Summary

Isoniazid (INAH) was studied on the formation of mixed complexes with chlorotetracycline(CT)-metal chelates with Th^{4+}, Fe^{3+}, Cu^{2+}, and Co^{2+} in acid medium. INAH-metal compounds were also studied. INAH-Cu(II) chelate, and INAH-Co(II) complex and its chelate were isolated as crystalline powder showing 1:1, 2:1, and 1:1 ratio of INAH to metal, respectively, from hydrous methanol. Molar ratio of the compounds in aqueous solution was measured by the continuous variation method. Iron(III) ion decomposed INAH to isonicotinic acid, but the mixed complex of CT-Fe(III) chelate with INAH was formed without decomposition. The mixed complex of CT-Cu(II) with INAH gave different compositions in accordance with the acidity of the preparation. CT-Co(II) chelate formed a mixed complex, but not CT-Th(IV) chelate. The molar ratio of the mixed complexes was mostly 1:1:1 (CT:metal:INAH). These mixed complexes were isolated in crystalline powder form and were soluble in methanol and hot water. Remarkable changes in color due to the formation of a mixed complex were not observed. Reaction between INAH-metal and CT-HCl was also studied.

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38. **Kiyomi Taguchi**: The Mixed Complex of Tetracycline-Metal Chelates. IV.[*2] Chlorotetracycline-Copper(II) Chelate with 4,4′-Bis(dimethylamino)-thiobenzophenone (Thio-Michler's Ketone).

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In the previous papers, mixed complexes of chlorotetracycline (CT)-metal chelates with penicillin G or isoniazid were reported. In the present work, thio-Michler's ketone (TMK) having a thione group as a complex functional group different from the ligands of the former was studied concerning the formation of mixed complexes with CT-Cu(II) chelate.

TMK-Cu(II) complexes were also investigated. TMK is a specific reagent for detection of copper, mercury, and precious metals.[1)]

TMK-Cu Complex—TMK produced a reddish purple color with copper(II) nitrate in acetone-methanol medium, showing a maximum absorption at 500 mμ (Fig. 2-Ⅲ). TMK produced, however, an indigo blue color with copper(II) chloride, and the absorption maxima moved to 530 and 645 mμ (Fig. 2-Ⅳ). This color change was not observed with halogen ion or copper(I) chloride.

The continuous variation method[2)] was applied for the determination of molar ratio of TMK to copper(II) nitrate in acetone. The result shown in Fig. 3 indicates a 2:1 ratio of TMK to copper(II).

TMK-Cu(II) (dusky brown) was prepared as a perchlorate by addition of sodium perchlorate solution to the solution of TMK and copper(II) nitrate in acetone-methanol.

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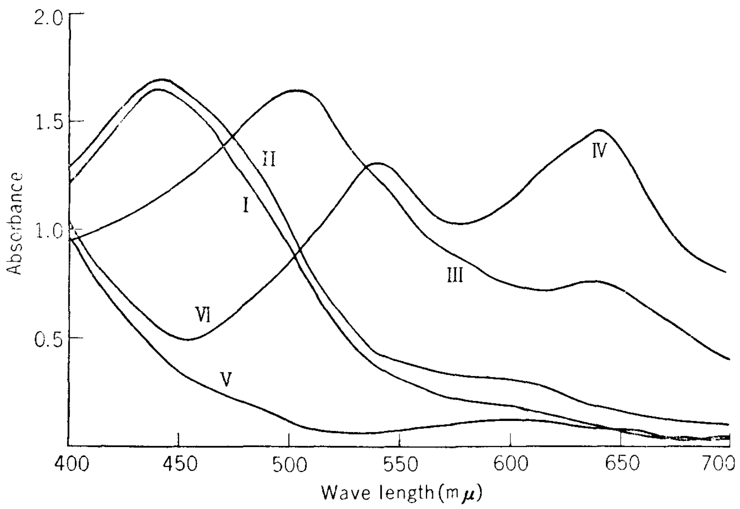


Fig. 1. Absorption Spectra of TMK and TMK-Cu(II) Complex

Concentration of TMK, $0.8\times10^{-4}M$ Solvent, $Me_2CO+EtOH$ (2:1)
I : TMK II : TMK+2*N* HNO_3 (1 drop/10 cc.) III : TMK+$Cu(NO_3)_2$ (1:1)
IV : TMK+$CuCl_2$(1:1) V : TMK+2*N* HCl (1 drop/10 cc.)

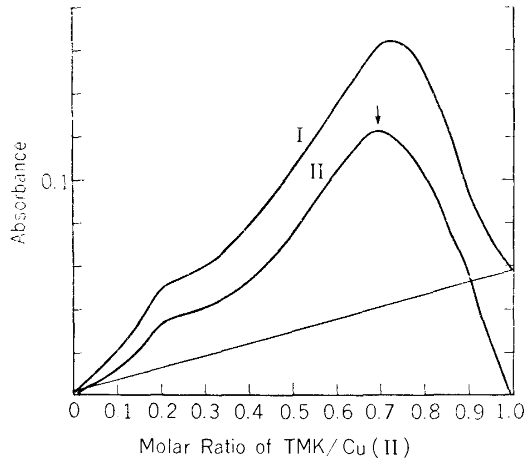
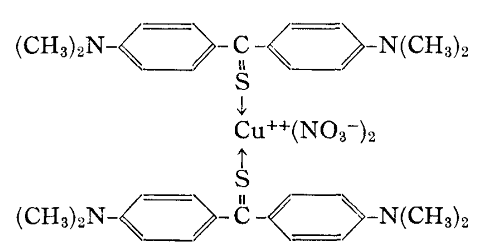


Fig. 2. Continuous Variation Study for Determination of Molar Ratio in TMK-Copper(II) Complex (measured 24 hr. after mixing)

Wave length, 530 mμ
Concentration of TMK+Cu(II), $10^{-4}M$ (in Me_2CO)
I Absorbance of TMK-Cu complex
II Absorbance related to TMK-Cu complex (Δ)
$Cu(NO_3)_2$ used for Cu^{2+}

A complex having indigo blue color was isolated by the reaction of TMK with copper(II) chloride. Content of copper in TMK-Cu(II) corresponds to a 2:1 ratio of TMK to copper similar to the result of the continuous variation method, so that TMK-Cu(II) may have a structure as illustrated. The substance isolated by reaction of TMK with copper(II) chloride contained 4.19% of copper and was considered to be a complex. The molar ratio, however, could not be determined because TMK changed to an unknown substance having indigo blue color. In the presence of halogens TMK was liable to be oxidized to a substance1) having similar color with TMK-$CuCl_2$ complex. Phenothiazine showed such a reaction and formed a complex with copper(II) chloride



Thio-Michler's Ketone-Cu(II) Complex

followed by discoloration as observed with the TMK-$CuCl_2$ complex.[3] Reduction of copper ion was reported in the reaction with thiourea[4] or ethylenethiourea[5] forming Cu(I)-thiourea or Cu(I)-ethylenethiourea. From these facts, it is considered that the formation of the indigo blue complex occurred through a reaction between copper(II) ion and oxidized TMK (indigo blue color) which were produced by the reduction of copper (II) chloride with TMK. Michler's ketone which has no thione group did not form a complex with copper(II) and copper(I) ions, and did not show any reaction with copper(II) chloride in acid medium. The color of TMK-Cu(II) prepared from copper(II) nitrate and TMK changed to indigo blue by the addition of chloride ion. This indicates that copper in the complex is in the copper(II) state, and the copper(II) ion will not be reduced by complexing with TMK in the absence of chloride ion.

Mixed Complex—The color of the solution of CT-Cu(II) chelate in methanol changed to reddish yellow by addition of TMK. The color varied from reddish yellow to indigo

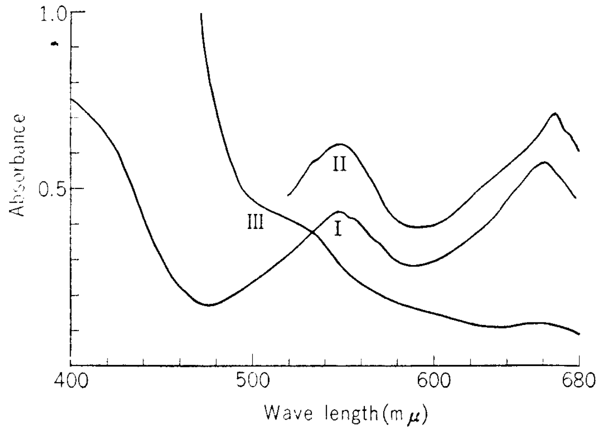


Fig. 3. Absorption Spectra of TMK-Cu(II)-CT Mixed Complex (in EtOH)

I Isolated sample
II Mixture of TMK+CT·HCl+$Cu(NO_3)_2$ (1:1:1) (indigo blue)
III Isolated sample using CT·HNO_3

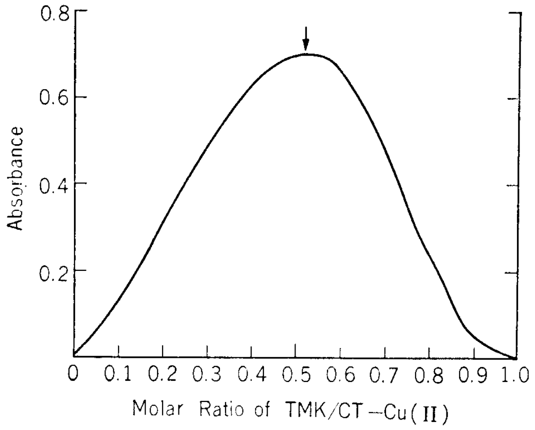


Fig. 4. Continuous Variation Study for Determination of TMK-(CT-Cu(II)) Ratio in Mixed Complex (measured 48 hr. after mixing)

Wave length, 650 mμ
Concentration of TMK+CT-Cu(II), $10^{-3}M$ (in Me_2CO:MeOH=1:1)
The mixed solutions were kept in a refrigerator.
CT·HCl and $Cu(NO_3)_2$ were used.

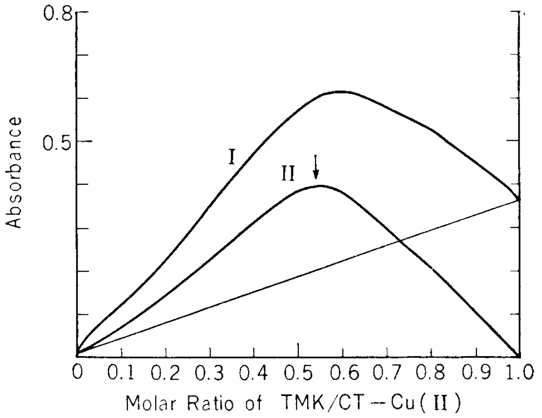


Fig. 5. Continuous Variation Study for Determination of TMK-(CT-Cu(II)) Ratio in Mixed Complex (measured 30 min. after mixing)

Wave length, 500 mμ
Concentration of TMK+CT-Cu(II) : $0.25\times10^{-3}M$ (in Me_2CO:EtOH=1:2).
CT·HNO_3 and $Cu(NO_3)_2$ were used.
I Absorbance of TMK+TMK-Cu-CT complex
II Absorbance related to TMK-Cu-CT complex

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blue in the presence of chloride ion as observed in the reaction of TMK and copper(II) chloride. This discoloration is specific to copper(II) chelate of tetracycline derivatives. With other copper(II) complexes, i.e. isoniazid–Cu(II), sulfadiazine–Cu(II), benzoylacetone–Cu(II), alizarine–Cu(II), and oxine–Cu(II), discoloration was not observed.

The mixture of CT–Cu(II) chelate with TMK in acetone–methanol medium had an absorption maxima at 515 mμ in the absence of chloride ion, and at 530 and 645 mμ in the presence of chloride ion (Fig. 4). Molar ratio between CT–Cu(II) chelate and TMK was determined by the continuous variation method[2] in both presence and absence of chloride ion (Figs. 5 and 6). Results showed a 1:1 ratio of CT–Cu(II) to TMK, which had a 1:1:1 ratio (CT:Cu:TMK). The measurement in the presence of chloride ion was carried out after keeping the mixture of CT–Cu(II) and TMK for 48 hours in a refrigerator for stabilization. The mixed complexes were prepared by the addition of ether to the solution of the components in acetone–methanol, and were purified by reprecipitation and washing with ether. The mixed complexes were soluble in methanol and ethanol, slightly soluble in acetone, and insoluble in ether and chloroform.

Results of assay of CT and copper indicated the same ratio as in solution, that is, the molar ratio of CT–Cu–TMK was 1:1:1.

Identification of Formation of the Mixed Complex—CT·HCl and $CT\cdot HNO_3$ did not form a compound with TMK and were separated from the mixture of CT salts and TMK by isolation and purification.

Paper chromatography was applied for the identification of the mixed complexes using benzene–butanol (1:2) as a developer. TMK and TMK–Cu complexes moved to the front of the developer, and CT·HCl, $CT\cdot HNO_3$, and CT–Cu(II) chelate remained at the starting point. The mixed complexes also remained at the starting point. The mixed complex will be separated by developing if the mixed complex isolated is a mixture. Separation, however, was not observed. The fact indicates that the mixed complexes isolated were not a mixture, but a new complex compound.

Experimental

Reagents—Thio-Michler's ketone was prepared from auramine by treating H_2S,[6] and freshly recrystallized substance was used for all experiments, m.p. 198~202°. (decomp.) $CT\cdot HNO_3$ was prepared from CT·HCl (Japan-Lederle Co. Ltd.) by treating with KNO_3 in HNO_3 (pH 2~3).[7] Analytical grades of $Cu(NO_3)_2\cdot 3H_2O$, $CuCl_2\cdot 2H_2O$, and purified solvents were used.

Analyses of Compounds—Assay of CT was carried out by BuOH-$Th(NO_3)_4$ extraction method[8] after dissolving 20~30 mg. of the sample into 100 cc. of water with the aid of 5% HCl and MeOH. As TMK was distributed to $Th(NO_3)_4$ solution, which was used for the final extraction, and hindered the measurement at 400 mμ, it was completely removed by extraction with 2 cc. of $CHCl_3$.

Determination of Cu was made by ashing to CuO at 800° in an electric furnace. Water of hydration was measured by vacuum drying at 90° over P_2O_5. NO_3^-, Cl^- and S were detected with phenylenediamine in H_2SO_4, $AgNO_3$ and $Pb(AcO)_2$ after decomposing with metallic Na, respectively.

Preparation of Complexes—CT-Cu(II) chelate was prepared by the method developed by the author and colleagues.[9]

TMK-Cu(II) Complex—A solution of 1 g. of $Cu(NO_3)_2$ in 4 cc. of water was added to a solution of 1 g. of TMK in 3 cc. of Me_2CO. A reddish purple syrupy solution was obtained and filtered. Three cc. of aqueous solution (20%) of $NaClO_4$ adjusted to about pH 4 with $HClO_4$, was added to the filtrate. The solution was then poured into 100 cc. of water. A dark purple precipitate was obtained by centrifugation. The precipitate was dissolved in 15 cc. of Me_2CO after washing with water. A little $NaClO_4$ solution was added to the solution and then the complex was reprecipitated by addition of water. The product was dried over silica gel. ClO_4^- was positive and NO_3^- was found to be slightly positive. *Anal.* Calcd. for $(TMK\cdot 2HClO_4)_2\text{-}Cu(ClO_4)_2\cdot H_2O$: Cu, 5.19; H_2O, 1.47.

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Found : Cu, 5.00; H_2O, 1.85.

TMK-Cu Complex (indigo blue) : A solution of 0.3 g. of $CuCl_2$ in 10 cc. of EtOH was mixed with a solution of 0.5 g. of TMK in 20 cc. of Me_2CO. The color changed instantly to indigo blue. Five cc. of $NaClO_4$ solution (20%) was added to the solution after keeping 1 hr. in a refrigerator and the mixture was poured into 50 cc. of water. Dark blue precipitate was collected on a sintered glass filter. The precipitate was dissolved in 30 cc. of Me_2CO. The Me_2CO solution was filtered, and mixed with 5 cc. of $NaClO_4$ solution and 70 cc. of water for reprecipitation. The precipitate was collected and washed with water until tests for Na^+ were negative with a solution of Zn-uranyl acetate. The product was dried over conc. H_2SO_4 in reduced pressure. S was positive. *Anal.* Found : Cu, 4.19; H_2O, 2.56.

TMK-Cu-CT mixed complex from $CT \cdot HNO_3$: 0.2 g. of $CT \cdot HNO_3$ and 0.1 g. of $Cu(NO_3)_2$ were dissolved in 10 cc. of EtOH. CT-Cu(II) chelate was precipitated by addition of 30 cc. of Et_2O and washed with Et_2O. The chelate was dissolved in 10 cc. of EtOH and mixed with 0.15 g. of TMK in 10 cc. of Me_2CO. A reddish brown solution of the mixed complex was obtained and filtered. Seventy cc. of Et_2O was added to the filtrate and the mixture was stored in an ice bath for 1 hr. A dark brown precipitate was collected on a sintered glass filter and washed with Et_2O. Reprecipitation was carried out by addition of Et_2O to the solution of the mixed complex in 10 cc. of EtOH. The product was dried over silica gel. S and NO_3^- were positive. *Anal.* Calcd. for $TMK\text{-}Cu(NO_3)\text{-}CT \cdot HNO_3 \cdot 4H_2O$: CT, 47.5; Cu, 6.29; H_2O, 7.12. Found : CT, 56.5; Cu, 6.29; H_2O, 7.12.

TMK-Cu-CT from $CT \cdot HCl$ (indigo blue color) : The solution of CT-Cu(II) chelate prepared from 0.2 g. of CT-HCl and 0.2 g. of $Cu(NO_3)_2$ in 5 cc. of MeOH was added to the solution of 0.2 g. of TMK in 10 cc. of Me_2CO. The solution was left to stand for about 10 min. The color changed from reddish brown to indigo blue. An indigo blue precipitate was obtained by the addition of 50 cc. of Et_2O and collected on a sintered glass filter. The product was dissolved into 5 cc. of MeOH, washed with Et_2O, and dried over conc. H_2SO_4. S was positive, and NO_3^- and Cl^- were slightly positive. *Anal.* Found : CT, 59.7; Cu, 7.71; H_2O, 3.45.

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Summary

The mixed complex of CT-Cu(II) chelate with thio-Michler's ketone (TMK) and its copper complex were studied concerning the properties and molar ratios in acid medium. Molar ratios of the complexes were measured by the continuous variation method. TMK formed a complex with copper(II) nitrate in a 2:1 ratio of TMK to copper, and the mixed complex had a 1:1 ratio of CT-Cu(II) to TMK, that is 1:1:1 (CT:Cu:TMK) ratio.

TMK is oxidized to an indigo blue substance by halogen. TMK was not changed to the indigo blue substance by chloride ion, but it was changed to the indigo blue substance by copper(II) ion in the presence of chloride ion. This phenomenon was caused by the reaction of TMK with chlorine which is produced from the reduction of copper(II) chloride by the formation of a complex. The indigo blue complex was isolated by reaction of TMK with copper(II) chloride. The complex of TMK with copper(II) nitrate was a reddish brown powder.

The mixed complex was prepared by the addition of ether to the solution of CT-Cu(II) chelate and TMK in acetone-methanol. The mixed complex prepared in the presence of chloride ion was an indigo blue substance. Color of the mixed complex was similar to that of TMK-Cu complex.

The formation of the mixed complex was identified by paper chromatography using benzene-butanol as a developer.

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