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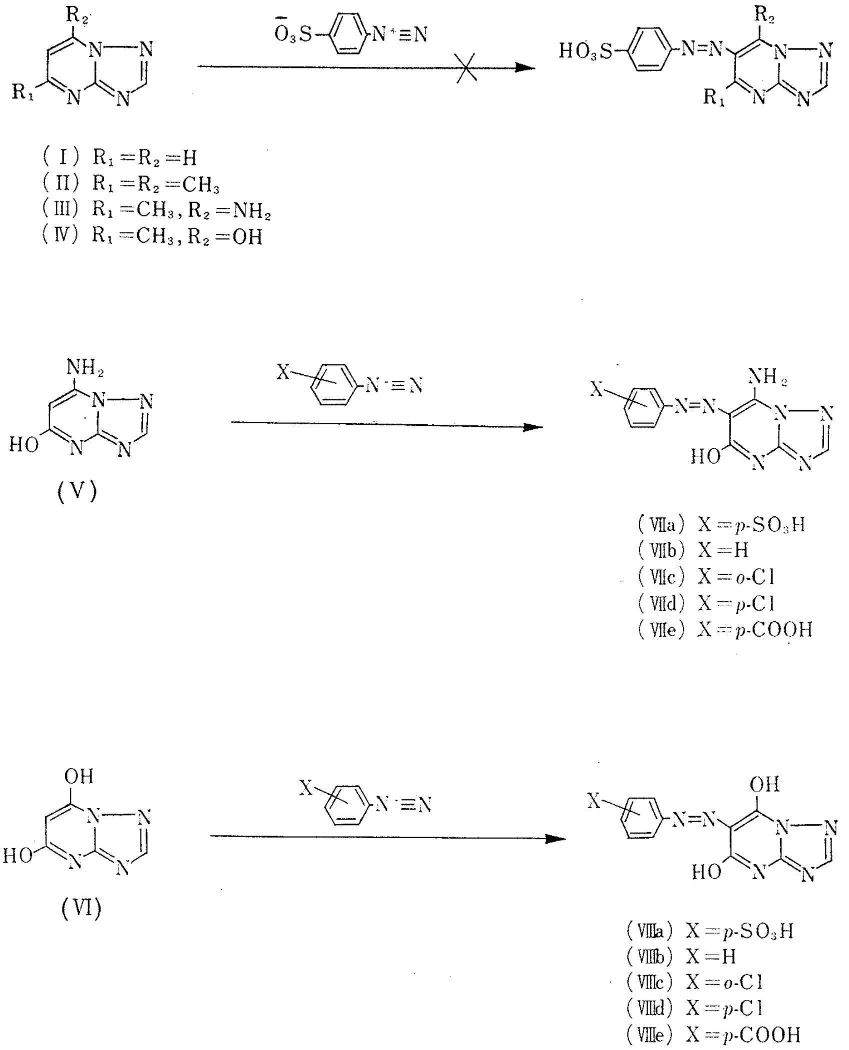
136. **Yasuo Makisumi** : Synthesis of Potential Anticancer Agents. VIII.[*2] 6-Phenylazo-*s*-triazolo[2,3-*a*]pyrimidines.

(*Research Laboratory, Shionogi & Co., Ltd.*[*1])

Various 5-phenylazopyrimidines were synthesized in the expectation of finding some with antagonistic activity upon nucleic acid metabolism. Many of these derivatives were found to have an anti-folic acid activity[1~3] and some derivatives[4] inhibited the growth of certain transplantable tumors in experimental animals.



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

(V)

(VIIa) X = *p*-SO_3H
(VIIb) X = H
(VIIc) X = *o*-Cl
(VIId) X = *p*-Cl
(VIIe) X = *p*-COOH

(VI)

(VIIIa) X = *p*-SO_3H
(VIIIb) X = H
(VIIIc) X = *o*-Cl
(VIIId) X = *p*-Cl
(VIIIe) X = *p*-COOH

Chart 1.

[*1] Fukushima-ku, Osaka (牧角徳夫).
[*2] Part Ⅶ : This Bulletin, 9, 873 (1961).
1) G. M. Timmis, D. G. I. Felton, H. O. J. Collier, P. L. Huskinson : J. Pharm. Pharmacol., **9**, 46 (1957).
2) E. J. Modest, N. Schlein, G. E. Foley : *Ibid.*, **9**, 68 (1957); Proc. Am. Assoc. Cancer Research, **2**, 134 (1956).
3) M. Kawashima : This Bulletin, **7**, 13, 17 (1959).
4) K. Tanaka, *et al.* : *Ibid.*, **7**, 7 (1959).

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In the present work, relative to the above 5-phenylazopyrimidines, 6-phenylazo-*s*-triazolo[2,3-*a*]pyrimidine derivatives were synthesized for testing antimetabolic and antitumor activities.

Data presented in the previous papers[5), *2] have demonstrated that *s*-triazolo[2,3-*a*]pyrimidine derivatives containing groups capable of tautomerism, such as hydroxyl or amino, at 5- or 7-position were displaced with electrophilic reagents at 6-position.

As one of the same electrophilic substitutions, coupling of *s*-triazolo[2,3-*a*]pyrimidine (I) and its 5,7-dimethyl (II), 5-methyl-7-amino (III), 5-methyl-7-hydroxy (IV), 5-hydroxy-7-amino (V), and 5,7-dihydroxy (VI) derivatives with *p*-diazobenzenesulfonic acid in various media was investigated. In the case of (V) and (VI), coupling reaction occurred smoothly and produced 6-phenylazo derivatives (VIIa and VIIIa), but the other derivatives (I～IV) did not react and the original materials were recovered.

Similarly, coupling of (V) and (VI) with benzenediazonium salt and its derivatives produced the 6-phenylazo-*s*-triazolo[2,3-*a*]pyrimidine derivatives listed in Chart 1 (VIIb～e and VIIIb～e).

To confirm the introduction of a phenylazo group into 6-position of the *s*-triazolo[2,3-*a*]pyrimidine ring by these reactions, the following independent synthesis was carried out.

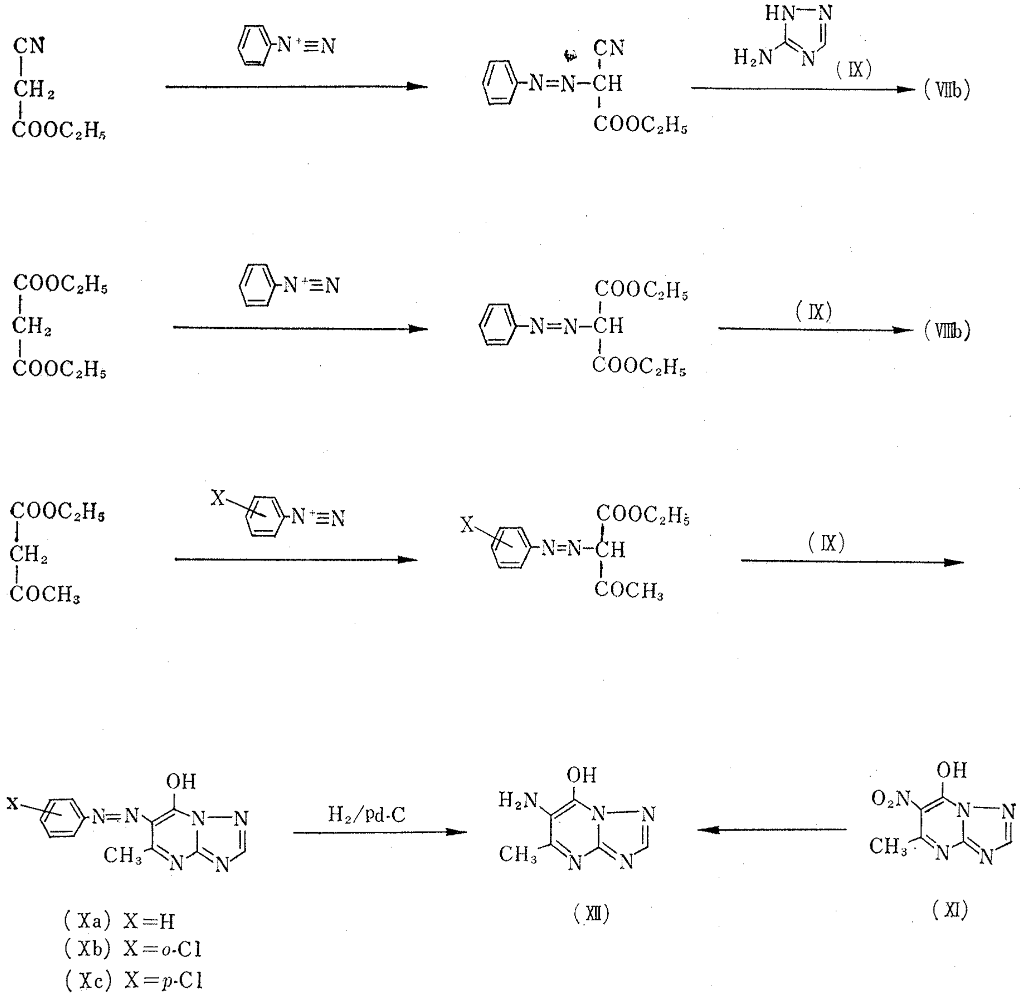

Chart 2.

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5) Part V. Y. Makisumi : This Bulletin, **9**, 808 (1961).

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Ethyl cyanoacetate or ethyl malonate was subjected to coupling with benzenediazonium salt and the resulting phenylazo compounds were allowed to condense with 5-amino-*s*-triazole (IX). 5-Hydroxy-6-phenylazo-7-amino- and 5,7-dihydroxy-6-phenylazo-*s*-triazolo-[2,3-*a*]pyrimidines were obtained and respectively identified with (VIIb) and (VIIIb) prepared by azo-coupling.

Similarly, the derivatives of ethyl 2-phenylazoacetoacetate, which resulted by coupling of ethyl acetoacetate with benzenediazonium salts, were condensed with (IX) in glacial acetic acid to 5-methyl-6-phenylazo-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (Xa) and its derivatives (Xb and Xc). 6-Amino derivative (XII) obtained by catalytic reduction of (Xa), was identified with 5-methyl-6-amino-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine prepared by the reduction of 6-nitro derivative (XI) in the preceding work.*[2]

From the results of these reactions, it was demonstrated that azo-coupling in the *s*-triazolo[2,3-*a*]pyrimidine ring always takes place at 6-position when both of its 5- and 7-positions are substituted with groups capable of tautomerism, such as hydroxyl and amino.

Results of biological screening of these compounds will be reported elsewhere.

Experimental*[3]

4-(5-Hydroxy-7-amino-*s*-triazolo[2,3-*a*]pyrimidin-6-ylazo)benzenesulfonic Acid (VIIa)——To a solution of 0.75 g. of 5-hydroxy-7-amino-*s*-triazolo[2,3-*a*]pyrimidine (V) in 15 cc. of 5% Na_2CO_3 and 50 cc. of H_2O, a solution of 0.92 g. of *p*-diazobenzenesulfonic acid (freshly prepared from sulfanilic acid) in 200 cc. of H_2O was added dropwise at room temperature with stirring. After the mixture was stirred for an additional 1 hr., this was acidified with AcOH and the precipitate was collected by filtration. The resulting product was dissolved in dil. NaOH solution with warming, filtered with charcoal, and the filtrate was acidified with dil. HCl solution, affording 1.0 g. of orange needles, m.p. 269~270°(decomp.). *Anal.* Calcd. for $C_{11}H_9O_4N_7S\cdot 2\frac{1}{2}H_2O$: C, 34.74; H, 3.68; N, 25.79. Found: C, 34.98; H, 3.69; N, 25.64.

4-(5,7-Dihydroxy-*s*-triazolo[2,3-*a*]pyrimidin-6-ylazo)benzenesulfonic Acid (VIIIa)——In the same way as above, 0.75 g. of 5,7-dihydroxy-*s*-triazolo[2,3-*a*]pyrimidine (VI) and 0.92 g. of *p*-diazobenzenesulfonic acid were reacted. The resulting precipitate was purified by reprecipitation with alkali and acid to yellow needles (1.0 g.), m.p. 270~271°(decomp.). *Anal.* Calcd. for $C_{11}H_8O_5N_6S\cdot 3H_2O$: C, 33.85; H, 3.59; N, 21.54. Found : C, 33.82; H, 3.85; N, 21.88.

5-Hydroxy-6-phenylazo-7-amino[2,3-*a*]pyrimidine (VIIb)——a) To a solution of 0.5 g. of (V) in 10 cc. of 5% NaOH, a benzenediazonium chloride solution (prepared from 0.31 g. of aniline by the usual method) was added dropwise with ice-cooling and stirring, by which an orange precipitate separated out. After stirring further for 30 min., the mixture was neutralized with AcOH, and the resulting precipitate was collected. The precipitate was dissolved in 5% NaOH solution with warming and reprecipitated by addition of AcOH as an orange-yellow powder (0.65 g.), which was recrystallized from hydr. EtOH to orange-yellow scales, m.p. 269~270°(decomp.). *Anal.* Calcd. for $C_{11}H_9ON_7$: C, 51.76; H, 3.53; N, 38.43. Found : C, 51.45; H, 3.98; N, 38.21.

b) A solution of 3.1 g. of aniline in 12 cc. of conc. HCl and 25 cc. of H_2O was diazotized by the customary method and the resulting solution was added to an aqueous solution of 4.5 g. of ethyl cyanoacetate and 20 g. of $AcONa\cdot 3H_2O$, by which ethyl phenylazocyanoacetate separated out as a yellow precipitate. The precipitate was collected and recrystallized from hydr. EtOH to yellow needles, m.p. 85°(7.8 g.).

A mixture of 5.5 g. of this product and 2.1 g. of (IX) added to a solution of 0.6 g. of Na in 40 cc. of dehyd. EtOH was refluxed for 10 hr. After cool, the resulting precipitate was collected, dissolved in H_2O, filtered with charcoal, and the filtrate was acidified with dil. AcOH. The resulting precipitate was collected and recrystallized from hydr. EtOH to orange-yellow needles, m.p. 269~270° (decomp.). *Anal.* Calcd. for $C_{11}H_9ON_7$: C, 51.76; H, 3.53; N, 38.43. Found : C, 51.56; H, 3.74; N, 38.28. This was identified by the infrared spectrum with the specimen obtained by the method (a).

5,7-Dihydroxy-6-phenylazo-*s*-triazolo[2,3-*a*]pyrimidine (VIIIb)——a) A solution of benzenediazonium chloride, prepared from 0.47 g. of aniline in the usual manner, was reacted with 0.75 g. of (VI) as above. The product was purified to give 0.8 g. of yellow needles, m.p. 269~270°(decomp.) (from EtOH). *Anal.* Calcd. for $C_{11}H_8O_2N_6$: C, 51.56; H, 3.15; N, 32.80. Found : C, 51.89; H, 3.22; N, 32.44.

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*[3] All melting points are uncorrected.

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b) A solution of 18.6 g. of aniline in 60 cc. of conc. HCl and 125 cc. of H_2O was diazotized by the usual method and the resulting solution was added to an aqueous solution of 32 g. of ethyl malonate and 175 g. of $AcONa \cdot 3H_2O$ in 300 cc. of H_2O, by which ethyl phenylazomalonate separated out as an orange oil. The oil was extracted with Et_2O and the extract was dried over Na_2SO_4. The solvent was evaporated and the residue was distilled in a diminished pressure to orange oil (46 g.), b.p$_7$ 197~199°.

This product (1.3 g.) and (IX) (0.4 g.) were added to a solution of 0.11 g. of Na in 15 cc. of dehyd. EtOH and the mixture was refluxed for 10 hr. After cool, the precipitate was collected, dissolved in H_2O, and filtered with charcoal. The filtrate was acidified with dil. HCl, the resulting precipitate was collected, and recrystallized from hydr. EtOH to orange-yellow needles, m.p. 269~270° (decomp.). *Anal.* Calcd. for $C_{11}H_8O_2N_6$: C, 51.56; H, 3.15; N, 32.80. Found : C, 51.68; H, 3.28; N, 32.95. This was identified by the infrared spectrum with the sample (VIIIb) prepared by the method (a).

5-Hydroxy-6-(*o*-chlorophenylazo)-7-amino-*s*-triazolo[2,3-*a*]pyrimidine (VIIc)——A solution of 0.7 g. of *o*-chloroaniline in 1.7 cc. of conc. HCl and 5 cc. of H_2O was diazotized by addition of a solution of 0.38 g. of $NaNO_2$ in 5 cc. of H_2O with ice-cooling and stirring. The reaction mixture was added to a solution of 0.75 g. of (V) in 45 cc. of 3% Na_2CO_3 solution with ice-cooling and stirring. After stirring for 30 min., the mixture was acidified with AcOH and the resulting precipitate was collected, washed with H_2O and EtOH, and dried to yield 1.3 g. of (VIIc), which was recrystallized from EtOH to orange-yellow needles, m.p. 314~315° (decomp.). *Anal.* Calcd. for $C_{11}H_8ON_7Cl$: C, 45.60; H, 2.67; N, 33.85. Fonnd : C, 45.77; H, 2.87; N, 34.08.

5,7-Dihydroxy-6-(*o*-chlorophenylazo)-*s*-triazolo[2,3-*a*]pyrimidine (VIIIc)——A solution of *o*-chlorobenzenediazonium chloride (prepared from 1.3 g. of *o*-chloroaniline as described above) was added to a suspension of 1.5 g. of (VI) in 45 cc. of 5% AcONa solution with ice-cooling and stirring, and a yellow precipitate separated out. After stirring for 30 min., the precipitate was collected, dissolved in dil. NaOH, and filtered with charcoal. The filtrate was acidified with dil. HCl and the resulting precipitate was collected, washed with H_2O, and dried to yellow crystals (2.5 g.), which was recrystallized from hydr. EtOH to yellow scales, m.p. 294° (decomp.). *Anal.* Calcd. for $C_{11}H_7O_2N_6Cl$: C, 45.44; H, 2.41; N, 28.92. Found : C, 45.84; H, 2.55; N, 29.09.

5-Hydroxy-6-(*p*-chlorophenylazo)-7-amino-*s*-triazolo[2,3-*a*]pyrimidine (VIId)——A solution of *p*-chlorobenzenediazonium chloride (prepared from 0.7 g. of *p*-chloroaniline in the same manner as above) was added dropwise to a solution of 0.75 g. of (V) in 45 cc. of 3% Na_2CO_3 solution with ice-coolig and stirring, and an orange precipitate separated out. After stirring for 30 min., the mixture was acidified with AcOH, and the precipitate was collected, washed with H_2O and EtOH, and dried to yield 1.4 g. of (VIId), which was recrystallized from hydr. EtOH to orange needles, m.p. 287~288° (decomp.). *Anal.* Calcd. for $C_{11}H_8ON_7$: C, 45.60; H, 2.76; N, 33.85. Found : C, 45.29; H, 2.95; N, 33.77.

5,7-Dihydroxy-6-(*p*-chlorophenylazo)-*s*-triazolo[2,3-*a*]pyrimidine (VIIId)——A solution of *p*-chlorobenzenediazonium chloride (prepared from 1.4 g. of *p*-chloroaniline by the same method as above) was added dropwise to a suspension of 1.5 g. of (VI) in 50 cc. of 10% AcONa with ice-cooling and stirring. The resulting precipitate was treated as above to form a yellow crystalline powder (2.6 g.) which was recrystallized from hydr. EtOH to yellow scales, m.p. 302° (decomp.). *Anal.* Calcd. for $C_{11}H_7O_2N_6Cl$: C, 45.44; H, 2.41; N, 28.92. Found : C, 45.58; H, 2.57; N, 29.20.

4-(5-Hydroxy-7-amino-*s*-triazolo[2,3-*a*]pyrimidin-6-ylazo)benzoic Acid (VIIe)——A solution of 0.82 g. of *p*-aminobenzoic acid in 1.5 cc. of conc. HCl and 5 cc. of H_2O was diazotized by addition of a solution of 0.4 g. of $NaNO_2$ in 3 cc. of H_2O with ice-cooling and stirring. The reaction mixture was added to a solution of 0.75 g. of (V) in 15 cc. of 10% Na_2CO_3 solution with ice-cooling and stirring. After stirring for 30 min., the mixture was diluted with H_2O and acidified with AcOH. The precipitate was collected and purified by reprecipitation with dil. NaOH and dil. HCl to an orange-yellow crystalline powder (0.9 g.), m.p. 311~312° (decomp.). *Anal.* Calcd. for $C_{12}H_9O_3N_7$: C, 48.18; H, 3.03; N, 32.77. Found : C, 48.23; H, 3.35; N, 32.63.

4-(5,7-Dihydroxy-*s*-triazolo[2,3-*a*]pyrimidin-6-ylazo)benzoic Acid (VIIIe)——An amount of 0.76 g. of (VI) and a solution of *p*-carboxybenzenediazonium chloride (prepared from 0.82 g. of *p*-aminobenzoic acid) was reacted by the same way as above. The resulting precipitate was purified by reprecipitation with alkali and acid to 1.25 g. of orange-yellow scales, m.p. 291~292° (decomp.). *Anal.* Calcd. for $C_{12}H_8O_4N_6 \cdot H_2O$: C, 45.29; H, 3.17; N, 26.41. Found : C, 45.45; H, 3.38; N, 26.03.

5-Methyl-6-phenylazo-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (Xa)——A solution of 1.9 g. of aniline in 6 cc. of conc. HCl and 25 cc. of H_2O was diazotized by the usual method and the resulting solution was added to an aqueous solution of 2.6 g. of ethyl acetoacetate and 10 g. of $AcONa \cdot 3H_2O$, by which ethyl phenylazoacetoacetate separated out as a yellow precipitate. The precipitate was collected and recrystallized from EtOH to yellow needles, m.p. 70~70.5° (4.4 g.). A mixture of 2.34 g. of this product and 0.84 g. of (IX) added to 8 cc. of glacial AcOH was refluxed for 3 hr. After cool, the resulting crystals were collected, washed with EtOH, and recrystallized from EtOH to scarlet needles

(0.9 g.), m.p. 212~213°(decomp.). *Anal.* Calcd. for $C_{12}H_{10}ON_6$: C, 56.68; H, 3.96; N, 33.06. Found: C, 56.71; H, 4.25; N, 32.90.

5-Methyl-6-(*o*-chlorophenylazo)-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (Xb)——Ethyl 2-(*o*-chlorophenylazo)acetoacetate, yellow needles, m.p. 76~78°, was prepared from 5.1 g. of *o*-chloroaniline and 5.2 g. of ethyl acetoacetate in the same manner as above, and 4.5 g. of this product was reacted with 1.45 g. of (IX). The product was recrystallized from EtOH to orange needles (0.8 g.), m.p. 226~227°(decomp.). *Anal.* Calcd. for $C_{12}H_9ON_6Cl$: C, 49.91; H, 3.12; N, 29.12. Found : C, 49.88; H, 3.36; N, 28.82.

5-Methyl-6-(*p*-chlorophenylazo)-7-hydroxy-*s*-triazolo[2,3-*a*]pyrimidine (Xc)——Ethyl 2-(*p*-chlorophenylazo)acetoacetate, yellow needles, m.p. 93~94°, was prepared from 5.1 g. of *p*-chloroaniline and 5.2 g. of ethyl acetoacetate in the same manner as above, and 2 g. of this product was reacted with 0.65 g. of (IX).

The product was recrystallized from EtOH to orange needles (1.4 g.), m.p. 225~226°(decomp.). *Anal.* Calcd. for $C_{12}H_9ON_6Cl$: C, 49.11; H, 3.12; N, 29.12. Found : C, 50.00; H, 3.55; N, 29.21.

Reductive Cleavage of the Azo-linkage in (Xa)——A solution of 1.0 g. of (Xa) in 50 cc. of EtOH containing few drops of conc. NH_4OH was shaken in H_2 atmosphere over 0.3 g. of 5% Pd-C. After the theoretical amount of H_2 was absorbed, the catalyst was filtered off, the filtrate was evaporated to dryness, and the residue was washed with Et_2O. The resulting crystals were recrystallized from EtOH to 0.6 g. of colorless pillars (XII), m.p. 281°(decomp.). *Anal.* Calcd. for $C_6H_7ON_5$: C, 43.63; H, 4.27; N, 42.41. Found : C, 43.82; H, 4.58; N, 42.39.

This was identified with the sample derived from (XI). The Et_2O solution was evaporated to dryness and the residual oil was acetylated with Ac_2O to colorless scales, m.p. 115°, which was identified with acetanilide.

The author wishes to express his gratitude to Dr. K. Takeda, Director of this Laboratory, and to Dr. H. Kanō of this Laboratory, for their continued guidance and encouragement. Thanks are also due to the members of the Analysis Room of this Laboratory for elemental analysis.

Summary

Several 6-phenylazo-*s*-triazolo[2,3-*a*]pyrimidine derivatives were synthesized by either of the following two methods :

1) Benzenediazonium salt was coupled with *s*-triazolo[2,3-*a*]pyrimidine derivatives at 6-position, by which the effect of 5- and 7-positions in *s*-triazolo[2,3-*a*]pyrimidine was clarified.

2) 2-Phenylazo-1,3-dicarbonyl compounds were condensed with 5-amino-*s*-triazole (IX).

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