left 0.66 g. of ammonium tosylate. The ethanolic extract was further treated as procedure b) after the isolation of ammonium tosylate. There was obtained 0.06 g. of crude picrate, m.p. 204~205°(decomp.), which was separated to (XII) picrate, m.p. 222°(decomp.), and a small amount of the mixture, m.p. 207°(decomp.), consisting presumably of (XII) and (XVII) picrates.

d) In dry AcOH: Isolations of cyclohexanone (IX), *dl-cis*-2-benzoyloxycyclohexylamine tosylate (XII), benzoic acid, and ammonium tosylate: Two grams of (VII) and 10 cc. of dry AcOH were refluxed for 3 hrs. and a reddish brown solution was obtained. The solution was evaporated to dryness and from the distillate 0.12 g. of cyclohexanone was isolated as 2,4-dinitrophenylhydrazone. The residue was extracted with 10 cc. of water and the oily remainder, after decantation, was dissolved in ether. After washing with 3 cc. of water, the ether solution was shaken with a sat. aq. solution of $NaHCO_3$. $NaHCO_3$ solution was acidified with conc. HCl and benozoic acid precipitated. Yield, 0.04 g. after filtration and drying. The ether liquor, after evaporation, gave a brownish gummy product which had a fragrant odor. The aq. extract was combined with the washing water and evaporated to dryness. The residue was treated with a small amount of EtOH and the undissolved substance, after filtration and recrystallization, was proved as ammonium tosylate.

Further treatment of the ethanolic extract was made as procedure b) after the isolation of ammonium tosylate. There were obtained (XII) picrate, m.p. 222~223°(decomp.), and crude picrate, m.p. 170~175°, which failed to be identified because of poor quantity.

Summary

dl-cis-2-Benzoylaminocyclohexyl tosylate was submitted to solvolysis in four of the sets of conditions: (a) In absolute ethanol, (b) in 60% ethanol, (c) in water, and (d) in dry acetic acid. The reactions gave rise to cyclohexanone and *dl-cis*-2-benzoyloxycyclohexylamine tosylate commonly in a, b, c, and d, *dl-trans*-2-benzoyloxycylohexylamine tosylate in b and c, and *dl*-2-ethoxycyclohexylamine tosylate only in a. The reaction mechanisms were discussed. By adaptation of Winstein's and McCasland's procedures the *dl-cis*-N-benzoyl-O-tosylate was synthesized and showed m.p. 174~175°, higher than those reported by these authors. Besides, by McCasland's procedure, *dl-cis*-2-tosylaminocyclohexyl benzoate was formed.

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70. Torizo Takahashi, Kan-ichi Ueda, and Toshiro Ichimoto: Sulfur-containing Pyridine Derivatives. XLIV.* Synthesis of β,γ-Disubstituted Pyridine Derivatives and Mercaptopyrido[3,4 : 4′,5′]thiazoles.

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The synthesis of β,γ-disubstituted pyridine derivatives containing a sulfur atom was undertaken with a view to investigating their anticancer activities. This paper describes pyridine derivatives prepared from 3-nitro-4-mercaptopyridine (I). Their results on anticancer activity will be reported elsewehere.

In one of the preceding papers of this series,[1] it was reported that 3-nitro-4-allylthiopyridine was formed by the condensation of the potassium salt of (I) with allyl bromide. Because of its insolubility in water, however, this compound was converted into its water-soluble quaternary ammonium derivatives (II~IV) by treatment with alkyl halides such as methyl or ethyl iodide, and allyl bromide.

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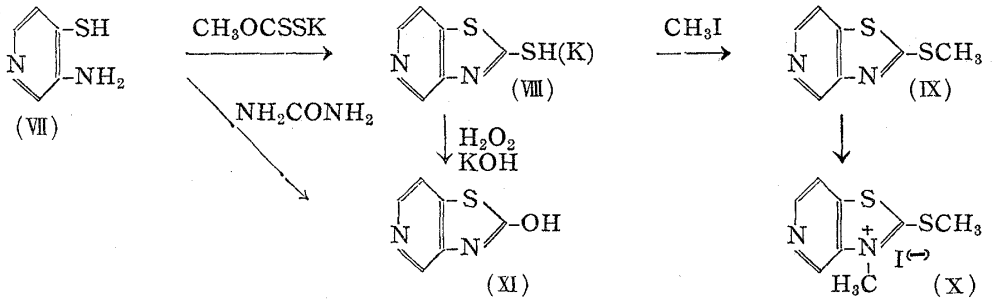
1) T. Takahashi, K. Ueda, T. Ichimoto: This Bulletin, **2**, 197(1954).

In view of the structural analogy with mustard gas, $S(CH_2-CH_2-Cl)_2$, which is a cell poison, 3-nitro-4-(β-chloroethylthio)pyridine hydrochloride (V) was prepared by the condensation of the potassium salt of (I) with ethylenechlorohydrin and by subsequent treatment of the hydrochloride of the condensate with thionyl chloride. (V), on reduction with stannous chloride and conc. hydrochloric acid, afforded 3-amino-4-(β-chloroethylthio)-pyridine (VI), which was isolated as the hydrochloride.

Since compounds possessing a mercapto group next to the amino group in aminopyridine are necessary not only as starting material for the synthesis of pyridothiazoles, but also are of much interest because of the anticancer action of its mercapto group, preparation of 3-amino-4-mercaptopyridine (VII) was undertaken. The reduction of (I) with stannous chloride was achieved in hydrochloric acid and led to (VII). This material, m.p. 213° (decomp.), soluble in aqueous alkali and precipitated by acids, could be recrystallized from water without change in the properties. This fact indicates that it is stable toward air oxidation as compared with 3-nitro-4-mercaptopyridine. On the other hand, 2′-aminopyrido[3,4 : 4′,5′]thiazole,[2] when allowed to react with 10% aqueous caustic alkali in the presence of arsenous oxide at boiling temperature, yielded a substance with its thiazole ring cleaved and this compound was shown to be identical with the reduction product of (I) by comparison of their properties and analytical data.

Recent communication[3] from this Laboratory included a preparation of 2-mercaptobenzothiazole derivatives, and since some of these were found to possess effective antitubercular activity, attempt was made to prepare 2′-mercaptopyridothiazole derivatives. In one[4] of this series, Yamamoto had carried out the synthesis of 2′-mercaptopyridothiazoles from 2-mercapto-3-aminopyridines using carbon disulfide or thiophosgene, and obtained 2′-mercapto-6-R-pyrido[2,3 : 5′,4′]thiazoles (R=H, Cl, or C_2H_5O). For the conversion of *o*-aminophenols into 2-mercaptobenzoxazoles, the literature cites three reagents, viz., carbon disulfide, thiophosgene, and potassium alkylxanthate.

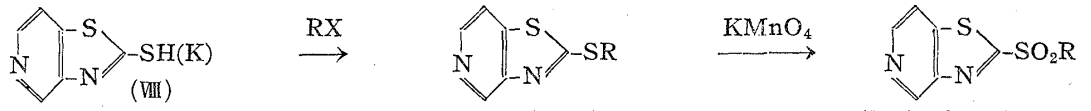
In 1954, Katz and Cohen[5] found the last reagent to be most successful although the reaction requires no less than 12~14 hours. The method used by Katz *et al.* was applied to 3-amino-4-mercaptopyridine and furnished 2′-mercaptopyrido[3,4 : 4′,5′]thiazole (VIII) in an almost quantitative yield. This material was obtained as a white crystalline powder which had no definite melting point but decomposed slowly at above 310°. It resisted various attempts at recrystallization because of its insolubility in ordinary solvents. Accordingly, the structural confirmation of this substance was provided by the following evidences, viz., the formation of the S-methylated ether and 2′-hydroxy compound.



On methylation with methyl iodide in alkaline medium, this substance gave the expected S-methyl ether (IX) whose analyses were acceptable. Treatment of (IX) with an excess of methyl iodide in ethanol afforded 2′-methylthiopyrido[3,4 : 4′,5′]thiazole

2) T. Takahashi, K. Ueda : This Bulletin, **2**, 35(1954).
3) T. Takahashi, J. Okada : J. Pharm. Soc. Japan, **71**, 902(1951).
4) Y. Yamamoto : *Ibid.*, **71**, 916(1951).
5) L. Katz, M. S. Cohen : J. Org. Chem., **19**, 758(1954).

monomethiodide (X). It seems questionable whether methyl iodide combined with nitrogen atom in the pyridine ring or that of the thiazole ring of the pyridothiazole molecule. In spite of the lack of direct evidence, it would be expected to have combined with the nitrogen atom of the thiazole ring by analogy with the cases of 2′-methylpyrido[2,3 : 5′,4′]thiazole monomethiodides and monoethiodides[6,7] forming trimethine cyanine dyes. Subsequently, Kitamura's reaction[8] was applied to the base and gave 2′-hydroxypyrido[3,4 : 4′,5′]thiazole (XI), which was found to be identical with the product obtained by the condensation of (VII) with urea.



(IX) R = CH_3- (XIII) R = C_3H_5- (XV) R = CH_3-
(XII) 〃 = C_2H_5- (XIV) 〃 = $C_6H_5CH_2$- (YVI) 〃 = $C_6H_5CH_2$-

Similarly, the reaction of the potassium salt of (VIII) with ethyl iodide, allyl bromide, and benzyl chloride gave the S-substituted derivatives (XII~XIV). Subsequently, methyl and benzyl pyrido[3,4 : 4′,5′]thiazolyl-2′-sulfones were prepared by the respective action of potassium permanganate on 2′-methylthio- and 2′-benzylthiopyrido[3,4 : 4′,5′]thiazole.

Several 2′-mercaptopyrido[3,4 : 4′,5′]thiazole derivatives described in this paper are now undergoing tests for antitubercular activity.

This work has been supported by a grant from the Ministry of Education to which we are greatly indebted.

Experimental[9]

3-Nitro-4-allylthiopyridine Methiodide (II)—A solution of 0.6 g. of 3-nitro-4-allylthiopyridine, 0.43 g. MeI, and 3 cc. EtOH in a sealed tube was heated in a boiling water bath. After 1 hr. of heating, separation of reddish purple crystals was observed. Heating was continued for a further 1 hr. and the solvent was distilled off. The residue was washed several times with ether and recrystallized from MeOH, yielding 0.63 g. of reddish orange scales, which decomposed at 182°. *Anal.* Calcd. for $C_9H_{11}O_2N_2IS$: N, 8.34. Found : N, 8.53.

3-Nitro-4-allylthiopyridine Ethiodide (III)—A mixture of 0.3 g. of 3-nitro-4-allylthiopyridine and 2 equimolar amounts of EtI in a sealed tube was heated in a boiling water bath for 3 hrs. After cooling, an oily substance, which soon solidified, was collected, washed with acetone, and purified by means of dilution of its MeOH solution with ether, yielding reddish scales, m.p. 180° (decomp.). Yield, 0.19 g. *Anal.* Calcd. for $C_{10}H_{13}O_2N_2IS$: N, 7.95. Found : N, 8.15.

3-Nitro-4-allylthiopyridine Allobromide (IV)—The reaction of 0.3 g. of 3-nitro-4-allylthiopyridine with C_3H_5Br was carried out in the same way as for (III). Recrystallization from MeOH–ether gave 0.2 g. of pale yellow needles, decomposing at 178°. *Anal.* Calcd. for $C_{11}H_{13}O_2N_2BrS$: N, 8.83. Found : N, 8.92.

3-Nitro-4-(β-chloroethylthio)pyridine Hydrochloride (V)—To 0.6 g. of (I) dissolved in aq. Na_2CO_3 solution (0.4 g. Na_2CO_3 in 15 cc. water), 0.3 g. of ethylenechlorohydrin was added, and the mixture was heated on a water bath for 1 hr. After cooling, yellow crystals were collected, washed with water, and dried. Recrystallization from $CHCl_3$ gave 0.4 g. of yellow crystals, m.p. 127~129° (presumably 3-nitro-4-(β-hydroxyethylthio)pyridine). This was converted to the chloro compound without being analysed. HCl was bubbled through a suspension of 0.7 g. of 3-nitro-

6) T. Takahashi, H. Goto : J. Pharm. Soc. Japan, **66 A**, 2(1946).
7) K. Satake : *Ibid.*, **72**, 46(1952).
8) R. Kitamura : *Ibid.*, 58, 29(1938). Kitamura's reaction is as follows : Organic sulfur compounds, having a double bond at the carbon atom adjacent to a sulfur atom, such as -N=C-SH, =NH-C=S, O=C-SH, -O-C=S, -C(SH)=S, S=C-SH, -N=C-S-C=N-, =N-C=S, and thioketo compounds >C=S, when allowed to react with alkaline hydrogen peroxide at room temperature, change to their corresponding oxygen compounds and their sulfur atoms undergo quantitative change to sulfuric acid, and differ from those sulfur compounds which possess thioether linkages or sulfur atoms inside the ring.
9) All melting points are uncorrected. The authors' thanks are due to the members of the Analysis Room of this Institute for the microanalyses.

4-(β-hydroxyethylthio)pyridine in 40 cc. $CHCl_3$ until saturation, after which the solvent was distilled off. To the residue was added 5 cc. of $SOCl_2$ and the mixture was heated under reflux for 30 min. The excess $SOCl_2$ was completely distilled off under a reduced pressure. The residue was dissolved in hot EtOH, decolorized with charcoal, and filtered. On cooling the filtrate, crystals separated out, which were recrystallized from EtOH to pale yellow scales, m.p. 198°(decomp.). Yield, 0.3 g. *Anal.* Calcd. for $C_7H_8O_2N_2Cl_2S$: N, 10.99. Found: N, 10.99.

3-Amino-4-(β-chloroethylthio)pyridine Hydrochloride (VI)—To a solution of 0.8 g. of $SnCl_2 \cdot 2H_2O$ in 5 cc. of conc. HCl was added 0.3 g. of (V) in small portions under stirring. After the initial exothermic reaction had subsided, the reaction mixture was heated on a boiling water bath for 30 mins. Upon cooling, the crystals which separated were collected by filtration, dissolved in 30 cc. of water, neutralized with solid Na_2CO_3, and extracted with ether. The ethereal extract was dried over Na_2SO_4, and the product was precipitated by passage of dry HCl gas through its ethereal solution. 0.2 g. of colorless plates, m.p. 172°(decomp.) were obtained. Recrystallization from MeOH caused little change in the m.p. *Anal.* Calcd. for $C_7H_{10}N_2Cl_2S$: C, 37.37; H, 4.45. Found: C, 37.70; H, 4.78.

3-Amino-4-mercaptopyridine (VII)—a) To a solution of 50 g. of $SnCl_2$ in 45 cc. of conc. HCl was added 5.5 g. of (I) in small portions, during 1 hr. of mechanical stirring. After continued stirring for several hrs., the deposited crystals were collected by filtration and the filtrate was evaporated to dryness under diminished pressure. The residue was combined with above crystals, dissolved in a large amount of water, and H_2S gas bubbled through the solution under occasional warming until no further precipitation occurred. The precipitate was removed by filtration while hot and washed several times with hot water. The filtrate and washings were combined, evaporated to dryness under diminished pressure, and the residue was extracted with aq. NH_3. Evaporation of NH_3 under diminished pressure and subsequent addition of a small amount of water gave pale yellow crystals, which were recrystallized from water to pale yellow granules, m.p. 213°(decomp.). Yield, 1.9 g. *Anal.* Calcd. for $C_5H_6N_2S$: 22.22. Found: N, 22.34.

b) A mixture of 0.5 g. of 2'-aminopyrido[3,4 : 4',5'] thiazole and 0.1 g. of As_2O_3 in 7 cc. of 10% NaOH was refluxed in an oil bath until evolution of NH_3 became barely evident. After cooling, the insoluble material which precipitated out was removed by filtration and the filtrate was adjusted to pH 6 with dil. HCl under cooling. After standing overnight, the pale yellow crystals obtained were recrystallized from water to 0.15 g. of (VII) whose analysis for nitrogen was acceptable.

2'-Mercaptopyrido[3,4 : 4',5']thiazole (VIII)—A solution of potassium methylxanthate was prepared by dissolving 0.3 g. of KOH in 2 cc. of H_2O and 10 cc. of MeOH and by subsequent addition of CS_2(0.32 g.) with shaking. To this solution, 0.5 g. of (VII) was added and the mixture was heated under reflux for 15 hrs. H_2S gas at first evolved rapidly. After being heated, the solution of K salt was decolorized with charcoal, filtered, and the clear filtrate was neutralized with AcOH. The product separated almost immediately as a white crystalline powder, m.p. >310°(decomp.), which weighed 0.6 g.

2'-Methylthiopyrido[3,4 : 4',5']thiazole (IX)—0.5 g. of (VIII) was dissolved in a solution of 0.2 g. of KOH in a small amount of water, 10 cc. MeOH and 0.45 g. CH_3I were added and the mixture was heated under reflux for 15 mins. Removal of the solvent left an oily product which solidified soon on addition of water. It was recrystallized from aq. MeOH to colorless pillars, m.p. 87°. Yield, 0.35 g. *Anal.* Calcd. for $C_7H_6N_2S_2$: C, 46.15; H, 3.30. Found: C, 45.82; H, 3.11.

2'-Methylthiopyrido[3,4 : 4',5']thiazole Monomethiodide (X)—A solution of 0.40 g. of (IX) and 1.0 g. CH_3I in 2 cc. of MeOH in a sealed tube was heated on a water bath for 1 hr. After the lapse of 5 mins., crystals began to separate out and soon the whole content solidified. The product (0.61 g.) was washed with ether and recrystallized twice from MeOH to pale yellow microneedles, m.p. 228°(decomp.). *Anal.* Calcd. for $C_8H_9N_2IS_2$: N, 8.64. Found: N, 8.79.

2'-Hydroxypyrido[3,4 : 4',5']thiazole (XI)—a) The conversion of (VIII) into (XI) was achieved by Kitamura's reaction as follows: To a solution of 0.20 g. of (VIII) in 3.5 cc. of *N* KOH solution was added 8 cc. of 30% H_2O_2, and, after being allowed to stand at room temperature for 3 hrs., the mixture was adjusted to pH 7. The product thus obtained was collected, washed with water, dried, and recrystallized from MeOH to colorless microneedles, m.p. 256°. Yield, 0.13 g. *Anal.* Calcd. for $C_6H_4ON_2S$: C, 47.37; H, 2.63. Found: C, 47.50; H, 2.73.

b) A mixture of 0.50 g. of (VIII) and 0.8 g. of urea was placed in a flask fitted with a gas outlet carrying a bubble tube. The flask was immersed in an oil bath at 150~160° until the evolution of gas ended (3~4 hrs.). The mixture was cooled and 3 cc. of water was added to dissolve unreacted urea. The insoluble material thereby precipitated was collected, dissolved in hot MeOH, and MeOH solution was treated with charcoal and filtered. Distillation of the solvent left 0.23 g. of colorless microneedles. After recrystallization from MeOH, this product

showed no m.p. depression on admixture with the above-mentioned sample.

2′-Ethylthiopyrido[3,4 : 4′,5′]thiazole Hydrochloride (XII)—To 0.1 g. of (VIII) dissolved in a solution of KOH in a small amount of water, 10 cc. EtOH and 1 g. of EtI were added and the mixture was heated under reflux for 30 mins. The solvent was removed by distillation and water added. The resulting oily substance was taken up in ether, the ether extract was washed with water, and dried over anhyd. Na_2SO_4. When dry HCl gas was introduced into the ethereal extract, an oily substance precipitated and soon solidified. The product (0.81 g.) was purified by dilution of its EtOH solution with ether, and formed white needles, m.p. 174°. *Anal.* Calcd. for $C_8H_9N_2ClS_2$: N, 12.04. Found: N, 12.09.

2′-Allylthiopyrido[3,4 : 4′,5′]thiazole Hydrochloride (XIII)—To a solution of 0.14 g. KOH in a small amount of water were added consecutively 0.43 g. of (VIII), 15 cc. EtOH, and 0.4 g. of allyl bromide. The treatment of the mixture was conducted in the same way as in the case of (XII). Recrystallization from MeOH-AcOEt mixture (1 : 5) gave 0.40 g. of pale yellow needles, m.p. 164°(decomp.). *Anal.* Calcd. for $C_9H_9N_2ClS_2$: C, 44.01; H, 3.67. Found: C, 43.97; H, 3.82.

2′-Benzylthiopyrido[3,4 : 4′,5′]thiazole (XIV)—To a solution of 0.4 g. KOH in a small amount of water, 1.0 g. of (VIII), 15 cc. EtOH, and 0.8 g. of benzyl chloride were added consecutively. The mixture was heated under reflux for 30 min. After cooling, the mixture was worked up as described in the case of (IX). Recrystallization from petr. ether yielded 1.2 g. of colorless needles, m.p. 91°. *Anal.* Calcd. for $C_{13}H_{10}N_2S_2$: C, 60.47; H, 3.88. Found: C, 60.73; H, 3.95.

Methyl Pyrido[3,4 : 4′,5′]thiazolyl-2′-sulfone (XV)—To a solution of 0.20 g. of (IX) in 5 cc. of glacial AcOH was added dropwise a sat. aq. solution of 0.25 g. $KMnO_4$ with stirring at room temperature. After continued stirring for several hrs., 30% H_2O_2 solution was added to the reaction mixture to dissolve the resulting precipitate of MnO_2. The clear solution thus obtained was evaporated to dryness under reduced pressure and addition of a small amount of water to the residue afforded the crude product (0.22 g.). It was recrystallized from MeOH to pale yellow plates, m.p. 152°. *Anal.* Calcd. for $C_7H_6O_2N_2S_2$: N, 13.08. Found: N, 13.00.

Benzyl Pyrido[3,4 : 4′,5′]thiazolyl-2′-sulfone (XVI)—To a solution of 0.50 g. of (XIV) in 30 cc. glacial AcOH, kept at 30~40°, was added dropwise a sat. aq. solution of $KMnO_4$ (0.56 g.) with stirring. After continued stirring for 7 hrs. at the above temperature, the mixture was treated in the same manner as in the case of (XV). Recrystallization of the crude product (0.42 g.) from MeOH yielded colorless needles, m.p. 164°. *Anal.* Calcd. for $C_{13}H_{10}O_2N_2S_2$: N, 9.66. Found: N, 9.42.

Summary

The preparations of β,γ-disubstituted sulfur-containing pyridine derivatives (group A) and 2′-mercaptopyrido[3,4 : 4′,5′]thiazole derivatives (group B) were undertaken in order to investigate the anticancer activity of group A and the antitubercular activity of group B.

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