.80. Found : C, 46.76; H, 4.81; N, 33.82.

This compound (0.5 g.) was heated with 5 cc. of 10% HCl on a water bath for 30 min. and the solution was neutralized with NH_4OH. The resulting crysrals were collected and recrystallized

from H_2O to 0.35 g. of colorless needles (VIb), m.p. 281°(decomp.). *Anal.* Calcd. for $C_6H_7ON_5$: C, 43.63; H, 4.27; N, 42.41. Found: C, 43.81; H, 4.68; N, 42.24.

5-Hydroxy-6-acetamido-7-amino-*s*-triazolo[2,3-*a*]pyrimidine (VIIc)——To a solution of 0.23 g. of Na in 30 cc. of dehyd. EtOH, 1.7 g. of ethyl acetamidocyanoacetate and 0.8 g. of (IX) were added and the mixture was refluxed for 8 hr. After cool, the resulting precipitate was collected, dissolved in H_2O, filtered with charcoal, and the filtrate was acidified with AcOH. The white precipitate that deposited was collected and recrystallized from hydr. EtOH to 1.2 g. of colorless scales, m.p. above 320°. *Anal.* Calcd. for $C_7H_8O_2N_6 \cdot H_2O$: C, 37.17; H, 4.46; N, 37.16. Found: C, 37.07; H, 4.57; N, 36.95.

This compound (0.1 g.) was heated on a water bath for 1 hr. with 1 cc. of 10% HCl. After cool, the reaction mixture was neutralized with dil. NH_4OH and the resulting crystals were recrystallized from hydr. EtOH to 0.07 g. of slightly yellow scales (VIIb), m.p. above 320°. *Anal.* Calcd. for $C_5H_6ON_6$: C, 36.14; H, 3.64; N, 50.59. Found: C, 36.29; H, 3.99; N, 50.48.

5,7-Dihydroxy-6-acetamido-*s*-triazolo[2,3-*a*]pyrimidine (VIIIc)——To a solution of 0.23 g. of Na in 30 cc. of dehyd. EtOH, 2.17 g. of ethyl acetamidomalonate and 0.84 g. of (IX) were added and the mixture was refluxed for 10 hr. After cool, the precipitate was treated as above and the resulting product was recrystallized from hydr. EtOH to 1.2 g. of white scales, m.p. 276~277°(decomp.). *Anal.* Calcd. for $C_7H_7O_3N_5$: C, 40.19; H, 3.37; N, 33.48. Found: C, 40.27; H, 3.75; N, 33.70.

This compound (0.7 g.) was heated with 7 cc. of 10% HCl at 70~80° for 2.5 hr. and the solution was neutralized with NH_4OH, The resulting crystals were collected and recrystallized from H_2O to 0.4 g. of colorless needles (VIIIb), m.p. 282°(decomp.). *Anal.* Calcd. for $C_5H_5O_2N_5 \cdot H_2O$: C, 32.43; H, 3.81; N, 37.83. Found: C, 32.60; H, 4.12; N, 37.81.

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Summary

By nitration of *s*-triazolo[2,3-*a*]pyrimidine derivatives, four kinds of 6-nitro derivatives (Va, VIa, VIIa, and VIIIa) were obtained. These nitro compounds were converted to the corresponding 6-amino derivatives by catalytic reaction and the nature of the amino group at 6-position was examined. 2-Acetamido-1,3-dicarbonyl compounds were condensed with 5-amino-*s*-triazole (IX) into 6-acetamido-*s*-triazolo[2,3-*a*]pyrimidines (VIc, VIIc, and VIIIc), which were converted into the corresponding 6-amino derivatives by hydrolysis.

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