Summary

The epoxide (II), the monomethyl ether (III), and the monodesoxy derivative (IV) of dihydrolycorine were obtained from dihydrolycorine (I) via its monotosylate (Ic). It has been determined from the results of the ring-opening reaction of the above-mentioned epoxide that the two vicinal hydroxyl groups in (I) are *trans* and diaxial. Furthermore, by the elimination reaction of the hydroxyl function, (III) and (IV) are easily convertible to the corresponding unsaturated compounds (V) and (VI), respectively. On the basis of these findings, it may be suggested that the stereochemical structure of dihydrolycorine (I) can be represented as shown in (XII).

(Received February 11, 1957)

U.D.C. 547.857.07

42. Morizo Ishidate and Hidetaka Yuki: The Synthesis of 8-Substituted Purine Derivatives. I. Some 8-Substituted Derivatives of Hypoxanthine.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo**)

Purine antagonists have been one of the subjects of intensive study on cancer chemotherapy during the past few years. 6-Mercaptopurine, 2,6-diaminopurine, and 8-azaguanine (5-amino-7-hydroxytriazolo[5,4-*d*]pyrimidine) have been demonstrated to act as the antagonist of precursors of nucleic acid components. These purine derivatives are in general sparingly soluble in water and in organic solvents. This lack of solubility is likely to bring about an unfavorable effect on the activity and a limitted application of the agents.

It seemed interesting to prepare more soluble purine derivatives which possess antagonizing activity.

Hitchings[1] demonstrated that the growth-promoting action of *Lactobacillus casei* produced by the addition of adenine was lost in the case of 9-methyladenine and that a marked growth inhibition in the rat and bacteria caused by 2,6-diaminopurine was not observed in the case of 9-methyl-2,6-diaminopurine. This is probably due to the fact that the hydrogen at 9-position of purine moiety is essential for the formation of nucleic acid *in vivo*.

Of purine derivatives only a few 8-substituted compounds are known in the literature. The present paper describes the preparation of some derivatives of hypoxanthine which are substituted at 8-position with hydrophilic groups such as -SH, -COOH, $-CH_2OH$, and $-CH_2CH_2COOH$, in order to investigate the biological property.

8-Mercaptohypoxanthine (II) was directly obtained in a good yield by the fusion of 4,5-diamino-6-hydroxypyrimidine (I) or its sulfate with thiourea. The compound (II) was further treated with monochloroacetic acid to give 8-carboxymethylthiohypoxanthine (III).

On the treatment of (I) with cyanoacetamide, 4-amino-5-cyanoacetamido-6-hydroxypyrimidine (IV) was produced. The cyclization of the latter to purine derivative by using alkali was unsuccessful, only to be hydrolysed to the starting material. The same

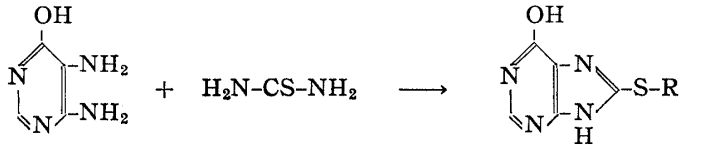
* Hongo, Tokyo (石館守三, 由岐英剛).

1) G.H. Hitchings: J. Biol. Chem., **192**, 505(1951).

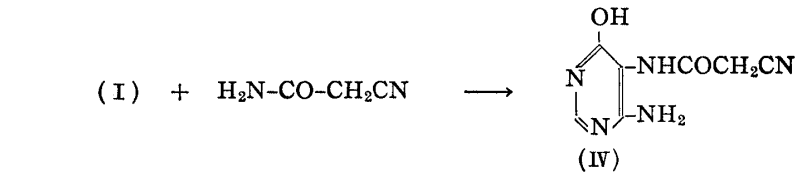
result was obtained in the case of a reaction product (VI) from 2-mercapto-4,5,6-triaminopyrimidine (V) with cyanoacetic acid.

By heating with oxalic acid, glycolic acid, succinic acid, or saccharic acid, 4,5-diamino-6-hydroxypyrimidine (I) yielded the corresponding 5-acyl derivatives, (VII), (IX), (XI), and (XIII). When they were heated with alkali or as the potassium salts, they underwent ring closure to form 8-substituted hypoxanthines, (VIII), (X) and (XII), except the glycosyl derivative (XIII).

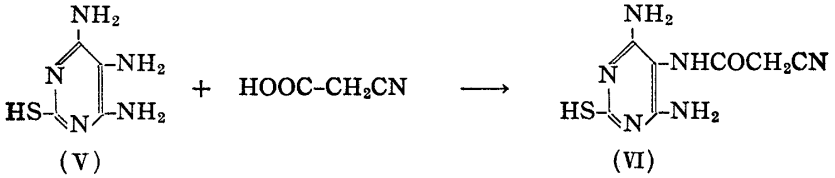
4-Amino-5-(3-methyl-2-thioureido)-6-hydroxypyrimidine (XIV), which was easily obtained by the treatment of (I) with methyl isothiocyanate in an aqueous solution, gave



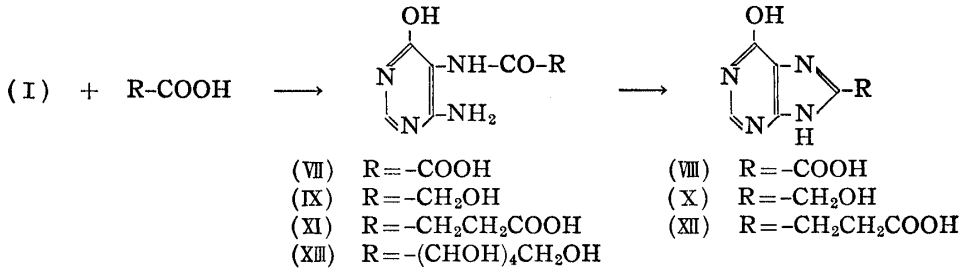
(I) (II) R=H (III) R=CH_2COOH



(IV)

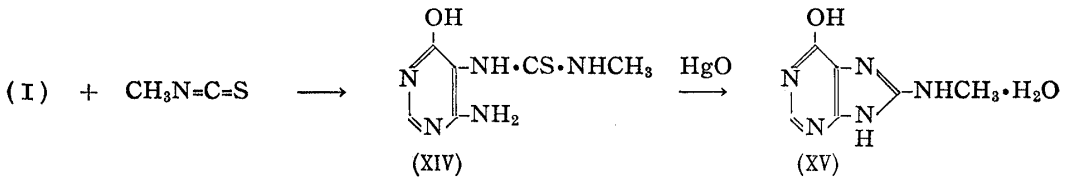


(V) (VI)

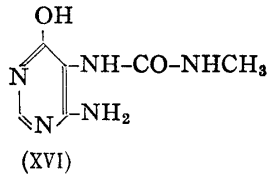


(VII) R=-COOH (IX) R=$-CH_2OH$ (XI) R=$-CH_2CH_2COOH$ (XIII) R=$-(CHOH)_4CH_2OH$

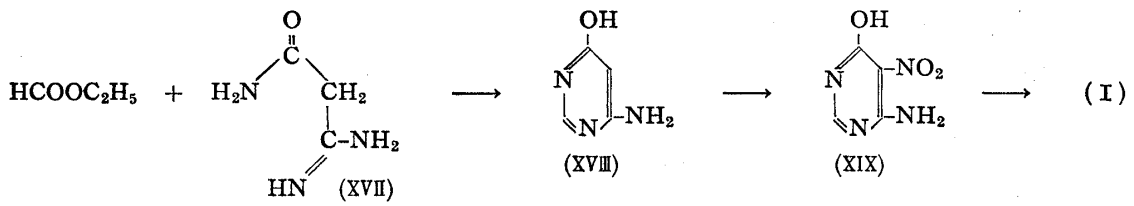
(VIII) R=-COOH (X) R=$-CH_2OH$ (XII) R=$-CH_2CH_2COOH$



(XIV) (XV)



(XVI)



(XVII) (XVIII) (XIX)

a product corresponding to either (XV) or (XVI). The structure (XV) was assigned for this product from the facts that the compound is sparingly soluble in water in contrast with the compound (XIV) and that its infrared absorption spectrum gave no characteristic band[2] (7.05 μ) indicating the presence of a 3-methylureido group (R-NH-CO-NH-CH_3). Moreover, the structure of (XV) was further comfirmed by its conversion to 6-mercapto-8-methylaminopurine[3] by treatment with phosphorus pentasulfide.

For the synthesis of hypoxanthine, Hitchings, *et al.*[4] recently described an improved method which involved the reduction of 2-mercapto-4-amino-6-hydroxypyrimidine with Raney nickel to obtain 4-amino-6-hydroxypyrimidine (XVIII) and followed by cyclization to a purine ring.

It is desirable to find a method of obtaining directly 2-unsubstituted 4-amino-6-hydroxypyrimidine with a considerable yield.

Todd, *et al.*[5] synthesized 4,6-diaminopyrimidine and Hull[6] obtained 4,6-dihydroxypyrimidine by the condensation of ethyl formate with malondiamidine and malondiamide, respectively.

To obtain 4-amino-6-hydroxypyrimidine directly the present authors condensed ethyl formate with malonamamidine (XVII). Without isolating the reaction product (XVIII), the mixture was treated with nitric acid. 4-Amino-5-nitro-6-hydroxypyrimidine (XIX) separated as plate crystals, but the yield was not more than 20% of the theoretical.

In the screening test for antitumor activity on Yoshida sarcoma rats, these 8-substituted hypoxanthines showed no possitive results.[7] Other biological properties will be reported elsewhere.

The authors wish to thank Messrs. E. Kimura and M. Endo for performing the analyses reported in this paper. For financial support of this work thanks are due to the Board of Pharmacological Research Foundation, Tokyo.

Experimental

8-Mercaptohypoxanthine (II)—A well-powdered mixture of 2.2 g. of 4,5-diamino-6-hydroxypyrimidine (I) and 4.5 g. of thiourea in a 25-cc. flask was heated in an oil bath for 15 mins. at the bath temperature of 220°. During heating at about 160~220° the mixture completely melted to a fluid and an evolution of NH_3 gas began. After a lapse of 15 mins. of heating at this temperature, the fluid resolidified gradually and the evolution of NH_3 gas ceased. After cooling, the mixture was dissolved in 10% NaOH, precipitated with AcOH, and recrystallized from 500 cc. of water, forming 1.5 g. of pale yellow plates, m.p. 235°(decomp.). *Anal.* Calcd. for $C_5H_4ON_4S \cdot \frac{1}{2}H_2O$: C, 33.90; H, 2.83; N, 31.64; S, 19.15. Found: C, 34.36; H, 2.70; N, 31.45; S, 19.32.

8-Carboxymethylmercaptohypoxanthine (III)—Two grams of finely-powdered (II) was suspended in 30 cc. of water containing 4 g. of monochloroacetic acid and boiled. The suspension became once clear and crystalline precipitate reformed. After cooling, the precipitate was recrystallized from 300 cc. of water to give 2 g. of (III), m.p. 265°(decomp.). *Anal.* Calcd. for $C_7H_6O_3N_4S$: C, 37.17; H, 2.66; N, 24.74. Found: C, 37.17; H, 3.14; N, 24.56.

4-Amino-5-cyanoacetamido-6-hydroxypyrimidine (IV)—A mixture of 11.5 g. of (I) and 17 g. of cyanoacetamide was well mixed and heated at a bath temperature of 190~200°. The reaction mixture melted once and resolidified during heating for 10~15 mins. After cooling, the product was dissolved in NaOH solution, reprecipitated with AcOH, and recrystallized from 400 cc. of water, giving 10.1 g. of (IV), m.p. 308°(decomp.). *Anal.* Calcd. for $C_7H_7O_2N_5 \cdot \frac{1}{2}H_2O$: C, 41.58; H, 3.96. Found: C, 41.82; H, 4.54.

This substance gave the starting material (I) on heating with conc. NaOH solution.

2) J. L. Boivin, P. A. Boivin: Can. J. Chem., **32**, 561(1954) (C. A. **48**, 1192(1954)).
3) See the following communication.
4) G. H. Hitchings, *et al.*: J. Am. Chem. Soc., **74**, 411(1952).
5) A. H. Todd, *et al.*: J. Chem. Soc., **1943**, 387.
6) R. Hull: *Ibid.*, **1951**, 2214.
7) The screening test was carried out by Dr. H. Sato of the Iatrochemical Institute, Tokyo, to whom we are indebted.

2-Mercapto-4,6-diamino-5-cyanoacetamidopyrimidine (VI)—A mixture of 1.7 g. of 2-mercapto-4,5,6-triaminopyrimidine (V) and the same amount of cyanoacetic acid was brought in 20 cc. of water and dissolved by heating. After cooling, orange yellow plates separated, which were recrystallized from dil. EtOH to give pale yellow plates, m.p. 250~300°(decomp.). *Anal.* Calcd. for $C_7H_8ON_6S \cdot H_2O$: C, 34.70; H, 4.13. Found: C, 35.20; H, 4.08.

This substance gave the starting material (V) on heating with conc. NaOH solution as in the case of (IV).

4-Amino-5-oxalylamino-6-hydroxypyrimidine (VII)—A mixture of 10 g. of oxalic acid and 2 g. of (I) was heated at 150° for 10 mins. The reaction mixture was boiled with water to remove unchanged oxalic acid and filtered. The product is insoluble in any other solvent but in alkali, so it was reprecipitated several times from its alkali solution with AcOH to give 2.4 g. of (VII), m.p. >350°. *Anal.* Calcd. for $C_6H_6O_4N_4$: C, 36.37; H, 3.05; N, 28.28. Found: C, 36.25; H, 3.63; N, 28.21.

8-Carboxyhypoxanthine (VIII)—The sodium salt obtained by addition of 2 moles of NaOH to 1.5 g. of (VII) was heated at 200° for 1 hr. under reduced pressure. After cooling, the residue was dissolved in water, filtered, and the filtrate was acidified with AcOH. One gram of (VIII) was obtained after the reprecipitation was repeated. m.p. >350°. *Anal.* Calcd. for $C_6H_4O_3N_4$: C, 40.00; H, 2.24; N, 31.10. Found: C, 40.50; H, 2.81; N, 30.30.

4-Amino-5-glycoloylamino-6-hydroxypyrimidine (IX)—By the condensation of 8 g. of (I) and 9 g. of glycolic acid at 170° for 20 mins., 9.5 g. of (IX) was obtained, which was recrystallized from 30 volumes of water, giving white plates of m.p. 290~305°(decomp.). *Anal.* Calcd. for $C_6H_8O_3N_4$: C, 39.13; H, 4.35; N, 30.43. Found: C, 39.28; H, 4.68; N, 30.23.

8-Hydroxymethylhypoxanthine (X)—The sodium salt obtained from 14 g. of (IX) was heated at 220° for 20 mins. under reduced pressure. The product was dissolved in water, filtered with carbon, and precipitated with AcOH, forming 8.4 g. of yellowish brown crystals of (X), m.p. 330~350°(decomp.). *Anal.* Calcd. for $C_6H_6O_2N_4$: C, 43.38; H, 3.61; N, 33.73. Found: C, 43.8; H, 4.2; N, 33.87.

4-Amino-5-succinylamino-6-hydroxypyrimidine (XI)—A mixutre of 3.2 g. of (I) and 8 g. of succinic acid was heated at 210~220° for 10 mins. The product was dissolved in NaOH, filtered, and acidified with AcOH. 3.8 g. of (XI) was obtained as white crystals, m.p. 268°(decomp.). *Anal.* Calcd. for $C_8H_{10}O_4N_4$: C, 42.48; H, 4.46; N, 24.77. Found: C, 42.87; H, 3.98; N, 24.89.

8-(2-Carboxyethyl)hypoxanthine (XII)—A solution of 2 g. of (XI) in 5 cc. of 15% NaOH was evaporated to dryness on a water bath under reduced pressure. The sodium salt thus obtained was heated at 210° for 1 hr. in a salt bath. After cooling, the product was dissolved in water and filtered. On acidification with AcOH a small amount of colored substance separated by filtration. By acidifying the filtrate with dil. H_2SO_4 a crude product of (XII) precipitated. Its recrystalliztion from 100 cc. of water gave 1 g. of (XII), m.p. 310°(decomp.). *Anal.* Calcd. for $C_8H_8O_3N_4$: C, 46.49; H, 3.95; N, 26.92. Found: C, 46.15; H, 3.87; N, 26.32.

4-Amino-5-gluconoylamino-6-hydroxypyrimidine(XIII)—A mixture of 1 g. of (I) and 5 cc. of aqueous solution of gluconic acid (2.5 g.) was heated to 140° for 15 mins. under reduced pressure. The product was recrystallized from a small amount of water. 2.5 g. of white crystals were obtained, m.p. 221°(decomp.). *Anal.* Calcd. for $C_{10}H_{16}O_7N_4 \cdot H_2O$: C, 37.27; H, 5.63; N, 17.39. Found: C, 37.21; H, 5.40; N, 17.79. The substance was dried at 120°. Calcd. for $C_{10}H_{10}O_7N_4$: C, 39.44; H, 5.26. Found: C, 39.01; H, 5.71.

An attempted cyclization of this substance to the corresponding purine derivative (XIV) was unsuccessful due to decomposition.

4-Amino-5-(3-methyl-2-thioureido)-6-hydroxypyrimidine (XIV)—To a solution of 3 g. of (I) in 20 cc. of hot water was added 2 g. of methyl isothiocyanate, and warmed on a water bath. After a lapse of about 10 mins., the oily layer of methyl isothiocyanate dissolved into solution and then the crystals began to precipitate. After heating further for 1 hr., the reaction mixture was cooled and the resulting precipitate was filtered. Four grams of (XIV) was obtained and recrystallized from water. This substance darkened at 270~280° and did not melt even at 350°. *Anal.* Calcd. for $C_6H_9ON_5S$: C, 36.18; H, 4.55; N, 35.18. Found: C, 36.11; H, 3.88; N, 34.54.

8-Methylaminohypoxanthine (XV)—Three grams of (XIV) and HgO, freshly prepared from 7 g. of $HgCl_2$, were suspended in 40 cc. of water and warmed on a water bath for 30 mins. The whole precipitate was collected, washed with water, and treated with 15 cc. of 10% NaOH to remove an insoluble sludge containing HgS. A white bulky precipitate was obtained by addition of AcOH to the filtrate, which was reprecipitated from its alkali solution, washed with water, and dried, giving 2.1 g. of (XV), m.p. >350°. *Anal.* Calcd. for $C_6H_7ON_5 \cdot H_2O$: C, 32.73; H, 4.54; N, 31.82. Found: C, 32.95; H, 3.97; N, 32.36.

4-Amino-5-nitro-6-hydroxypyrimidine (XIX)—To a solution of metallic Na (12 g.) in EtOH (300 cc.) was added malonamamidine hydrochloride (35 g.), refluxed for 30 mins., and precipitated NaCl was filtered off. Ethyl formate (50 cc.) was added to the filtrate and refluxed for 1 hr. After cooling the solution, the precipitate obtained was collected, dissolved in 50 cc. of water, acidified with AcOH,

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.

(Received January 26, 1957)

U. D. C. 547.853.7'789.07

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

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo**)

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

* Hongo, Tokyo (石館守三, 由岐英剛).

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).