Synthesis of Derivatives of Thiazolo[4,5-*d*]pyrimidine. Part I

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Reaction of 2,4-diamino-5-bromo-6-hydroxypyrimidine with thiourea gave the isothiouronium salt, which with alkali afforded di-(2,4-diamino-6-hydroxypyrimidin-5-yl) disulphide and not the thiazolo[4,5-*d*]pyrimidine claimed by an earlier worker. Kaufmann thiocyanation of 2,4-diamino-6-hydroxypyrimidine yielded the 5-thiocyanato-compound, which did not readily cyclise to a thiazolo[4,5-*d*]pyrimidine as previously claimed by other workers. Cyclisation of the thiocyanato-compound has been effected with acetic anhydride. Deacetylation of the resulting diacetamido-derivative gave 2,5-diamino-7-hydroxythiazolo[4,5-*d*]pyrimidine.

THE claim by Horiuchi [1] to have prepared 2,5-diamino-7-hydroxythiazolo[4,5-*d*]pyrimidine (IX) by the action of alkali on the product from thiourea and 2,4-diamino-5-bromo-6-hydroxypyrimidine (II) is surprising, although not impossible, since the ease with which isothiouronium bases generate thiols is well known. Horiuchi based his conclusion on a comparison of the i.r. spectrum of his compound with that of one prepared according to the method of Maggiolo and Hitchings,[2] who claimed to obtain the thiazolopyrimidine (IX) by the thiocyanation of 2,4-diamino-6-hydroxypyrimidine (I).

We have confirmed that 2,4-diamino-5-bromo-6-hydroxypyrimidine (II) [prepared by the bromination of 2,4-diamino-6-hydroxypyrimidine (I) in alkaline solution] reacts smoothly with thiourea. The product of this reaction, with ethanol as solvent, was the slightly impure isothiouronium bromide (III), which, when crystallised from concentrated hydrobromic acid, gave pure isothiouronium bromide hydrobromide. The reaction also proceeded readily with boiling water as solvent, and, in this case, a small amount of white solid was collected from the hot solution, and identified as di-(2,4-diamino-6-hydroxypyrimidin-5-yl) sulphide (IV) by analysis and comparison of its i.r. spectrum with that of a product obtained by the reaction of sodium hydrogen sulphide with the bromopyrimidine (II). The filtrate, when cooled, deposited a hydrobromide, assumed to be the isothiouronium salt (III), which gave a pale yellow solid on treatment with aqueous sodium hydrogen carbonate. This product was presumably the isothiouronium base. An attempt to purify it by crystallisation from water failed because of decomposition.

During other runs no attempt was made to isolate the isothiouronium compounds. The sulphide (IV) was removed, the filtrate adjusted to a pH of about 8 by the addition of sodium hydrogen carbonate, and refluxing continued for 1·5 hours. The pale yellow, crystalline solid, which deposited, was shown to be the disulphide (V) by analysis and comparison of its i.r. spectrum with that of a product obtained by the reaction of sodium hydrogen sulphide with the bromopyrimidine (II). Its sulphur content indicated that it could not be the thiazolopyrimidine (IX). It is obvious that the reaction of sodium hydrogen sulphide with the bromopyrimidine (II) could not give a thiazolopyrimidine.

In earlier attempts to effect decomposition of the isothiouronium base we used sodium hydroxide solution as employed by Horiuchi.[3] This led to impure specimens of the disulphide (V) which were difficult to purify, because of the low solubility of the disulphide in all common solvents except aqueous alkali. The pure disulphide (V) when treated with hot sodium hydroxide solution for 45 hours, gave the sulphide (IV) identified by its i.r. spectrum. Acidification of the solution, remaining after removal of the sulphide, gave sulphur dioxide, but neither sulphide ion nor sulphate was detected. Decomposition of the disulphide (V) by cold aqueous sodium hydroxide in the presence of hydrogen peroxide resulted in the extrusion of about half of the sulphur as sulphate, but the fate of the rest of the molecule was not elucidated. This behaviour of the disulphide (V) was taken as evidence that the compound had been assigned the correct structure, and that it was not the corresponding thiol (X).

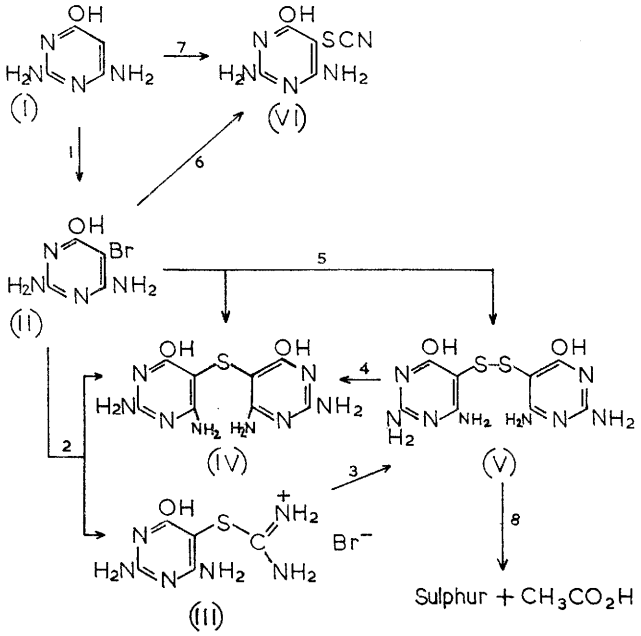
Chemical reduction of the disulphide (V), using Devarda's alloy and cold N-hydrochloric acid, was followed by u.v. absorption and appeared to be successful, since changes were noted. Oxygenation of the solution

[1] Mahiko Horiuchi, *Chem. and Pharm. Bull.* (*Japan*), 1959, **7**, 393.

[2] Allison Maggiolo and G. H. Hitchings, *J. Amer. Chem. Soc.*, 1951, **73**, 4226.

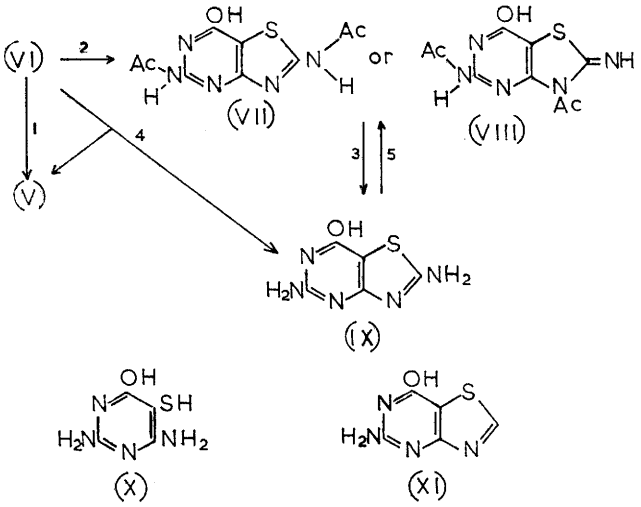
[3] Y. Sawa and M. Horiuchi, Jap. Pat. 7486/1962 (*Chem. Abs.*, 1963, **59**, 2835).

then gave further changes, but the original spectrum was not reproduced. Attempts at catalytic reduction of the disulphide (V) failed owing to its insolubility in suitable solvents. The yellow solution of the disulphide (V) in aqueous sodium hydroxide became colourless on



Reagents: 1, Br_2, NaOH; 2, thiourea; 3, $NaHCO_3$, pH 8; 4, hot NaOH or Zn/HCO_2H; 5, NaSH; 6, KSCN; 7, Br_2, KSCN; 8, hot dilute HCl.

treatment with sodium dithionite, and the colour was restored on leaving the solution in air. Treatment of the disulphide (V) with zinc dust and formic acid, which, it was hoped, would lead to reduction followed by cyclisation to the thiazolopyrimidine (XI), furnished the sulphide (IV).

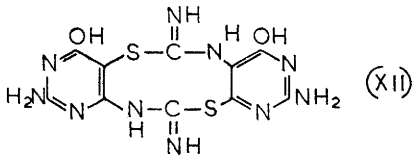


Reagents: 1, Cold NaOH; 2, Ac_2O; 3, Na_2CO_3; 4, phosphate buffer, pH 8; 5, Ac_2O.

The disulphide (V) was unstable to hot 10% hydrochloric acid, and decomposed with the formation of sulphur and with degradation of the ring, as shown by the detection of acetic acid.

Application of the Kaufmann thiocyanation reaction to 2,4-diamino-6-hydroxypyrimidine (I) gave 2,4-diamino-6-hydroxy-5-thiocyanatopyrimidine (VI). The i.r. spectrum of the latter compound showed a sharp medium-to-strong band at about 4·65 μ characteristic of the thiocyanato-group. The preparation of the thiocyanato-compound (VI) by reaction of the bromopyrimidine (II) with potassium thiocyanate was found more convenient. Either water or dimethylformamide was found to be a suitable solvent, the latter giving a better yield of purer material. Isomerisation of the thiocyanato-compound (VI) to the thiazolopyrimidine (IX) did not occur in water, pentanol, hexanol, propane-1,2-diol, ethyl digol, or tetralin, all of which were employed at the boiling point, or in 95% acetic acid at 70°.

Treatment of the thiocyanatopyrimidine (VI) with cold sodium hydroxide solution for 40 hours gave the disulphide (V) as shown by i.r. spectra. Cyclisation of the thiocyanato-compound (VI) was effected by boiling acetic anhydride (72 hours) with the formation of 2(3),5-diacetamido-7-hydroxythiazolo[4,5-*d*]pyrimidine, (VII) or (VIII), which was soluble in cold sodium hydroxide solution. Upon acidification of the alkaline solution the compound was recovered unchanged, thus proving the absence of *O*-acetylation. Boiling sodium carbonate solution readily hydrolysed the diacetamido-derivative, (VII) or (VIII), to 2,5-diamino-7-hydroxy-



thiazolo[4,5-*d*]pyrimidine (IX), which was also obtained in small yield, along with the disulphide (V), by isomerisation of the thiocyanato-compound (VI) in phosphate buffer (pH 8). The i.r. spectrum of the thiazolopyrimidine (IX) showed no absorption at about 4·65 μ. The cyclisation of the thiocyanato-compound (VI), using acetic anhydride and subsequent hydrolysis, could have given the dimeric structure (XII). A molecular weight determination was not attempted, because of the high insolubility of the product, and such a determination might not be unequivocal, because of the extensive hydrogen bonding to be expected in these compounds. The product, to which we have assigned structure (IX), was recovered almost quantitatively (i.r. spectra) after being boiled with dilute hydrochloric acid for 18 hours. The centre ring of structure (XII) should rupture under such treatment.

The u.v. absorption data for the disulphide (V) are compared in the Table with recalculated ε values for the products obtained by Horiuchi and Maggiolo, and claimed by them to be the thiazolopyrimidine (IX). Since the absorption is slightly time-dependent when determined in acid solution, and much more so when determined in alkaline solution, these comparisons are

U.v. absorption data for the disulphide (V). The results of Horiuchi and Maggiolo have been recalculated to convert ε values into those for the disulphide

		Horiuchi	Maggiolo	Present work
0·1N-NaOH	$\lambda_{max.}$	271, 345 mμ		267, 347 mμ
	ε	13,210, 6180		13,540, 5040 after 2·5 hr.
pH 11	$\lambda_{max.}$		265, 330 mμ	
	ε		14,510, 4540	
0·1N-HCl	$\lambda_{max.}$			267, 318 mμ
	ε			21,380, 6610 after 96 hr.
pH 1	$\lambda_{max.}$		262, 313 mμ	
	ε		20,900, 5900	

not as unequivocal as one would wish. It does, however, appear that the products obtained by the earlier workers were samples of the disulphide (V). The data shown are quite different from those for the compound which we describe as the thiazolopyrimidine (IX).

EXPERIMENTAL

All i.r. spectra were run in KCl discs on an Infracord spectrophotometer.

2,4-Diamino-5-bromo-6-hydroxypyrimidine.—A solution of 2,4-diamino-6-hydroxypyrimidine (I) (25·2 g.) in 4% aqueous sodium hydroxide (400 ml.) was treated with charcoal, filtered, and bromine (10 ml.) added, with stirring, during 2·5 hr., the temperature being held at 23°. The solution was left overnight, filtered, and slowly acidified with concentrated hydrochloric acid (21 ml.), with stirring. After leaving the mixture for 30 hr., the precipitated *bromo-compound* (II) was collected, washed until acid-free, and dried to yield 34 g. of product. Crystallisation from water (2·5 l.), followed by drying at 100°, gave 32 g. (78%), m.p. 264·5—265° (decomp.) [lit., 244° (decomp.),[1] 255° (decomp.) [4]] (Found: C, 23·7; H, 2·6; Br, 39·3. Calc. for $C_4H_5BrN_4O$: C, 23·4; H, 2·5; Br, 39·0%); $\lambda_{max.}$ (in 0·1N-HCl) 275 mμ (log ε 4·20), (in 0·1N-NaOH) 270 mμ (log ε 3·94).

In subsequent runs the periods of leaving were omitted with only a small decrease in yield.

Reaction of 2,4-Diamino-5-bromo-6-hydroxypyrimidine with Thiourea.—(a) *Isolation of 2,4-diamino-6-hydroxypyrimidine-5-isothiouronium bromide and bromide hydrobromide.* A mixture of the bromo-compound (II) (2 g.), thiourea (0·8 g.), and ethanol (50 ml.) was refluxed with stirring for 0·5 hr. After leaving overnight, the solid was collected, washed with ethanol, and dried to give 2·5 g. of the *isothiouronium bromide* (III), m.p. 273—274° (decomp.) (Found: C, 21·4; H, 3·4; Br, 27·3; S, 11·3. $C_5H_9BrN_6OS$ requires C, 21·35; H, 3·2; Br, 28·4; S, 11·4%). Crystallisation from concentrated hydrobromic acid afforded the *bromide hydrobromide*, m.p. >350° (Found: C, 16·7; H, 3·0; Br, 43·9; N, 23·7; S, 8·9. $C_5H_{10}Br_2N_6OS$ requires C, 16·6; H, 2·8; Br, 44·2; N, 23·2; S, 8·9%), $\lambda_{max.}$ (in 0·1N-HCl) 259 mμ [log ε 4·18(5)].

(b) *Decomposition of the isothiouronium base in alkaline solution.* A mixture of the bromo-compound (II) (5·1 g.), thiourea (2·1 g.), and water (125 ml.) was refluxed with stirring for 1·25 hr., and the almost white precipitate (130 mg.) collected. This precipitate was identified as *di-(2,4-diamino-6-hydroxypyrimidin-5-yl) sulphide* (IV) *dihydrate*, m.p. >270°. It was hygroscopic (Found, after drying at 100°: C, 30·1; H, 4·4; N, 35·3; S, 10·1. $C_8H_{10}N_8O_2S,2H_2O$ requires C, 30·2; H, 4·4; N, 35·2; S, 10·1%); $\lambda_{max.}$ (in 0·1N HCl) 258 mμ (log ε 4·41), (in 0·1N-NaOH) 266 mμ (log ε 4·545). After removal of the sulphide, the filtrate plus washings (total volume 200 ml.) was clarified with kieselguhr, sodium hydroxide (10 g.) added, and the solution boiled for 0·5 hr. The cooled solution was neutralised with concentrated hydrochloric acid, and the orange solid collected (2 g.). Purification was effected as follows. The crude material (300 mg.) was extracted with boiling water (25 ml.) for 10 min., dissolved in hot aqueous 2·5% sodium hydroxide solution (20 ml.), and this solution gradually added without delay to stirred, boiling glacial acetic acid (20 ml.). After cooling, the pale yellow precipitate was collected, resuspended in warm water (50 ml.), collected, and washed successively with water and ethanol to afford 200 mg. of *di-(2,4-diamino-6-hydroxypyrimidin-5-yl) disulphide* (V) *hydrate*, m.p. >350° (Found, after drying at 60°: C, 28·95; H, 3·9; N, 33·9; S, 19·2. $C_8H_{10}N_8O_2S_2,H_2O$ requires C, 28·9; H, 3·65; N, 33·7; S, 19·3%). The spectral data for this compound are in the Table.

(c) *Decomposition of the isothiouronium base at pH* 8. A solution of the isothiouronium bromide (III) was prepared from the bromo-compound (II) (2·55 g.), thiourea (1·0 g.), and water (75 ml.) as previously described. After removal of the sulphide (IV), sodium hydrogen carbonate (0·5 g.) was added, and the solution refluxed for 1·75 hr., then cooled. The pH was 8. The pale yellow, crystalline product (0·68 g.) was collected, washed, and dried at 100°. Analysis and comparison of i.r. spectra showed it to be the *disulphide* (V) *hydrate* (Found: C, 29·0; H, 3·85; S, 19·1%). A further quantity (0·46 g.), slightly less pure, was obtained from the filtrate after the addition of more sodium hydrogen carbonate (0·25 g.) and the continuation of refluxing for 3·5 hr. Total yield, 70%.

Reaction of 2,4-Diamino-5-bromo-6-hydroxypyrimidine with Sodium Hydrogen Sulphide.—A solution of sodium hydrogen sulphide was prepared according to the method of Hodgson and Ward.[5] Sodium hydrogen carbonate (1·7 g.) was slowly added to a solution of sodium sulphide nonahydrate (4·8 g.) in water (12 ml.). Methanol (12 ml.) was then slowly added, the mixture was stirred for 0·5 hr., and the sodium carbonate filtered off and washed with methanol (6 ml.). During the whole of these operations the temperature was kept below 20°. The filtrate was mixed with phosphate buffer (pH 8; 200 ml.), and the bromo-compound (II) (1 g.), dissolved in 10% aqueous sodium hydroxide (5 ml.) and water (145 ml.) slowly added. The pH was maintained at about 8·5 by the gradual addition of 0·067M-sodium dihydrogen phosphate (80 ml.). The white precipitate, which formed, was collected after neutralisation of the solution, and was identified as the *sulphide* (IV) by comparison of its i.r. spectrum with that of the product obtained by reaction of the bromo-compound (II) with thiourea. On leaving the filtrate for 3 days, pale yellow needles separated. These were collected (100 mg.), and purified by crystallisation from saturated sodium carbonate solution (6 ml.) followed by reprecipitation in boiling glacial acetic acid from a solution in aqueous sodium hydroxide as described previously. The product (25 mg.) was identified as the *disulphide* (V) by comparison of its i.r. spectrum with that of the product obtained by reaction of the bromo-compound (II) with thiourea.

Conversion of the Disulphide (V) *into the Sulphide* (IV).—A solution of the disulphide (V) (0·5 g.) in 10% aqueous

[4] T. L. V. Ulbricht and C. C. Price, *J. Org. Chem.*, 1956, **21**, 567.

[5] H. H. Hodgson and E. R. Ward, *J. Chem. Soc.*, 1948, 242.

sodium hydroxide (30 ml.) was heated to 100° in a plastic beaker (f.e.p.) for 45 hr. The cooled solution was decolourised with charcoal, heated to the b.p., and neutralised with glacial acetic acid. The white product (250 mg.) was collected from the cold mixture, and identified as the sulphide (IV) by comparison of i.r. spectra. Acidification of the filtrate with dilute hydrochloric acid gave sulphur dioxide (odour, dichromate paper) but no hydrogen sulphide. The filtrate contained no sulphate, but this was detected after acidification with dilute hydrochloric acid in the presence of hydrogen peroxide.

Degradation of the Disulphide (V) *with Hot, Dilute Hydrochloric Acid.*—A mixture of the disulphide (V) (0·5 g.) and dilute hydrochloric acid (25 ml.) was refluxed for 12 hr. A trace of hydrogen sulphide was evolved possibly along with a little sulphur dioxide. The yellow solid, which formed in the condenser, was removed and identified as sulphur (Found: S, 98·8%). The solution was distilled to half volume and the distillate neutralised with an excess of lime and taken to dryness. On being heated the residue gave vapours which turned *o*-nitrobenzaldehyde paper (moistened with 10% sodium hydroxide) green, indicating complete breakdown of the ring with the formation of acetic acid.

Preparation of 2,4-*Diamino*-6-*hydroxy*-5-*thiocyanatopyrimidine.*—(a) *Thiocyanation of* 2,4-*diamino*-6-*hydroxypyrimidine.* Potassium thiocyanate (80 g.) and 2,4-diamino-6-hydroxypyrimidine (I) (25·2 g.) were dissolved with heat in 95% acetic acid (1600 ml.), and the solution cooled to 10°. Bromine (10 ml.) in glacial acetic acid (40 ml.) was slowly added with stirring during 20 min., the temperature being kept at 10°. Stirring was continued for a further 40 min. The mixture was then neutralised with concentrated aqueous ammonia below 30°, allowed to stand overnight, and the precipitate (28 g.) collected. Four recrystallisations of 1·8 g. from water (500 ml.) afforded the monohydrate of the *thiocyanato-compound* (VI), which was dried at 60°, m.p. 258° (decomp.) when placed in the bath at 256°. (Found: C, 29·9; H, 3·4; N, 35·0; S, 15·9. $C_5H_5N_5OS,H_2O$ requires C, 29·8; H, 3·5; N, 34·8; S, 15·9%); $\lambda_{max.}$ (in 0·1N-HCl) 259 mμ (log ε 4·18), $\lambda_{max.}$ (KCl disc) about 4·65 μ (SCN). The monohydrate lost water on prolonged drying at 100°.

(b) *Reaction of* 2,4-*diamino*-5-*bromo*-6-*hydroxypyrimidine with potassium thiocyanate.* A solution of potassium thiocyanate (5·5 g.) in water (50 ml.) was added during 5 min. to a solution of the bromo compound (II) (5·1 g.) in boiling water (300 ml.) and the mixture refluxed for 1 hr. The *thiocyanato-compound* (VI) (4·1 g.) was collected from the hot solution. The filtrate, on cooling, afforded a further 0·8 g. of less pure material. The i.r. spectrum of the pure product was identical with that of the compound prepared by thiocyanation of 2,4-diamino-6-hydroxypyrimidine. A slightly better yield of pure product was obtained with dimethylformamide as solvent. A solution of potassium thiocyanate (50 g.) in dimethylformamide (300 ml.) was heated to 80°, stirred, and the solid bromo-compound (II) (51 g.) added in portions during 25 min. The mixture was heated at 60—80° and stirred for a further 25 min., and then filtered, whilst hot, to remove potassium bromide, which was washed with dimethylformamide (2 × 20 ml.). Addition of hot water (60°) (3 l.) to the filtrate, followed by cooling, precipitated the thiocyanato-compound (45 g.), which was collected, washed with water and acetone, and dried at 60°.

Reaction of 2,4-*Diamino*-6-*hydroxy*-5-*thiocyanatopyrimidine with Cold, Aqueous Sodium Hydroxide.*—A solution of the thiocyanato-compound (VI) (1·9 g.) in 10% aqueous sodium hydroxide (30 ml.) was kept at room temperature for 40 hr., the reaction being followed by u.v. absorption, which, initially, changed rapidly. The deep yellow solution was treated with charcoal, filtered, quickly heated, and added rapidly to stirred, boiling glacial acetic acid (50 ml.). After heating the mixture for 5 min., it was filtered and the microcrystalline precipitate washed and dried to yield 0·8 g. of the disulphide (V) identified by its i.r. spectrum.

Cyclisation of 2,4-*Diamino*-6-*hydroxy*-5-*thiocyanatopyrimidine with Acetic Anhydride.*—A mixture of the thiocyanato-compound (VI) (45 g.) and acetic anhydride (1·5 l.) was refluxed with stirring for 72 hr. The solid was collected from the hot solution, washed successively with acetic anhydride, glacial acetic acid, water, and ethanol to give 49 g. (75%) of 2(3),5-*diacetamido*-7-*hydroxythiazolo*[4,5-*d*]-*pyrimidine* (VII) or (VIII), m.p. >350° (Found: C, 40·3; H, 3·6. $C_9H_9N_5O_3S$ requires C, 40·4; H, 3·4%). Recrystallisation from water, followed by drying at 60°, gave the *monohydrate*, m.p. >350° (Found: C, 37·9; H, 3·9; N, 24·5; S, 11·1. $C_9H_9N_5O_3S,H_2O$ requires C, 37·9; H, 3·9; N, 24·6; S, 11·2%). The i.r. spectrum (KCl disc) showed no absorption at about 4·65 μ.

Preparation of 2,5-*Diamino*-7-*hydroxythiazolo*[4,5-*d*]-*pyrimidine.*—A solution of the foregoing anhydrous diacetamido compound (VII) or (VIII) (7 g.) in saturated aqueous sodium carbonate (400 ml.) and water (20 ml.) was refluxed for 1 hr. and cooled. The white needles were collected, washed successively with water, alcohol, and acetone, and dried at 100° to afford 5·1 g. of the *thiazolopyrimidine* (IX) monohydrate, m.p. >350° (Found: C, 29·7; H, 3·6; N, 34·3; S, 15·8. $C_5H_5N_5OS,H_2O$ requires C, 29·8; H, 3·5; N, 34·8; S, 15·9%); $\lambda_{max.}$ (in 0·1N-HCl) 223, 255sh, 305 mμ (log ε 4·40, 3·78, 4·11). Crystallisation of the thiazolopyrimidine from 10% aqueous sodium hydroxide gave a sodium derivative (Found: Na, 10·1. $C_5H_4N_5NaOS,H_2O$ requires Na, 10·3%). The sodium derivative, when crystallised from nearly saturated sodium carbonate solution, yielded the free thiazolopyrimidine.

Acetylation of 2,5-*Diamino*-7-*hydroxythiazolo*[4,5-*d*]*pyrimidine.*—The thiazolopyrimidine (IX) (100 mg.) was refluxed with acetic anhydride for 1 hr., and the mixture cooled. The product was collected and crystallised from water (80 ml.) to give 80 mg. of a compound having an i.r. spectrum identical with that of the diacetamido-derivative prepared by cyclisation of the thiocyanato-compound (VI) with acetic anhydride.

Isomerisation of 2,4-*Diamino*-6-*hydroxy*-5-*thiocyanatopyrimidine in Phosphate Buffer* (pH 8).—The thiocyanato-compound (VI) (470 mg.) was added to 200 ml. of boiling phosphate buffer (Sörensen; pH 8), and the mixture refluxed with stirring for 75 min. Ammonia was evolved. The pale yellow needles (330 mg.) were collected, washed, and dried. The i.r. spectrum indicated that the product was a mixture of the *disulphide* (V) and the *thiazolopyrimidine* (IX). Crystals of the sodium derivative (100 mg.) of the thiazolopyrimidine deposited from a hot solution of the product in the minimum of 10% aqueous sodium hydroxide. Crystallisation (charcoal) of the sodium compound from saturated aqueous sodium carbonate (5 ml.) and water (2 ml.) gave the pure thiazolopyrimidine (60 mg.) identified by comparison of its i.r. spectrum with that of the product obtained as described earlier.

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