5-Ethylaminothiazolo(5,4-*d*)pyrimidine (VIa)——A solution of 0.7 g of Va in 20 ml of ethyl orthoformate was refluxed for 2 hr. The reaction mixture was evaporated to almost dryness in vacuum and the residue was treated with 5% NaOH, and the insoluble crystalline product was taken up in benzene. Removal of the solvent provided 0.64 g of VIa, mp 136—137°. Purification data for this compound and the similarly prepared **5**-benzylamino- (VIb) and *p*-chloroanilino- (VIc)derivatives were shown in Table IV.

5-Ethylamino-2-mercaptothiazolo(5,4-*d*)pyrimidine (VIIa)——To a solution of MeOCSSK (prepared from 3.3 g of KOH, 3.5 g of CS_2 and 120 ml of MeOH) was added 5 g of Va and the mixture refluxed for 18 hr. After cooling, the reaction mixture was treated with charcoal and filtered, and the filtrate was concentrated to almost dryness. To the residue was added H_2O and the solution was acidified with AcOH to give yellow precipitate. Reprecipitation from 2% NH_4OH and AcOH gave yellow amorphous solid. mp 240—241° (decomp.). *Anal.* Calcd. for $C_7H_8N_4S_2$: C, 39.62: H, 3.77. Found: C, 39.66; H, 3.51. The other 2-mercaptothiazolopyrimidines, shown in Table IV, were prepared by the same method.

5-Amino- or 5-Alkylamino-2-alkylthiothiazolo(5,4-*d*)pyrimidines (VIIIa—k)——The potassium salt of 2-mercaptothiazolopyrimidines and a slight excess of alkyl halide in EtOH was heated for a short time. The solvent was removed, a small amount of H_2O was added to the residue, and the separated crystals were collected. All 2-alkylthio derivatives thus prepared were listed in Table V.

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UDC 547.857.2.07

Studies on Pyrimidine Derivatives. X[1]

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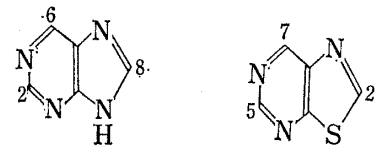
Present study was undertaken to examine the relation between the order of reactivity to nucleophilic substitutions of chlorine atoms in 2,5,7-trichlorothiazolo(5,4-*d*)pyrimidine (II) and the proton chemical shifts of thiazolo(5,4-*d*)pyrimidine nucleus.



purine thiazolo(5,4-*d*)pyrimidine.

Chart 1

It is usually considered that electron density on each of the carbon atoms in the aromatic nucleus is roughly parallel to the chemical shift of the protons attached to the carbon atom, and ease of the nucleophilic aromatic substitutions is also in relation to the electon density of the carbon atom. In the case of purine, such a parallel relation could be observed; thus, the order of chemical shifts is H_6–H_2–H_8,[3,4] and the order of reactivity to nucleophilic substitutions of chlorine atoms[5] in 2,6,8-trichloropurine by means of diethylamine and sodium ethoxide was C_6–C_2–C_8. In the thiazolo(5,4-*d*)pyrimidine (XVI), a sulfur analog of purine, the same relation would be expected, but in fact no such simple relation could be observed (Table I and Fig. 1).

Assignments of signals to the protons in thiazolo(5,4-*d*)pyrimidine (XVI) can be easily done by inspection of the signal shapes (Fig. 1). Thus, the sharpest peak at 569.0 cps is assigned to the proton at C_2 and the broadest peak at 553.0 cps to that at C_5, since the proton at C_5

1) Part IX: *Chem. Pharm. Bull.* (Tokyo), **16**, 745 (1968).
2) Location: *Yagotourayama, Tenpaku-cho, Showa-ku, Nagoya.*
3) S. Matsuura and T. Goto, *Tetrahedron Letters*, **1499** (1963); *J. Chem. Soc.*, **1965**, 623.
4) ^{13}C chemical shifts of purine were also determined; the order is H_2–H_8–H_6. R.J. Pugmire, D.M. Grant, R.K. Robins, and G.W. Rhodes, *J. Am. Chem. Soc.*, **87**, 2225 (1965).
5) R.K. Robins and B.E. Christensan, *J. Am. Chem. Soc.*, **74**, 3624 (1952).

Table I

No.	Compound	Chemical shift (cps from TMS) H_2	H_5	H_7
XVI	thiazolo(5,4-*d*)pyrimidine[a]	569.0	553.0	551.5
XVII	5-chloro-[b]	559.0	—	549.5
XVIII	7-chloro-	554.0	538.5	—
XIX	2,5-dichloro-[c]	—	—	546.0
XX	2,7-dichloro-	—	535.0	—
XXI	5,7-dichloro-[d]	552.0	—	—

Condition: 5% solution in $CDCl_3$ at room temp. (60 Mc). Acuracy ±0.5 cps
a) S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 349 (1958).
b) S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 675 (1958).
c) S. Sugiura, E. Suzuki, T. Naito, and S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **16**, 741 (1968).
d) S.J. Childless and R.L. Mckee, *J. Am. Chem. Soc.*, **73**, 3862 (1951).

is broadened by the adjacent two nitrogen atoms and small coupling with the proton at C_7, whereas sharpness of the signal of H_2 is comparable to that of TMS. When H_5 is substituted with chlorine atom the signal for H_7 becomes sharper. It is evident from this result that chlorine atom has rather electron-donating effect on the aromatic ring.

The order of chemical shift of thiazolo(5,4-*d*)pyrimidine (XVI) was H_2–H_5–H_7, but the order of chemical shift of dichloro derivatives, *e.g.* (XIX), (XX) and (XXI), was H_2–H_7–H_5. However, reactivity of chlorine atoms towards nucleophilic substitutions with N-methylaniline, sodium ethylmercaptide, and sodium ethoxide was C_7–C_2–C_5 as shown in Chart 2.

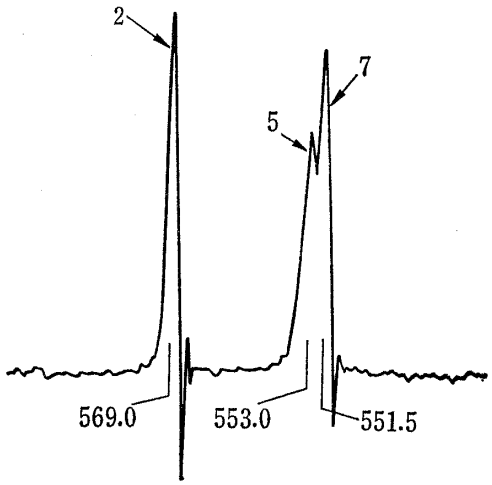


Fig. 1. NMR Spectrum of Thiazolo-(5,4-*d*)pyrimidine (XVI) in $CDCl_3$
5% solution, c/s from internal standard, TMS

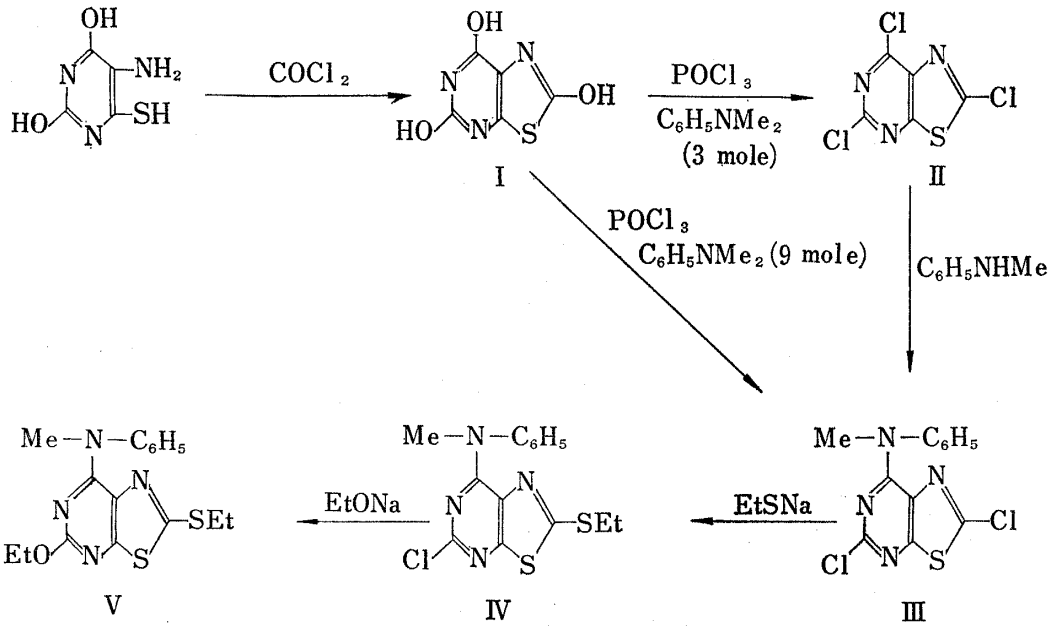


Chart 2

2,5,7-trichlorothiazolo(5,4-*d*)pyrimidine(II) was obtained by chlorination of 2,5,7-trihydroxythiazolo(5,4-*d*)pyrimidine (I), prepared from thiouramil and phosgene, with phosphorus oxychloride in the presence of three moles of dimethylaniline. Reaction of the trichloro derivative (II) with two moles of N-methylaniline afforded N-methylanilino-dichloro derivative (III). This compound (III) was also produced when nine moles of dimethylaniline was used in the above chlorination (I→II). To confirm which chlorine atom in II was replaced by N-methylaniline or by dimethylaniline (presumably formed by loss of methyl chloride from the quaternary intermediate during the chlorination), the N-methylanilino-dichloro derivative (III) was treated successively with sodium ethylmercaptide and sodium ethoxide. The final product was proved to be 5-ethoxy-2-ethylthio-7-N-methylanilinothiazolo(5,4-*d*)pyrimidine (V) by comparison with an authentic specimen obtained by the following procedure.

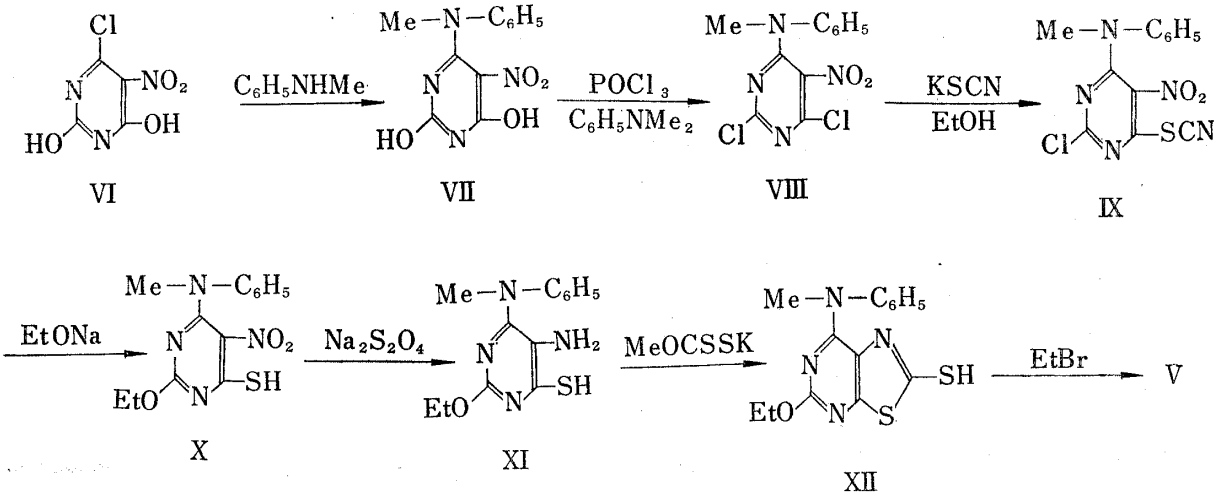


Chart 3

Wood, *et al.*[6] reported the three step preparation of 6-chloro-2,4-dihydroxy-5-nitropyrimidine(VI) from barbituric acid. Reaction of VI with N-methylaniline gave 2,4-dihydroxy-6-N-methylanilino-5-nitropyrimidine (VII), which was converted by treatment with phosphorus oxychloride in the presence of dimethylaniline to 2,5-dichloro-6-N-methylanilino-5-nitropyrimidine (VIII). The improved procedure developed by us[7] was applied for the preparation from VIII of 5-amino-2-ethoxy-4-mercapto-6-N-methylanilinopyrimidine (XI), which could be cyclized further to the fully substituted thiazolo(5,4-*d*)pyrimidine (XII). Thus, replacement of the chlorine atom only at C_4 in VIII by a thiocyanato group was effected with potassium thiocyanate by using ethanol as a solvent. Treatment of IX with three moles of sodium ethoxide in ethanol under cooling gave 2-ethoxy-4-mercapto-5-nitro derivative (X), reduction of which with sodium hydrosulfite in an alkaline medium afforded 5-amino-2-ethoxy-4-mercapto-6-N-methylanilinopyrimidine (XI). Heating of XI with potassium methylxanthate in methanol resulted in ring closure to afford, in good yield, 5-ethoxy-2-mercapto-7-N-methylanilinothiazolo(5,4-*d*)pyrimidine (XII). Subsequent S-ethylation of XII gave 5-ethoxy-2-ethylthio-7-N-methylanilinothiazolo(5,4-*d*) pyrimidine (V).

On the other hand, 5,7-dihydroxy-2-mercaptothiazolo(5,4-*d*)pyrimidine (XIII) prepared by the same method as in the case of XII was S-ethylated with ethyl bromide to give 2-ethylthio derivative (XIV) which was converted to 5,7-dichloro-2-ethylthiothiazolo(5,4-*d*)-

6) R.M. Creswell and H.C.S. Wood, *J. Chem. Soc.*, **1960**, 4768.
7) T. Takahashi, T. Naito, and S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 334 (1958).

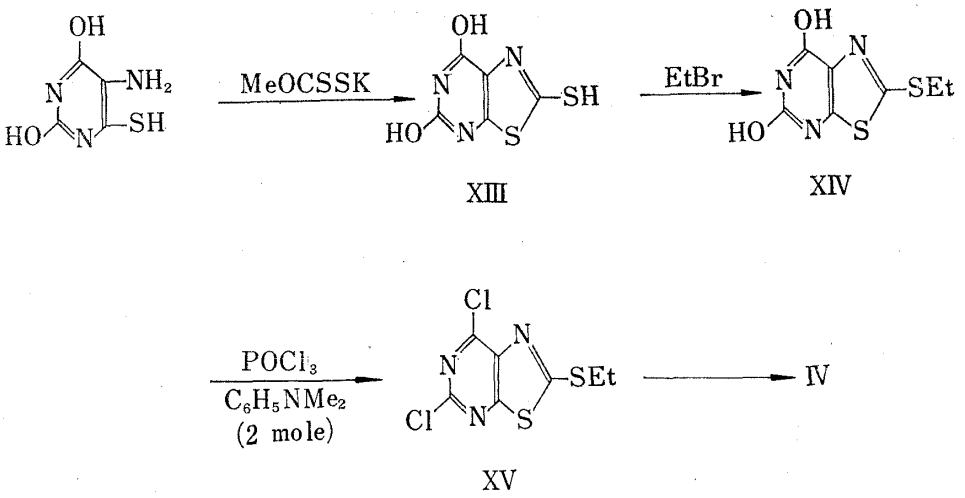


Chart 4

pyrimidine (XV) by chlorination with phosphorus oxychloride. The reaction product of XV with two moles of N–methylaniline was also found to be identical with IV obtained above.

It is interesting that reactivity of chlorine atoms in the thiazolo(5,4–*d*)pyrimidine nucleus towards nucleophilic reagents decreases in the order of C_7–C_2–C_5 as compared with the order of chemical shifts, H_2–H_5–H_7 and further studies of this problem are now in progress.

Experimental[8]

2,5,7-Trihydroxythiazolo(5,4-*d*)pyrimidine (I)——To a suspension of 2 g of thiouramil in 50 ml of dioxane was introduced $COCl_2$ gas with stirring for 15 min under heating. After the solvent was removed in vacuum, the residue was dissolved in dil. NH_4OH, decolorized with charcoal, filtered, and the filtrate was acidified with AcOH to yield 2 g of I. Recrystallization from a large volume of EtOH gave colorless crystalline solid, which did not melt above 300°. *Anal.* Calcd. for $C_5H_3O_3N_3S$: C, 32.43; H, 1.62; N, 22.70. Found: C, 32.63; H, 2.02; N, 23.02.

2,5,7-Trichlorothiazolo(5,4-*d*)pyrimidine (II)——A mixture of 2 g of I, 3.9 g of dimethylaniline and 20 ml of $POCl_3$ was refluxed for 3 hr. After removal of the excess $POCl_3$ in vacuum, the residue was poured on crushed ice and extracted with ether. The extract was washed with H_2O, dil. NaOH, and H_2O successively, and dried over Na_2SO_4. Evaporation of the solvent gave an oily product which was crystallized from petr. ether to colorless scales of II, mp 85°. Yield, 0.7 g. *Anal.* Calcd. for $C_5N_3Cl_3S$: N, 17.43. Found: N, 17.68.

2,5-Dichloro-7-N-methylanilinothiazolo(5,4-*d*)pyrimidine (III)——From I: A mixture of 1.85 g of I, 10.2 g of dimethylaniline and 40 ml of $POCl_3$ was refluxed for 4 hr. The further treatment was followed by the same way as described the above experiment. Recrystallization from EtOH gave 0.54 g of III as colorless needles, mp 133—134°. *Anal.* Calcd. for $C_{12}H_8N_4Cl_2S$: C, 46.30; H, 2.57; N, 18.01. Found: C, 45.86; H, 2.18; N, 18.00.

From II: To a solution of 1 g of II in 10 ml of EtOH was added 0.88 g of N–methylaniline and the mixture was refluxed for 20 min. The reaction mixture was evaporated to almost dryness, the residue was washed with dil. HCl and then H_2O. The product thus obtained was recrystallized from EtOH to give 1 g of colorless needles. This compound was identified as 2,5–dichloro–7–N–methylanilinothiazolo(5,4–*d*)-pyrimidine (III) by the mixed mp determination with the product obtained by the above experiment.

5-Chloro-2-ethylthio-7-N-methylanilinothiazolo(5,4-*d*)pyrimidine (IV)——From III: A mixture of EtSNa (prepared from 0.05 g of Na, 5 ml of EtOH and 0.14 g of EtSH) and 0.6 g of III in 20 ml of EtOH was refluxed for 45 min. After removal of the solvent, a small amount of H_2O was added to the reisdue. The crystals deposited were collected and recrystallized from MeOH to give IV as colorless needles, mp 137°. Yield, almost theoretical. *Anal.* Calcd. for $C_{14}H_{13}N_4ClS_2$: C, 49.85; H, 3.86; N, 16.62. Found: C, 50.02; H, 3.82; N, 16.69. IR ν_{max}^{KBr} cm^{-1}: 2960, 1600, 1553, 1500, 1448, 1330, 1014, 772, 700.

From XV: A solution of 1.3 g of XV and 1.1 g of N–methylaniline in 20 ml of EtOH was heated under reflux for 1.5 hr. After cooling, the solvent was removed and the resulting crystals were recrystallized from MeOH to afford colorless needles (1.25 g). The mixed mp and IR of the product were identical with those of a sample obtained from III.

8) All melting points are uncorrected.

5-Ethoxy-2-ethylthio-7-N-methylanilinothiazolo(5,4-*d*)pyrimidine (V)——From IV: A solution of 0.2 g of IV in 20 ml of EtOH containing 0.015 g of Na was refluxed for 15 min. Removal of the solvent left an oily residue, which was solidified upon addition of cold H_2O. Recrystallization from petr. ether provided the compound (V), colorless prisms, mp 96—98°. Yield, 0.2 g. *Anal.* Calcd. for $C_{16}H_{18}ON_4S_2$: C, 55.49; H, 5.20; N, 16.22. Found: C, 55.48; H, 5.07; N, 16.17. IR ν_{max}^{KBr} cm^{-1}: 2960, 1593, 1536, 1500, 1427, 1386, 1295, 1069, 1010, 771, 700.

From XII: XII (0.06 g) was dissolved in a solution of 0.012 g KOH in 2 ml of dil. MeOH and followed 0.02 g of EtBr. The reaction mixture was refluxed for one hour. Removal of the solvent left an oily residue, which solidified upon cooling. Recrystallization from petr. ether gave colorless prisms. This was proved to be identical with the compound (V) prepared from IV by mixed mp and comparison of the IR spectrum.

6-Chloro-2,4-dihydroxy-5-nitropyrimidine (VI)——Preparation of this product was followed by Wood's method.[6] mp *ca.* 227° (decomp.).

2,4-Dihydroxy-6-N-methylanilino-5-nitropyrimidine (VII)——To a hot solution of 22 g of VI in 500 ml of EtOH was added 20 g of N-methylaniline with stirring. Heating was continued for 15 min and the deposited crystals were recrystallized from AcOH to afford 20 g of yellow needles, mp 252—253° (decomp.). *Anal.* Calcd. for $C_{11}H_{10}O_4N_4$: C, 50.38; H, 3.82; N, 21.37. Found: C, 50.02; H, 3.99; N, 21.47.

2,4-Dichloro-6-N-methylanilino-5-nitropyrimidine (VIII)——A mixture of 10 g of VII, 10 ml of dimethylaniline and 50 ml of $POCl_3$ was refluxed for one hour. After removal of the excess of $POCl_3$ in vacuum, the residue was poured on crushed ice to separate the dark brown lump which was extracted with ether. The extract was washed with dil. NaOH, H_2O and dried over Na_2SO_4. Evaporation of the solvent afforded reddish brown crystals of VIII, which were recrystallized from petr. ether to give 9 g of yellow needles, mp 141—143°. *Anal.* Calcd. for $C_{11}H_8O_2N_4Cl_2$: N, 18.73. Found: N, 18.72.

2-Chloro-6-N-methylanilino-5-nitro-4-thiocyanatopyrimidine (IX)——A mixture of 8.6 g of VIII and 5 g of KSCN in 250 ml of EtOH was refluxed for 4 hr. After cooling, the deposited yellow crystals were collected by filtration and the filtrate was concentrated to almost dryness, and a small amount of H_2O was added to the residue. The crystals thus obtained were combined, washed with H_2O and MeOH, and dried. The crude product (7.1 g) of IX was recrystallized from AcOEt to yellow grains, mp *ca.* 200° (decomp.). *Anal.* Calcd. for $C_{12}H_8O_2N_5ClS$: C, 44.72; H, 2.48; N, 21.74. Found: C, 45.07; H, 2.71; N, 21.71.

2-Ethoxy-4-mercapto-6-N-methylanilino-5-nitropyrimidine (X)——To a stirred solution of 90 ml of EtOH containing 0.86 g of Na was added 3 g of IX at below 10°. The reaction mixture was warmed gradually to 60° during 30 min. After removal of the solvent, a small amount of H_2O was added to the residue and the separated oily by-product was extracted with ether. The by-product obtained from the ether extract was identified as 2,4-diethoxy-6-N-methylanilino-5-nitropyrimidine (mp 75—76° from EtOH, *Anal.* Calcd. for $C_{15}H_{18}O_4N_4$: C, 56.60; H, 5.66. Found: C, 56.78; H, 5.76), which was also prepared from VIII with two moles of EtONa. The aqueous layer was acidified with AcOH to give 2-ethoxy-4-mercapto-6-N-methylanilino-5-nitropyrimidine (X). Recrystallization from EtOH afforded yellow crystalline solid, mp 174—175° (decomp.). Yield, 0.85 g. *Anal.* Calcd. for $C_{13}H_{14}O_3N_4S$: C, 50.98; H, 4.58; N, 18.30. Found: C, 51.48; H, 4.44; N, 18.82.

5-Amino-2-ethoxy-4-mercapto-6-N-methylanilinopyrimidine (XI)——The 4-mercapto-5-nitro derivative (X) (0.8 g) was dissolved in 3 ml of 10% NaOH. To this solution was added $Na_2S_2O_4$ until the red color changed to yellow. Stirring was continued for a few minutes, cooled, and the separated crystals were collected (0.7 g). The product was decomposed around 163—166°. Since this compound underwent change during purification, it was used for the next experiment without purification.

5-Ethoxy-2-mercapto-7-N-methylanilinothiazolo(5,4-*d*)pyrimidine (XII)——A solution of CH_3OCSSK was prepared by adding 0.64 g of CS_2 to 0.65 g of KOH in 15 ml of MeOH, and 0.8 g of XI was added to this solution. The reaction mixture was refluxed for 20 hr, the solvent was then removed by distillation. A small amount of H_2O was added to the residue, treated with charcoal, filtered, and the filtrate was acidified with AcOH to give 0.8 g of the crude product of XII. Recrystallization from MeOH gave pale yellow needles, mp 166—168°. *Anal.* Calcd. for $C_{14}H_{14}ON_4S_2$: C, 52.83; H, 4.40; N, 17.61;. Found: C, 52.92; H, 4.12; N, 17.65.

5,7-Dihydroxy-2-mercaptothiazolo(5,4-*d*)pyrimidine (XIII)——This compound was prepared from 10 g of thiouramil by the same way as described above XII. Yellow crystalline solid from H_2O, mp above 300°. *Anal.* Calcd. for $C_5H_3O_2N_3S_2$: C, 29.85; H, 1.49; N, 20.90. Found: C, 30.07; H, 1.71; N, 20.50.

5,7-Dihydroxy-2-ethylthiothiazolo(5,4-*d*)pyrimidine (XIV)——To a solution of 1.45 g of XIII in 10 ml of EtOH containing 1.5 g of KOH was added 0.4 g of EtBr. After standing the reaction mixture for few hours, the deposited precipitate was collected by filtration. The crude precipitate was dissolved in hot H_2O, treated with charcoal, and acidified with AcOH to afford the 2-ethylthio derivative(XIV)as a pale yellow crystalline solid. Recrystallization from EtOH gave 1.6 g of pale yellow needles, mp 280° (decomp.). *Anal.* Calcd. for $C_7H_7O_2N_3S_2$: C, 36.68; H, 3.06; N, 18.34. Found: C, 36.84; H, 3.49; N, 17.92.

5,7-Dichloro-2-ethylthiothiazolo(5,4-*d*)pyrimidine (XV)——This compound was prepared from 3 g of XIV and 30 ml of $POCl_3$ in the presence of 3 ml of dimethylaniline by the same method as in the case of II

Colorless needles from petr. ether, mp 61°. *Anal.* Calcd. for $C_7H_5N_3Cl_2S_2$: C, 31.58; H, 1.88; N, 15.79. Found: C, 31.47; H, 1.73; N, 15.90.

7-Chlorothiazolo(5,4-*d*)pyrimidine (XVIII)——A suspension of 2 g of 5-amino-4-chloro-6-mercaptopyrimidine[9] in 20 ml of ethyl orthoformate was refluxed for 2 hr. After cooling, the deposited crystals were collected by filtration and concentration of the filtrate to almost dryness gave another crop of crystals which were combined to the above deposited crystals. Recrystallization from EtOH afforded colorless plates of XVIII, mp 156°. Yield, almost theoretical. *Anal.* Calcd. for $C_5H_2N_3ClS$: C, 34.99; H, 1.17. Found: C, 34.85; H, 1.35.

2,7-Dichlorothiazolo(5,4-*d*)pyrimidine (XX)——This compound was prepared from 1 g of 7-chloro-2-hydroxythiazolo(5,4-*d*)pyrimidine[10] and 5 ml of $POCl_3$ in the presence of 0.64 g of dimethylaniline by the same way as in the case of II. Recrystallization from benzene-hexane gave colorless needles, mp 100—101°. Yield, 1.1 g. *Anal.* Calcd. for $C_5NH_3Cl_2S$: C, 29.27; H, 0.49; N, 20.49. Found; C, 29.38; H, 0.36; N, 20.57.

Acknowledgement The authors wish to express their gratitude to Prof. T. Goto, Nagoya University, for his advice on NMR spectra. The authors gratefully acknowledge the technical assistance of Mr. K. Kato.

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Gas Chromatographic Separation of L-Idonate and D-Gluconate

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Catalytic hydrogenation of calcium D-xylo-5-hexulosonate (calcium 5-oxo-D-gluconate) by Raney nickel produces a mixture of two diastereoisomers calcium D-gluconate and calcium L-idonate.[2,3] The composition of the products has been determined so far by gravimetric,[4] polarimetric[5] or polarographic method.[6] We have found that the two diastereoisomers are completely separable by a gas chromatographic method when the sample is lactonized and trimethylsilylated. The method and result will be communicated here.

Experimental

A mixture of calcium L-idonate and D-gluconate was converted to acid by passage through Amberlite CG-120 (H^+) resin column and the sample eluted was lactonized after Morrison and Perry.[7] A conc. hydrochloric acid was added to the eluted solution adjusting its concentration to 2 N. The solution was then evaporated to dryness under a reduced pressure at 75—85°. The lactone sample was dissolved in 0.5 ml pyridine and trimethylsilylated by adding 0.2 ml hexamethyldisilazane and 0.1 ml trimethylchlorosilane followed by shaking for about 10 minutes at 75—85°. The sample was then evaporated to dryness and the

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