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Yoshinori Kidani*1 and Kenji Ukai*2 : Syntheses of Sulfur-containing Phenazine Derivatives. Synthesis of α- and β-Mercaptophenazines.*3

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It was already reported[1] that oxine showed a fortifying action against bacteria by an addition of certain metallic ions. Yosioka, *et al.*[2] confirmed that the antibacterial activities of α-hydroxyphenazines were increased when either copper or iron ion was added to the culture media. One of the authors found that the fortifying action by an addition of metallic ions had a very close relation to the metal chelate formation and therefore the formation of metal chelates and stability in solution were reported,[3] suggesting the mode of action of metal chelate of α-hydroxyphenazine derivatives against bacteria. When an oxygen atom of the hydroxy group was replaced by a sulfur atom, that is, thiooxine prepared showed a remarkably stronger antibacterial activity[2] than oxine itself and it would be explained by the stronger stability of thiooxine metal chelate compound. Therefore, in the case of α-hydroxyphenazine derivatives, a similar phenomenon might be expected by replacing an oxygen atom with a sulfur atom.

As it is also well-known that pyocyanin[4] may form semi-quinone in living system, phenazine nucleus has a very significant role on the electron transfer mechanism in oxidation and reduction reaction in living system and moreover the reductive mercapto group has an important effect on the redox conditions prevailing cellular systems.

The mercapto group has an important contribution in enzymatic activity, at the same time, maintaining in the steric structure by the formation of disulfide bridge lincages.

In order to investigate the mode of action of phenazine metal chelate compounds, the synthesis of α-mercaptophenazine derivatives was attempted.

In regard to the syntheses of sulfur-containing phenazine derivatives, Maffei[5] attempted sulfonation of phenazine according to the usual method in oleum but it was unsuccessful at the beginning. He finally succeeded after many trials in obtaining 2-phenazine sulfonic acid, only when mercuric sulfate was used in oleum as a catalyst in a very poor yield. However, no sulfonation had been taken place at the α-position of phenazine ring, even if mercuric sulfate was added as a catalyst.

Cherenetskii[6] tried to react potassium hydrosulfide with 2-chlorophenazine in ethanol and obtained yellowish di(2-phenazyl) disulfide, m.p. 248~249°. When 2-chlorophenazine 10-oxide was treated similarly with potassium hydrosulfide, was afforded

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1) A. Albert, *et al.* : J. Biochem., **41**, 534 (1947); Brit. J. Exptl., **28**, 69 (1947); *Ibid.*, **31**, 4125 (1950).
2) I. Yosioka, *et al.* : Yakugaku Zasshi, **78**, 351, 353 (1958).
3) Y. Kidani : This Bulletin, **6**, 556, 563 (1958).
4) L. Michaelis : J. Biol. Chem., **92**, 211 (1931). L. Michaelis, E.S. Hill, M.P. Schubert : Biochem. Z., **255**, 66 (1932). L. Michaelis : Chem. Reviews, **16**, 243 (1935).
5) Silvio Maffei : Gazz. chim. ital., **80**, 651 (1950).
6) V.P. Cherenetskii : Zhur. Obshchei Kim., **27**, 2833 (1957).

yellowish 2-mercaptodihydrophenazine, m.p. 195~200°, which was soon air-oxidized to di(2-phenazyl) disulfide, through greenish phenazhydrine derivatives. However, no α-mercaptophenazine had been prepared, having the chelating functional group.

The authors were this time successful in obtaining newly chelatable α-mercaptophenazine derivatives in a very good yield and the synthesis of both α- and β-mercaptophenazine derivatives was dealt in this paper.

Following to the synthetic method by Tischler[7] and Furst,[8] 1-chlorophenazine afforded stable greenish yellow 1-phenazine isothiuronium hydrochloride, m.p. 208°, even when it may be reacted in either ethanol or acetone,[9] though there was a difference in the reaction period of time. Various methods of the alkali decomposition to mercapto derivatives were attempted. At first, according to the method reported by Furst, *et al.*, it was carried out in ethanolic alkaline solution, but no reaction was proceeded, simply recovering the starting material. Therefore, secondly sodium amide or metal-sodium was reacted respectively in absolute ethanol and the reaction mixture was evaporated to dryness. After the acidification of the water solution, lemon yellow precipitates were deposited, and either collected by filtration or extracted with ether, but it turned itself into greenish phenazhydrine derivatives immediately and finally brownish yellow disulfide, m.p. 274~276°(decomp.). Therefore, the authors obtained mercaptophenazines in potassium salt form and the decomposition was attempted with potassium hydroxide in ethanol. The initial yellow coloration turned reddish and finally violet blue for about three hours' reflux on a water bath. The coloration indicates a completion of the reaction.

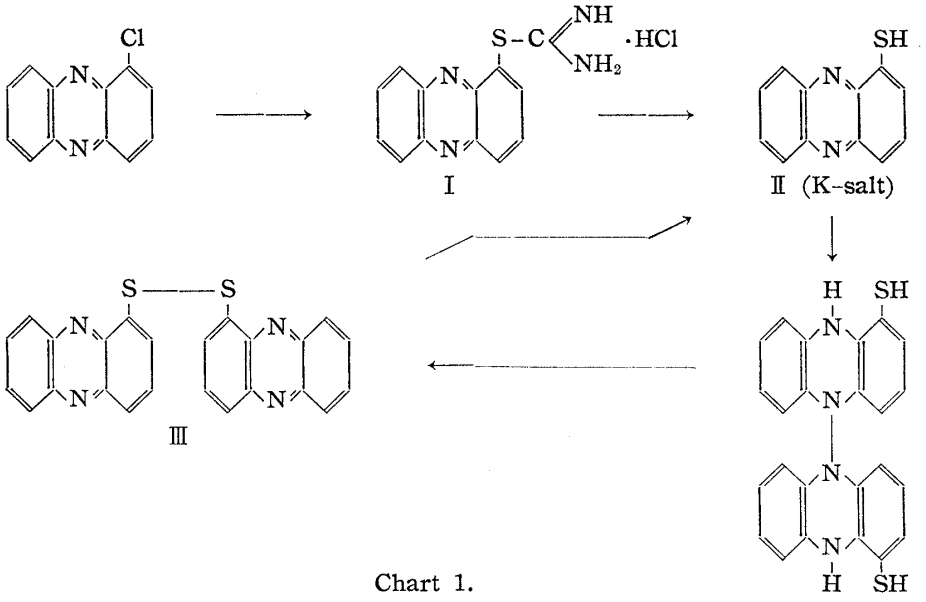

Chart 1.

After an evaporation of ethanol, benzene was added to remove unreacted materials and reddish violet needles were collected by filtration. The potassium salt of 1-mercaptophenazine, m.p. >300°, is very hygroscopic, easily soluble in water in red (λ_{max}=510 mμ) and pretty soluble in ethanol in violet coloration (λ_{max}=560 mμ). It is positive to the iodine-azide reaction.[10] The potassium salt of 1-mercaptophenazine was confirmed by the syntheses of mercaptomethyl and 2,4-dinitrophenyl ether derivatives, as an elemental analysis of the potassium salt being impossible.

7) W. Wilson, Jr., Tischler : J. Am. Chem. Soc., **76**, 2266 (1954).
8) D.C. Morrison, A. Furst : J. Org. Chem., **21**, 470 (1956).
9) D.G. Markees : J. Org. Chem., **28**, 2530 (1963).
10) F. Feigl : Z. anal. Chem., **74**, 369 (1928).

On the other hand, 2-chlorophenazine afforded very unstable 2-phenazine isothiuronium hydrochloride, m.p. 157~158°, when it was reacted in acetone. When similar reaction was carried out in ethanol, precipitates were deposited in the clear reaction solution, after about three hours' reflux, producing unexpected thioether, m.p. 263°. The unstable 2-phenazine isothiuronium hydrochloride was simply evaporated to dryness *in vacuo* without further purification and then subjected to the alkali decomposition. The similar treatment with potassium hydroxide gave the potassium salt of 2-mercaptophenazine, very hygroscoptic, orange needles. It is very soluble in water in orange red and soluble in ethanol in red coloration. It is also easily air-oxidized to disulfide, m.p. 248~249°, through greenish phenazhydrine derivative.

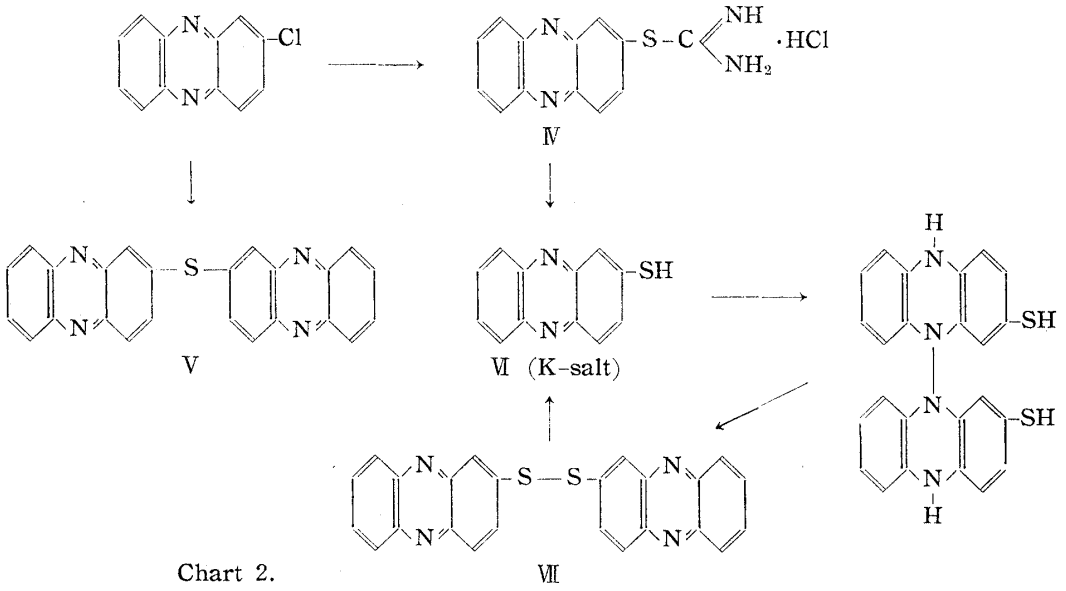

Chart 2.

2-Mercaptophenazine has been also confirmed by the mercaptomethyl and 2,4-dinitrophenyl ether derivatives. The potassium salts are so hygroscopic that the elemental analysis did not afford satisfactory data. Both α- and β-disulfides were reduced easily to their potassium salts respectively, by refluxing with potassium hydrosulfide in ethanol.

Experimental*4

1-Phenazine Isothiuronium Hydrochloride (I)——i) One gram of 1-chlorophenazine and 2 g. of thiourea were refluxed for about five hours in 30 ml. of EtOH on a water bath. The reaction mixture afforded greenish yellow needles, recrystallized from acetone-H_2O, m.p. 208°. *Anal.* Calcd. for $C_{13}H_{10}N_4S \cdot HCl$: C, 53.70; H, 3.81; N, 19.29. Found: C, 53.35; H, 3.68; N, 19.07.

ii) A mixture of 1 g. of 1-chlorophenazine and 2 g. of thioure was reacted in EtOH for 16 hr. Similar treatment gave greenish yellow needles, m.p. 208°. No melting point depression was observed by an admixture of both compounds.

1-Mercaptophenazine Potassium Salt (II)——A half gram of I was dissolved in 30 ml. of EtOH and to this was added 1 g. of KOH pellets. It was refluxed for 3 hr. The initial yellow coloration turned violet to blue, which indicated a completion of the reaction. EtOH was evaporated to dryness *in vacuo* and benzene was added to remove unreacted materials and filtered. The K-salt is very hygroscopic, reddish violet needles, m.p. >300°. It is very soluble in water in red, $\lambda_{max}=510$ mμ and easily soluble in EtOH in violet, $\lambda_{max}=560$ mμ. It shows a positive iodine-azide reaction.

1-Mercaptomethylphenazine, yellow needles, m.p. 172~173°, recrystallized from hydrated EtOH. *Anal.* Calcd. for $C_{13}H_{10}N_2S$: C, 69.02; H, 4.42; N, 12.38. Found: C, 68.80; H, 4.78; N, 12.15.

*4 All melting points were uncorrected.

1-Mercaptophenazine 2,4-dinitrophenyl ether, yellow leaflet crystals, m.p. 247~248°, recrystallized from MeOH. *Anal.* Calcd. for $C_{18}H_{10}O_4N_4S$: C, 57.14; H, 2.70; N, 14.81. Found : C, 57.37; H, 2.79; N, 14.52.

Di(1-phenazyl)disulfide (III)——II was dissolved in either EtOH or water, and it was acidified under nitrogen stream. Lemon yellow precipitates were deposited, melted nearly at 113°, which turned greenish dimeric phenazhydrine derivatives, melted at around 178°. It finally turned to di(1-phenazyl) disulfide, yellow needles, m.p. 274°(decomp.), recrystallized from EtOH. *Anal.* Calcd. for $C_{24}H_{14}N_4S_2$: C, 68.24; H, 3.31; N, 13.26. Found : C, 67.83; H, 3.49; N, 12.83.

Reduction of the Disulfide to Potassium Salt——III was dissolved in EtOH, to this was added KSH and refluxed for 1 hr. Evaporation of EtOH *in vacuo* gave II.

Copper Chelate of 1-Mercaptophenazine[11]——Water solution of II was acidified with acetic acid and $CuSO_4$ solution was added. Greenish yellow precipitates were deposited. They were collected and washed with water, EtOH and benzene, successively, affording yellowish green chelate, m.p. 248°(decomp.).

2-Phenazine Isothiuronium Hydrochloride (IV)——A mixture of 1 g. of 2-chlorophenazine and 2 g. of thiourea was refluxed for about 3 hr. in 30 ml. of acetone. The reaction mixture afforded yellow needles, m.p. 157~158°, unstable and not recrystallized. For further syntheses, the reaction mixture was simply evaporated to dryness *in vacuo*.

Di(2-phenazyl)sulfide (V)——One gram of 2-chlorophenazine and 2 g. of thiourea were refluxed for about 3 hr. in 30 ml. of EtOH. Then the initial yellow clear solution produced yellow precipitates. Separation of the precipitates gave yellow powder, m.p. 263°(decomp.). This is hardly soluble in organic solvents. *Anal.* Calcd. for $(C_{12}H_7N_2)_2S$: C, 71.79; H, 3.58; N, 14.35. Found : C, 72.67; H, 3.75; N, 14.27.

2-Mercaptophenazine Potassium Salt (VI)——The simply evaporated IV was warmed with KOH in EtOH and treated quite similarly to the case of II. Orange red needles, >300° were afforded, being very hygroscopic and very soluble in water in orange and easily soluble in EtOH in red. It is positive to the iodine-azide reaction.

2-Mercaptomethylphenazine, yellow needles, m.p. 159.5~160°, recrystallized from hydrated EtOH. *Anal.* Calcd. for $C_{13}H_{10}N_2S$: C, 69.02; H, 4.42; N, 12.38. Found : C, 69.43; H, 4.68; N, 12.27.

2-Mercaptophenazine 2,4-dinitrophenyl ether, yellow leaflets, recrystallized from MeOH, m.p. 211°. *Anal.* Calcd. for $C_{18}H_{10}O_4N_4S$: C, 57.14; H, 2.70; N, 14.81. Found : C, 57.04; H, 2.88; N, 14.80.

Di(2-phenazyl)disulfide (VII)——By the air-oxidation, it gave greenish dimeric phenazhydine, m.p. 160~165° and finally yellowish disulfide, m.p. 248~250°(decomp.).

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Summary

Syntheses of 1- and 2-mercaptophenazine derivatives were carried out by reacting chlorophenazine derivatives with thiourea and its potassium salts were confirmed as their mercaptomethyl and 2,4-dinitrophenyl ether derivatives.

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11) Y. Kidani : This paper was presented at the 14th Symposium on Coördination Chemistry at Fukuoka, Nov., 1964.