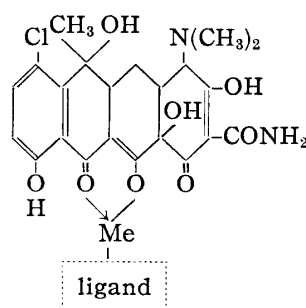


36. Kiyomi Taguchi : The Mixed Complex of Tetracycline-Metal Chelates. II.*²
Chlorotetracycline-Metal Chelates with Penicillin G.

(Pharmaceutical Faculty, University of Chiba*¹)

Tetracycline derivatives behave as bidentate chelating agents similar to alizarine toward metal ion, including Th^{4+} , Zr^{4+} , Al^{3+} , Fe^{3+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Mn^{2+} , and UO_2^{2+} .¹⁾ The metal chelates are cationic chelates consisting mainly of 1:1 ratio of tetracycline derivatives to metal in an isolated state and in an aqueous solution.²⁾

The normal chelates are not formed probably due to steric hindrances. Metals in chelates have remaining valencies and have a space to coordinate with other ligands, forming mixed complexes. Though there are many ligands for formation of mixed



Mixed Complex of CT-Metal Chelate

complexes with tetracycline-metal chelates, antibiotics and chemical therapeutics, i.e. penicillin G, isoniazid, sulfanilamide derivatives, and dihydrostreptomycin, which form metal complexes having an interesting bactericidal action, were chosen.

When studying the assay of tetracycline derivatives by means of chelate formation with thorium,³⁾ acetate ion interfered with chelate extraction. This interference is probably due to formation of a mixed complex. Ritter⁴⁾ reported on the complexes of tetracycline derivatives with aluminum and α -hydroxy acids. Penicillin-Al(III) salt compounds with sulfanilamide derivatives having high therapeutic efficacy were prepared by Dale and Bennett.⁵⁾ These compounds would be mixed complexes.

The formation, specific ability, and characteristics of mixed complexes will be of interest in the field of enzymosis and pharmacology.

In the present series of work, the isolation, molar ratio, and properties of the mixed complex of chlorotetracycline(CT)-metal chelates with penicilline G(PenG) were studied using thorium(IV), iron(III), and cobalt(II) ions, which had affinity to oxygen, and copper(II) ion having affinity to nitrogen. Addition of strong acid or alkali and heating were avoided because of the lability of ligands used. The experiments were carried out under acid conditions. PenG-metal complexes were also studied for use in the identification of mixed complexes.

*¹ Inohana-cho, Chiba (田口清水).

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1) M. Ishidate, T. Sakaguchi : This Bulletin, **3**, 147(1955).

2) T. Sakaguchi, K. Taguchi, S. Fukushima, N. Obi : Yakugaku Zasshi, **78**, 177(1958); T. Sakaguchi, K. Taguchi : *Ibid.*, **79**, 1381(1959).

3) T. Sakaguchi, K. Taguchi : This Bulletin, **3**, 303(1955); Bunseki Kagaku, **6**, 787(1957); T. Sakaguchi, K. Taguchi, K. Suzuki : *Ibid.*, **6**, 782(1957).

4) L. Ritter : U. S. Pat. 2,736,725 (February 28, 1956) (C. A., **50**, 10348(1956)).

5) J. K. Dale, M. E. Bennett : U. S. Pat. 2,752,335 (June 26, 1956) (C. A., **50**, 15029(1956)).

Various metal salts of PenG have been reported,^{6,7)} but nothing is known of the reaction under acid conditions. Recently, Szymanski and Panzica⁸⁾ studied the iron(III) complex of penicillin G procaine using infrared spectroscopy.

PenG-Metal Complexes—PenG-metal complexes were mainly prepared by the addition of water to the methanol solution of PenG-K and metal salt. Analytical results show compositions having a 2:1 ratio of PenG to iron(III) or cobalt(II), and a 1:1 ratio of PenG to thorium(IV). The thorium(IV) and iron(III) complexes will be a cationic complex because of the existence of the anion and molar ratio. Copper(II) ion decomposed PenG to penicillamine and PenG-Cu(II) complex was not obtained. PenG-Th(IV) complex (white) is very insoluble in water and common organic solvents, but soluble in dimethylformamide and dilute mineral acids below pH 2. PenG-Fe(III) (light brown) and PenG-Co(II) (pink) complexes are soluble in ethanol, methanol, dioxane, and dimethylformamide, and insoluble in water and ether. The cobalt(II) and iron(III) complexes were not precipitated at pH 4~6 directly from aqueous solution in spite of their insolubility in water.

PenG undergoes a rearrangement forming the isomeric benzylpenillic acid which has a typical absorption maximum at 320 m μ on standing in an aqueous solution below pH 5,^{9,10)} and the isomerization is promoted catalytically in the presence of metal ion such as Zn²⁺ or Al³⁺ (Fig. 2). Absorption curves of PenG-Co(II) and PenG-Fe(III) complexes isolated in methanol medium are shown in Fig. 2-1 and 2-2. Absorption curves of PenG fraction of PenG-metal complexes in ether extract are shown in Fig. 3. There is no absorption maximum at 320 m μ . This fact indicates that the complexes isolated may be the PenG-metal complex and not a complex of penillic acid. Penillic acid, however, was found in the mother liquor of the preparation (Fig. 3-3 and 3-4).

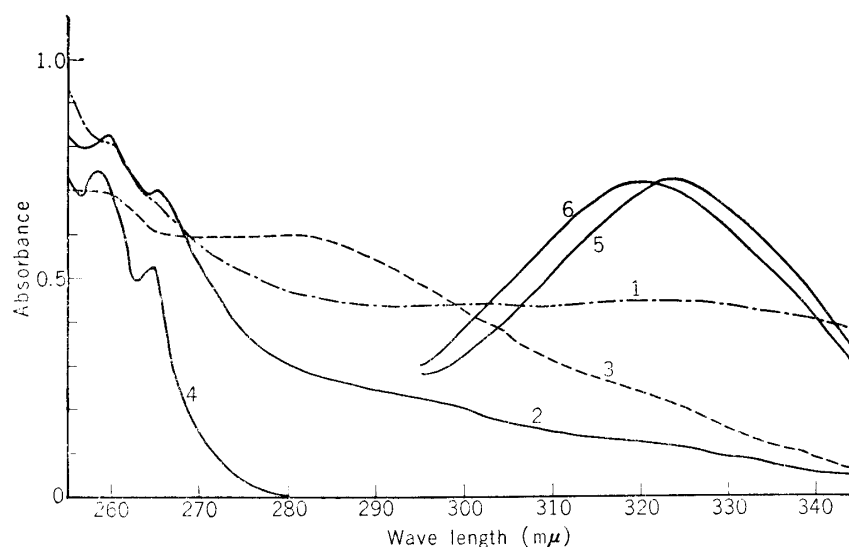


Fig. 1. Absorption Curves of Penicillin G-Metal Complexes

1. (PenG)₂-FeCl₃. 2. (PenG)₂-Co(II). 3. Methyl-PenG-Co(II), crude. 4. PenG-K. 5. Penillic acid produced by acid degradation of PenG with 0.01N HCl. 6. Mixture of PenG-K and alum solution left for 20 min. at 25°.

1, 2, and 3; MeOH solution. 4, 5, and 6; H₂O solution. Reference solution: H₂O.

- 6) H. Malissa : Mikrochemie ver. Mikrochim. Acta, **38**, 120(1951).
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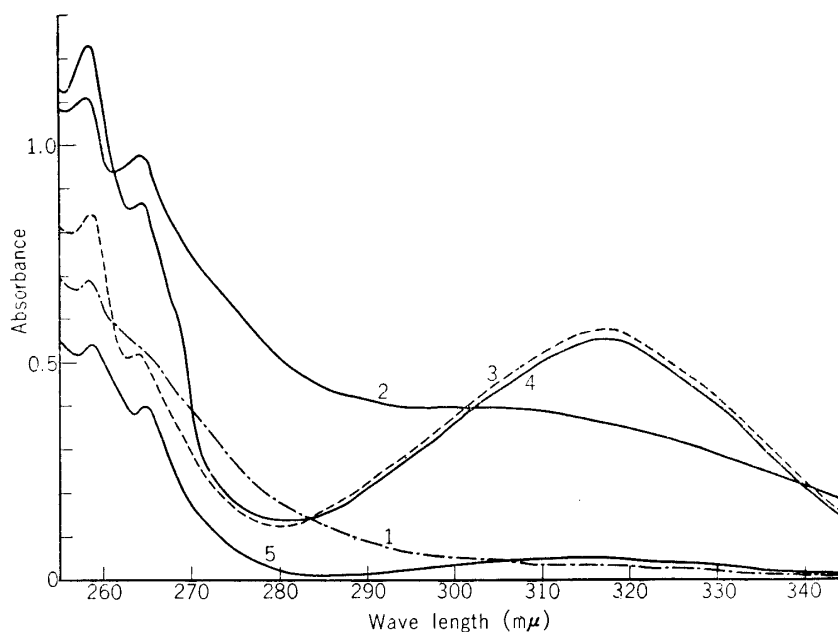


Fig. 2. Absorption Spectra of PenG Fraction of PenG-K and PenG-Metal Complexes

1. PenG-Co(II) complex isolated. 2. PenG-Th(IV) complex isolated. 3. Mother liquor from the preparation of PenG-Co(II) complex. 4. Mother liquor from the preparation of PenG-Th(IV) complex. 5. PenG acid. Method: Aqueous solution of samples, prepared by decomposing into components with EDTA-Na₂ and 0.01N HCl, and extracted with Et₂O at pH 3.0. PenG fraction was separated into ether layer and absorbancy of the extract was measured within a few minutes in order to avoid further decomposition. Reference solution: H₂O.

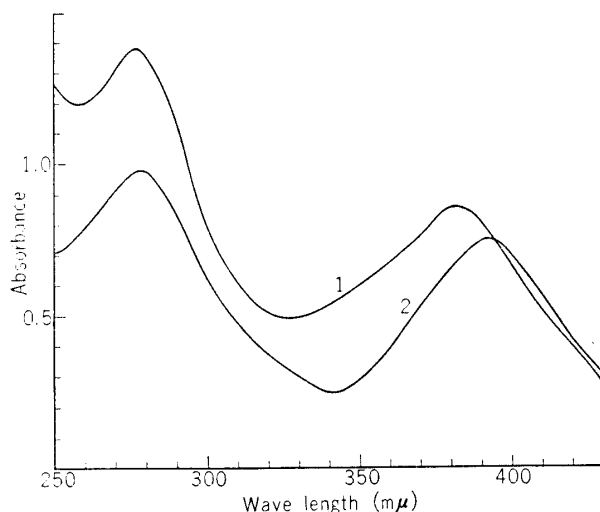


Fig. 3. Absorption Spectra of Mixed Complex of CT-Th(IV)-PenG

1. CT-Th(IV)-PenG mixed complex
2. CT-Th(IV) chelate
Solvent: MeOH
Reference Solution: MeOH

Benzylpenicillin methyl ester (Methyl-PenG) with inactivated carboxyl group did not form a complex with thorium(IV) and iron(III) ions in methanol-ether medium. A pinkish solid was obtained by addition of water to the solution of Methyl-PenG and cobalt(II) nitrate in ethanol. Cobalt(II) ion was not removed by repeated reprecipitation. The fact suggests the formation of a complex of Methyl-PenG with cobalt(II). The complex formation of PenG with cobalt(II) may take place at both carboxyl group and another group (nitrogen of the thiazolidine group), and PenG-Co(II) complex will be a chelate compound. It is assumed that the functional group of PenG to thorium(IV) and iron(III)

ions is the carboxyl group.

Mixed Complexes—Mixed complexes were mainly prepared by the addition of water to the solution of PenG-K and CT-metal chelate in methanol or water at pH 4~6. The purification of mixed complexes was carried out by washing and repeated reprecipitation as recrystallization could not be applied. Preparation of mixed complexes by reaction between PenG-metal complex and CT·HCl was also attempted. PenG-Fe(III) and PenG-Co(II) complexes react with CT·HCl in methanol, but the PenG-Th(IV) complex did not react with CT·HCl. It is considered that the insolubility of PenG-Th(IV) interferes with this reaction. The same phenomenon was observed in the case of the reaction between insoluble isoniazid-Cu(II) chelate and CT·HCl.

Ratio of CT-Th(IV)-PenG and CT-Fe(III)-PenG mixed complexes was 1:1:1 (CT:metal:PenG). CT-Cu(II) and CT-Co(II) chelates did not form a mixed complex at pH 4 but at pH 5.5. The content of PenG in the latter two mixed complexes was rather poorer than in the mixed complexes with thorium(IV) and iron(III). The molar ratios were not confirmed. The ratio of mixed complex prepared from PenG-Fe(III) and CT-HCl was 1:1:1 and the mixed complex of PenG-Co(II) with CT-HCl had no definite molar ratio.

These mixed complexes were soluble in methanol, ethanol, dioxane, Methylcellosolve, dimethylformamide, 0.1*N* hydrochloric acid, and 0.1*N* sodium hydroxide. They were slightly soluble in butanol and insoluble in water, ether, acetone, and benzene. The mixed complexes did not show a melting point, similar to CT-metal chelate.

Absorption curves of the mixed complexes in methanol are shown in Fig. 4. There is no typical difference from the characteristic absorption of CT-metal chelate in the region of 250~400 mμ.

Desdimethylaminochlorotetracycline (DACT) was used for determination of the mixed complex as will be mentioned in the next section. DACT-Th(IV) chelate which was a cationic complex having 1:1 ratio of DACT to thorium formed a mixed complex with PenG. DACT-Th(IV) was soluble in water, but the complex was insoluble in water and soluble in dimethylformamide.

Discrimination of the Mixed Complex—1) Two reactions of PenG with CT-metal chelate can be considered, one being the salt formation between PenG and CT base, and the other the mixed complex formation through metal, upon which emphasis is laid in this work.

PenG-K reacted with CT·HCl forming PenG-CT salt,^{11,12)} but DACT from which the basic group ($-N(CH_3)_2$) of CT was eliminated did not form additional compounds with PenG. Consequently the compound formed by the reaction of DACT-metal with PenG must have been the mixed complex. As the chelating functional group of CT remains in DACT, the compound of CT-metal chelate with PenG is considered to be a mixed complex.

2) Furthermore, if PenG in the isolated mixed complexes caused the formation of a simple salt with CT, amount of PenG will be constant at a definite pH, and will be indifferent to the metal used. However, the amount of PenG actually varied with the metal used. This fact indicates that PenG combines with CT-metal through the metal forming a mixed complex.

3) If the reaction between CT-metal chelates and PenG was only a displacement reaction, the product would be similar in solubility and in color to the single compound, such as CT-metal or PenG-metal. The mixed complexes isolated were different from the single compound.

11) S.L. Ruskin: Brit. Pat. 772,573 (April 17, 1957) (C. A., **51**, 10847(1957)).

12) E.H. Gans, T. Higuchi: J. Am. Pharm. Assoc., **46**, 458(1957).

Experimental

Reagent—Crystalline penicillin G potassium (J.P.), crystalline chlorotetracycline hydrochloride (Japan-Lederle Co. Ltd.), guaranteed reagent of metal salts, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and purified solvents.

Analyses of Complexes and Mixed Complexes—For the PenG-metal complexes, assays of metal, water of hydration, C, H, and N were conducted, and metal, CT, water of hydration, and some elements in the mixed complexes were assayed. NO_3^- and Cl^- ions, and PenG were detected by qualitative procedures. Molar ratios of the complexes were assumed from these results, assay of CT was carried out by the $\text{BuOH-Th}(\text{NO}_3)_4$ extraction established by the author and colleagues³⁾ after dissolving 15~20 mg. of powdered sample into 100 cc. of water by the aid of 5% HCl and 10% EDTA-Na_2 . PenG and metals did not interfere with this method. Metal content was obtained by ashing to constant weight in an electric furnace ($\text{Cu(II)} \rightarrow \text{CuO}$ at 700~800°; $\text{Co(II)} \rightarrow \text{CoO}$ at 1000~1200°; $\text{Fe(III)} \rightarrow \text{Fe}_2\text{O}_3$ at 700~800°; $\text{Th(IV)} \rightarrow \text{ThO}_2$ at 900~1000°). Water of hydration was determined on a sample dried over silica gel. by vacuum drying to constant weight at 90~100° over P_2O_5 . CT·HCl and PenG-K did not show loss of weight on drying. NO_3^- was detected with diphenylamine in conc. H_2SO_4 . PenG did not interfere with the reaction, but CT interfered slightly. PenG was detected with NH_2OH and $\text{NH}_4\text{Fe}(\text{SO}_4)_2$ ¹³⁾ or by a test of S as PbS. Cl^- was detected as AgCl in HNO_3 acid medium.

Preparation of the Complex and Mixed Complex—Preparation of the CT-metal chelates was made by addition of Et_2O to the solution of CT·HCl and metal salt in MeOH.²⁾ Desiccation of the samples was eliminated, but was used for preparation of the mixed complexes.

PenG-Metal Complex—PenG-Th(IV): A solution of 0.35 g. (0.0006 mole) of $\text{Th}(\text{NO}_3)_4$ in 4 cc. of MeOH was gradually added to a solution of 0.25 g. (0.0007 mole) of PenG-K in 4 cc. of MeOH with stirring. A white precipitate, formed instantly, was filtered through a sintered glass filter, washed with 3 portions of 5 cc. each of MeOH and water, and dried over silica gel. Tests for NO_3^- were positive. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{17}\text{O}_8\text{N}_3\text{STh} \cdot 3\text{H}_2\text{O}$ (PenG-ThO(NO_3)·3 H_2O): C, 27.50; H, 3.31; N, 6.00; Th, 33.2; H_2O , 7.72. Found: C, 32.71; H, 3.63; N, 5.76; Th, 34.9; H_2O , 6.90.

(PenG)₂-Co(II): A solution of 0.3 g. (0.001 mole) of $\text{Co}(\text{NO}_3)_2$ was added to a solution of 0.4 g. (0.001 mole) of PenG-K in 10 cc. of MeOH. A precipitate of KNO_3 was formed within a few min. by adding 1 cc. of Et_2O and keeping in a refrigerator. The precipitate was removed by centrifuging and the solution poured into 30 cc. of water. A pinkish precipitate occurred, which was collected on a sintered glass filter and washed with several portions of water until the filtrate showed negative results for K^+ ion. The product was dried over silica gel. *Anal.* Calcd. for $\text{C}_{32}\text{H}_{34}\text{O}_8\text{N}_4\text{Co} \cdot 2\text{H}_2\text{O}$ ((PenG)₂-Co·2 H_2O): C, 50.62; H, 5.02; N, 7.38; Co, 7.74; H_2O , 4.74. Found: C, 49.14; H, 5.65; N, 6.57; Co, 7.86; H_2O , 4.46.

PenG-Fe(III): A solution of 0.3 g. (0.0011 mole) of FeCl_3 was mixed with a solution of 0.25 g. (0.00054 mole) of PenG-K in 5 cc. of MeOH. The mixture was stored in a refrigerator for 0.5 hr. after which 30 cc. of water was added. A light brown precipitate was formed, which was collected on a sintered glass filter. The precipitate was washed with water until the filtrate showed negative results for K^+ ion. The product was dried over silica gel. When the mixture emulsified on addition of water, a precipitate was obtained by adding 3 cc. of saturated NaCl solution. Cl^- ion was positive. *Anal.* Calcd. for $\text{C}_{32}\text{H}_{34}\text{O}_8\text{N}_4\text{S}_2\text{ClFe} \cdot 3\text{H}_2\text{O}$ ((PenG)₂-FeCl·3 H_2O): C, 47.40; H, 4.95; N, 6.91; Fe, 6.87; H_2O , 6.65. Found: C, 50.62; H, 5.54; N, 6.59; Fe, 6.66; H_2O , 6.48.

Preparation of the Mixed Complexes—CT-Th(IV)-PenG: A solution of 0.1 g. of PenG-K in 2 cc. of water was added gradually to the solution of CT-Th(IV) chelate prepared from CT·HCl (0.2 g.) and $\text{Th}(\text{NO}_3)_4$ (0.3 g.) in 2 cc. of water with stirring. The pH of the mixture was about 4. An orange-yellow precipitate occurred in a short time and was collected on a sintered glass filter. The product was washed with water, EtOH, and Et_2O in turn, and dried over silica gel. Cl^- ion and PenG were positive. *Anal.* Calcd. for $\text{C}_{33}\text{H}_{37}\text{O}_{12}\text{N}_4\text{SClTh} \cdot \text{HCl} \cdot 6\text{H}_2\text{O}$ (PenG-CT·HCl·6 H_2O): CT·HCl, 42.7; Th, 19.25; H_2O , 8.99. Found: CT·HCl, 43.0; Th, 21.0; H_2O , 8.88.

CT-Fe(III)-PenG: The solution of CT-Fe(III) chelate prepared from CT·HCl (0.3 g.) and FeCl_3 (0.2 g.) in 4 cc. of MeOH was mixed gradually with a solution of 0.2 g. of PenG-K in 3 cc. of MeOH. A small amount of precipitate was removed by centrifuging after keeping in a refrigerator for 0.5 hr. A brown precipitate was obtained by addition of 50 cc. of NaCl solution (10%) and collected on a sintered glass filter. The product was washed with water until tests were negative for Na^+ ion using a solution of zinc-uranyl acetate. It was washed with Et_2O and dried over silica gel. Use of NaCl solution was necessary for breaking emulsification of the mixed complex. Cl^- ion and PenG were positive. *Anal.* Calcd. for $\text{C}_{33}\text{H}_{37}\text{O}_{12}\text{N}_4\text{Cl}_2\text{FeS} \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$ (PenG-FeCl-CT·HCl·3 H_2O): C, 46.60; H, 4.46; N, 5.65; CT·HCl, 51.9; Fe, 5.63; H_2O , 5.45. Found: C, 47.83; H, 4.86; N, 5.36;

13) G. E. Boxer, P. M. Everett: *Anal. Chem.*, **21**, 670(1949).

CT·HCl, 59.4; Fe, 6.20; H₂O, 5.95.

CT-Cu(II)-PenG: A solution of 0.3 g. of PenG-K in 5 cc. of MeOH was added to a solution of CT-Cu(II) prepared from CT·HCl (0.2 g.) and Cu(NO₃)₂ (0.2 g.) in 5 cc. of Methylcellosolve with stirring. The pH of the mixture was adjusted to about 6 with KOH(0.2N). Insoluble substances were filtered off after standing in a refrigerator for 0.5 hr. The filtrate was poured into 15 cc. of water. A yellow green precipitate formed was collected on a sintered glass filter, and the product was washed with Et₂O-MeOH (4:1), then dried over silica gel. NO₃⁻ ion and PenG were positive. *Anal.* Found: CT, 62.8; Cu, 5.58; H₂O, 7.19.

The mixed complex of CT-Co(II) chelate with PenG was prepared by a similar method to that of CT-Cu-PenG. *Anal.* Found: CT, 67.3; Co, 5.83; H₂O, 4.67.

CT-Fe(III)-PenG from PenG-Fe(III): PenG-Fe(III) complex freshly prepared from PenG-K (0.25 g.) and FeCl₃ (0.3 g.) was dissolved in 10 cc. of MeOH. 0.3 g. of CT·HCl was added to the solution and the mixture was kept at room temperature for 0.5 hr. with stirring. Insoluble substances were removed by centrifugation. A brown precipitate formed on addition of 70 cc. of Et₂O. The product was washed with Et₂O and a little water, and then dried over silica gel. *Anal.* Calcd. for C₃₈H₃₇O₁₂N₄FeCl₂S·HCl·3H₂O (PenG-FeCl-CT·HCl·3H₂O): CT·HCl, 51.9; Fe, 5.63; H₂O, 5.45. Found: CT·HCl, 55.4; Fe, 5.63; H₂O, 4.96.

DACT-Th(IV)-PenG: 0.1 g. of DACT-Th(IV) chelate prepared by the method mentioned below and 0.15 g. of PenG-K were dissolved in 10 cc. of dimethylformamide-MeOH (2:1). A yellow precipitate was obtained on the addition of 20 cc. of Et₂O to the solution after separating a small amount of insoluble substance. The product was washed with water and EtOH. Reprecipitation was carried out by the addition of Et₂O to a solution of 5 cc. of dimethylformamide-MeOH (2:1). The product was washed with water, EtOH, and Et₂O in turn, and then dried over silica gel. PenG was positive and NO₃⁻ ion was slightly positive. *Anal.* Calcd. for PenG-ThO-DACT·5H₂O: Th, 21.0; H₂O, 8.14. Found: Th, 22.28; H₂O, 8.28. Assay of DACT could not be performed by the method used for CT. DACT-Th(IV): To a solution of DACT-MeOH (0.2 g.) and Th(NO₃)₄ (0.25 g.) in 5 cc. of water, 50 cc. of EtOH-Et₂O (1:2) was added. A pasty substance was obtained. Reprecipitation was performed by the addition of Et₂O to the solution of the product in 5 cc. of EtOH. The product was washed with Et₂O-EtOH(3:1) and dried over silica gel. The occurrence of a pasty substance was not observed in the preparation of CT-Th(IV) chelate. The product when dried was a vitreous amber solid, soluble in water, MeOH, EtOH, and dimethylformamide, and insoluble in Et₂O and Me₂CO. The chelate had a UV: $\lambda_{\max}^{\text{H}_2\text{O}}$ 400 mμ (log ε 4.325) at pH 4.4 (acetate buffer). NO₃⁻ ion was positive. *Anal.* Calcd. for C₂₀H₁₅O₈-NCITh(NO₃)₃·3H₂O (DACT-Th(NO₃)₃·3H₂O): Th, 25.5; H₂O, 5.94. Found: Th, 25.1; H₂O, 5.33.

Desdimethylamino-chlorotetracycline(DACT) was prepared in accordance with method proposed by Woodward¹⁴) with slight modifications as follows: Reduction with Zn dust in AcOH was carried out in atmosphere of AcOH instead of N₂ gas, and lyophilization was replaced by drying *in vacuo* at 40°. The test for secondary amine with Na₂(Fe(NO)(CN)₅) and CH₃CHO was negative on the distillate which

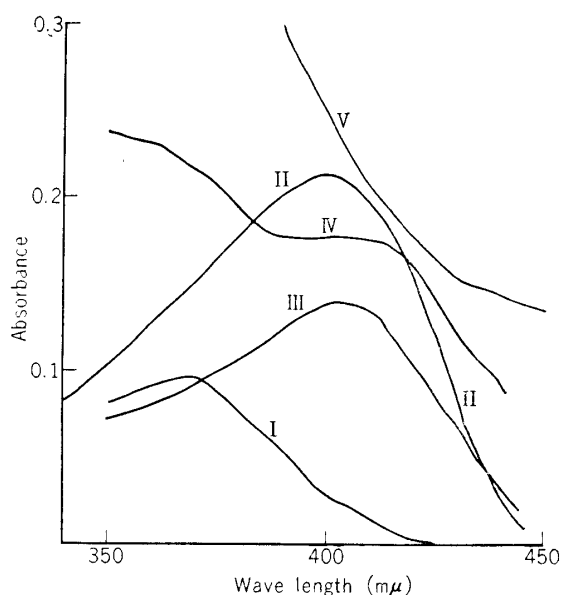


Fig. 4. Absorption Spectra of DACT-Metal Chelates (in aqueous solution at pH 4.4 acetate buffer)

I. DACT, II. DACT-Th(IV), III. DACT-Zr(IV), IV. DACT-UO₂(II), V. DACT-Fe(III).

Reference solution: H₂O

14) R.B. Woodward, C.R. Stephens, *et al.*: J. Am. Chem. Soc., **76**, 3573(1954).

was obtained by distillation of the sample in NaOH solution (10%), m.p. 157~160° (160~161° in the literature¹⁴⁾). UV : $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 270 and 358 m μ . The absorption caused by anhydro-DACT at about 440 m μ was not observed. The absorption spectra of DACT-metal chelates were similar to that of CT-metal chelates (Fig. 4).

Methyl benzylpenicillinate (Methyl-PenG) was synthesized by methylation of PenG acid with CH_2N_2 .¹⁵⁾

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Summary

The existence of cationic complex of chlorotetracycline(CT)-metal chelate having 1:1 ratio of CT to metal suggested the possibility of formation of a mixed complex combined with other ligands. Preparation and properties of the mixed complexes with penicillin G(PenG) and PenG-metal complexes were studied using Th^{4+} , Fe^{3+} , Co^{2+} , and Cu^{2+} ions in acid medium.

Th(IV) , Co(II) , and Fe(III) complexes with PenG which have 1:1, 2:1, and 2:1 ratios of PenG to metal, respectively, were isolated by addition of water to a solution of PenG-K and metal salt in methanol. Copper(II) complex was not obtained. Methyl-PenG was used for the determination of the functional group of PenG. Methyl-PenG- Co(II) complex was also formed.

Mixed complexes of CT-metal chelates with PenG were prepared from methanolic solution. Both CT- Th(IV) -PenG and CT- Fe(III) -PenG mixed complexes have a 1:1:1 ratio. Mixed complexes with Co(II) and Cu(II) did not show a definite composition. Desdimethylamino-chlorotetracycline- Th(IV) chelate formed a mixed complex with PenG. This indicates the formation of mixed complexes through the metal. The mixed complexes were soluble in methanol and insoluble in water and ether. Color of the mixed complexes was similar to that of corresponding CT-metal chelates.

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15) H. T. Clark, J. R. Johnson, R. Robinson : "The Chemistry of Penicillin," (1948). Princeton University Press, Princeton, N. J., U.S.A.