and evaporated to dryness under reduced pressure. The product was added in small portions to a mixture of HNO_3(sp. gr., 1.4, 120 cc.) and conc. H_2SO_4(100 cc.) at room temperature under stirring during 35 mins. After further agitation for 30 mins. at 50°, the reaction mixture was poured onto crushed ice. Eight grams of the pale yellow precipitate obtained was washed with water and recrystallized from 300 volumes of water, forming colorless plate crystals, m.p. >350°. *Anal.* Calcd. for $C_4H_4O_3N_4$: C, 30.78; H, 2.58; N, 35.89. Found: C, 30.78; H, 2.26; N, 35.15.

Summary

Some hypoxanthine derivatives which were substituted at C_8-position by hydrophilic groups such as -SH, $-S-CH_2-COOH$, -COOH, $-CH_2OH$ and $-CH_2-CH_2-COOH$ were synthesized for testing purine-antagonist action. They were prepared by condensation of 4,5-diamino-6-hydroxypyrimidine (I) with the corresponding acid or amide compounds through the acyl derivatives of (I). A direct preparation of 4-amino-6-hydroxypyrimidine by reaction of malonamamidine with ethyl formate was also described.

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43. Morizo Ishidate and Hidetaka Yuki: The Synthesis of 8-Substituted Purine Derivatives. II.[1] Some 8-Substituted 6-Mercaptopurines.

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The present work was undertaken in order to prepare new 8-sustituted 6-mercaptopurines which possibly exhibit a more hydrophilic property than the original 6-mercaptopurine retaining purine activity in biological systems. For the synthesis of these compounds, 4,5-diamino-6-mercaptopyrimidine (I)[2] was conveniently employed as the starting compound.

6,8-Dimercaptopurine (II) was directly obtained in a good yield by the fusion of 4,6-diamino-6-mercaptopyrimidine (I) with thiourea. This compound is readily soluble in an alkali solution. On treatment of (II) with monochloroacetic acid both thiol groups were attacked to give 6,8-bis(carboxymethylthio)purine.

When (I) was fused with succinic acid or glycolic acid, 5-succinylamino- or 5-glycolylamino-6-mercaptopyrimidine (III or V) was formed, respectively. By further heating of their sodium salts at 250° the ring closure was effected to produce 8-(2-carboxyethyl)-6-mercaptopurine (IV) and 8-hydroxymethyl-6-mercaptopurine (VI), respectively. From (I) and gluconic acid, 5-gluconyl-4-amino-6-mercaptopyrimidine (VII) was likewise obtainable, but the cyclization to purine ring by heating in the presence of alkali or in form of its alkali salt was not successful without decomposition.

Unexpectedly, when (I) was heated with oxalic acid at ca. 180~200°, with evolution of carbon dioxide, a non-acidic compound of needle crystals (m.p. 209~210° from water) was isolated in good yield (66%). The product was insoluble in a bicarbonate solution and gave negative tests for thiol group. From the ultraviolet absorption spectrum (λ_{max} 264 mμ at pH 1), which can be differentiated easily from that of 6-mercaptopurine, and from analytical data, the compound was found identical with 7-aminothiazolo[5,4-*d*]-pyrimidine (XII) which has recently been described by Hichings, *et al.*[3] as one of the

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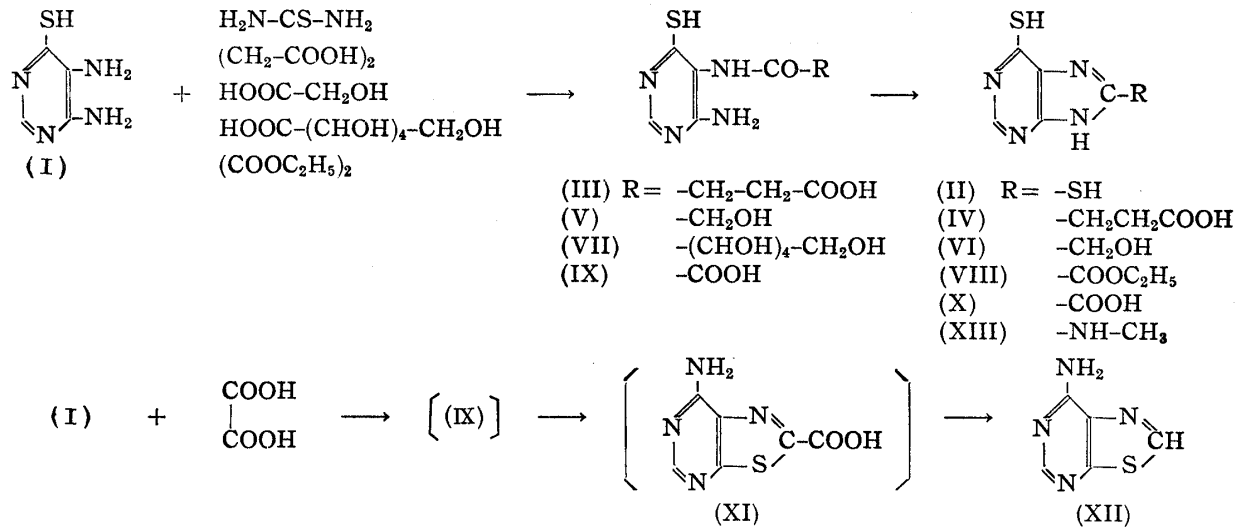
1) Part I. M. Ishidate, H. Yuki: This Bulletin, **5**. 240(1957).
2) G. H. Hitchings, G. B. Elion: J. Am. Chem. Soc., **76**, 4027(1954).
3) G. B. Elion, W. H. Lange, G. H. Hitchings: *Ibid.*, **78**, 2858(1956).

condensation products of (I) with formic acid. The reaction process might be so explained that the intermediate 4-amino-5-oxalylamino-6-mercaptopyrimidine (IX) undergoes ring closure in acidic medium more appreciably toward the thiol group than to the amino group yielding 2-carboxy-7-aminothiazolo[5,4-*d*]pyrimidine (XI) as an intermediate, whose decarboyxlation gives the product (XII). Another example of this reaction process will be reported in a forthcoming communication.

In order to obtain 6-mercapto-8-carboxypurine (X) the compound (I) was condensed with diethyl oxalate by heating and 6-mercapto-8-ethoxycarbonylpurine (VIII) was directly produced, which by saponification easily gave the compound (X).

6-Mercapto-8-methylaminopurine (XIII) was prepared from 8-methylaminohypoxanthine[1] by treatment with phosphorus pentasulfide in tetralin medium, since the synthesis of the compound from 5-(3-methyl-2-thioureido)-6-mercaptopyrimidine by means of mercuric oxide was hopeless.

The result of biological test of the above described compounds with regard to possible antagonist of precursor of nucleic acid or to the antitumor effect will be reported elsewhere.



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Experimental

6,8-Dimercaptopurine (II)—A well-powdered mixture of 4,5-diamino-6-mercaptopyrimidine (I) (3 g.) and thiourea (6 g.) was heated gradually to melt at about 180°. Under evolution of ammonia gas it changed to a solid. After further heating for 10 mins. the product was dissolved in 10% NaOH and precipitated with AcOH to give 3.4 g. of (II). Pale yellow crystals were formed by recrystallization from 200 volumes of water, m.p. 310~350°(decomp.). *Anal.* Calcd. for $C_5H_4N_4S_2$: C, 32.61; H, 2.19; N, 30.43; S, 34.83. Found : C, 33.09; H, 1.88; N, 29.44; S, 33.90.

6,8-Bis(carboxymethylthio)purine—A suspension of finely powdered (II) (0.7 g.) in 15 cc. of water containing 1 g. of monochloroacetic acid was refluxed for 1 hr. The reaction mixture was filtered hot with activated carbon and stood over night. Orange yellow crystals (0.8 g.) separated, which were recrystallized from water, giving m.p. 230~245°(decomp.). *Anal.* Calcd. for $C_9H_8O_4N_4S_2$: C, 36.01; H, 2.69; N, 18.67; S, 21.31. Found : C, 36.12; H, 2.71; N, 18.04; S, 21.22.

4-Amino-5-succinylamino-6-mercaptopyrimidine (III)—A mixture of 3 g. of (I) and 6 g. of succinic acid was heated at 210~220° for 15 mins. The product after cooling was dissolved in 10% NaOH, filtered, and the filtrate was acidified with AcOH to give 3.4 g. of (III). Recrystallization from water gave colorless crystals which darkened at ca. 245° and decomposed. *Anal.* Calcd. for $C_8H_{10}O_3N_4S$: C, 39.67; H, 4.16; N, 23.14. Found : C, 39.87; H, 3.78; N, 23.53.

8-(2-Carboxyethyl)-6-mercaptopurine (IV)—A solution of 1.5 g. of (III) in 5 cc. of 10% NaOH was evaporated to dryness under a reduced pressure and heated at 250° for 30 mins. After cooling the

product was dissolved in water, filtered, acidified with AcOH, and stood over night. Crystals separated were dissolved in bicarbonate solution, filtered with activated carbon, reprecipitated with AcOH, and recrystallized from water to give white needles, m.p. 296~298°(decomp.). *Anal.* Calcd. for $C_5H_3O_2N_4S$: C, 42.86; H, 3.60; N, 24.99; S, 14.28. Found: C, 43.24; H, 3.44; N, 24.61; S, 14.18.

4-Amino-5-glycolylamino-6-mercaptopyrimidine(V)—A solution of 2 g. of (I) and 1.5 g. of glycolic acid in 100 cc. of water was evaporated to dryness and heated for 20 mins. at a bath temperature of 180~190° under a reduced pressure. After cooling, the product was dissolved in NaOH solution and acidified with AcOH to give 1.6 g. of pale yellow precipitate. Recrystallization from water gave colorless needles, m.p. 262°(decomp.). *Anal.* Calcd. for $C_6H_8N_4O_2S$: C, 36.00; H, 4.03; N, 27.99. Found: C, 36.74; H, 3.73; N, 28.14.

8-Hydroxymethyl-6-mercaptopurine (VI)—The sodium salt obtained from 2 g. of (V) was heated gradually to ca. 220° in an oil bath. The reaction mixture thereby partially decomposed. The product was dissolved in water, filtered, acidified with AcOH, and filtered with activated carbon, and the filtrate was condensed to about 10 cc. and stood in a refrigerator to give a small amount of pale yellow crystals, m.p. 270~300°(decomp.). *Anal.* Calcd. for $C_6H_6ON_4S$: C, 39.56; H, 3.32; N, 30.76; S, 17.57. Found: C, 39.80; H, 3.29; N, 30.11; S, 16.73.

4-Amino-5-gluconylamino-6-mercaptopyrimidine (VII)—A mixture of 1 g. of (I) and 5 cc. of 50% gluconic acid solution was heated at 150~155° for 20 mins. under a reduced pressure. The product was dissolved in 20 cc. of hot water and cooled. The precipitate was separated and recrystallized from 20 cc. of 30% EtOH to give 0.8 g. of (III), m.p. 170~200°(decomp.). *Anal.* Calcd. for $C_{10}H_{16}O_6N_4S \cdot H_2O$: C, 35.50; H, 5.33; N, 16.57; S, 9.47. Found: C, 35.77; H, 5.48; N, 16.01; S, 8.57.

7-Aminothiazolo[5,4-*d*]pyrimidine (XII)—A mixture of 10 g. of oxalic acid and 2.5 g. of (I) was heated in an oil bath at about 180~190° under a reduced pressure. The heating was stopped when the sublimation of oxalic acid started after an evolution of CO_2. The product was dissolved in hot water and stood under cooling. The precipitate obtained was recrystallized from 100 cc. of water to give 2 g. of white needles, m.p. 209~210°. *Anal.* Calcd. for $C_5H_4N_4S$: C, 39.77; H, 2.47; N, 36.25; S, 21.04. Found: C, 39.48; H, 2.65; N, 36.84; S, 20.99.

7-Acetamidothiazolo[5,4-*d*]pyrimidine—A mixture of 2.5 cc. Ac_2O, 0.2 g. of (XII), and 1 drop of conc. H_2SO_4 was refluxed for several mins. until the solid substance dissolved. After cooling, the reaction mixture was poured into crushed ice and brought to pH 7 to precipitate white crystals, which were recrystallized from water, m.p. 186.5~188°. *Anal.* Calcd. for $C_7H_6ON_4S$: C, 43.76; H, 3.42; N, 28.51; S, 16.48. Found: C, 43.30; H, 3.12; N, 28.56; S, 16.05.

8-Ethoxycarbonyl-6-mercaptopurine (VIII)—A suspension of 0.5 g. of finely powdered (I) in 5 cc. of ethyl oxalate was refluxed for 3 hrs. After cooling, the precipitate formed was filtered and recrystallized from 300 cc. of water to give pale yellow needles, m.p. 280~300°(decomp.). *Anal.* Calcd. for $C_8H_8O_2N_4S$: C, 42.86; H, 3.60; N, 25.00; S, 15.08. Found: C, 42.67; H, 3.06; N, 24.72; S. 15.13.

8-Carboxy-6-mercaptopurine (X)—One gram of (VIII) was dissolved in an equimolar amount of 10% NaOH and evaporated to dryness. A small amount of water was added to the residue, filtered, and acidified with AcOH. The precipitate formed was recrystallized twice from 150~200 cc. of water to give 0.3 g. of yellow amorphous powder. *Anal.* Calcd. for $C_6H_4O_2NS$: C, 36.74; H, 2.06; N, 28.52. Found: C, 36.45; H, 2.03; N, 27.95.

8-Methylamino-6-mercaptopurine (XIII)—A mixture of 8-methylaminohypoxanthine (2 g.) and P_2S_5 (8 g.) in tetralin (50 cc.) was refluxed gently for 3.5 hrs. The reflux was continued for further 5.5 hrs. after 2 g. of P_2S_5 was added to the reaction mixture and stood over night. Precipitate formed was filtered, washed with ligroine, dissolved in warm 10% NaOH, filtered, and reprecipitated with AcOH. The precipitate was boiled with 150 cc. of water, filtered with activated carbon, and cooled. The product was amorphous even by repeated recrystallization from water. *Anal.* Calcd. for $C_6H_7N_5S$: C, 39.60; H, 4.10; N, 38.66; S, 20.36. Found: C, 39.78; H, 3.89; N, 37.78; S, 20.13.

Summary

Some 6-mercaptopurine derivatives substituted at 8-position with hydrophilic groups such as -SH, $-S-CH_2-COOH$, $-CH_2-CH_2-COOH$, $-CH_2OH$, -COOH, and $-NH-CH_3$ were synthesized for testing purine-antagonist action. They were prepared by the condensation of 4,5-diamino-6-mercaptopyrimidine (I) with thiourea, succinic acid, glycolic acid, or ethyl oxalate, except in the case of 6-mercapto-8-methylaminopurine.

In the condensation of (I) with oxalic acid, 7-aminothiazolo[5,4-*d*]pyrimidine was directly obtained in considerable amount under alternative ring closure.

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