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Analysis of *d*-Ethambutol by Circular Dichroism of Its Copper Chelates[1)]

Tsunehiro Kitagawa, Shigeharu Oie, and Hyozo Taniyama

Faculty of Pharmaceutical Sciences, Nagasaki University[2)]

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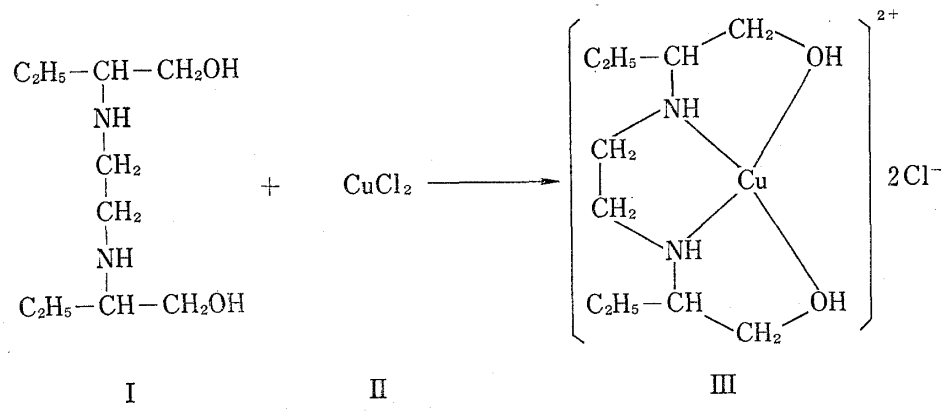
Antituberculostatic agent ethambutol, possessing a little molar specific rotation, was converted to several metal chelated derivatives. Among them, the copper chelate showed a large ellipticity value in its circular dichroism (CD) spectrum. A quantitative analysis of ethambutol by means of the copper chelation followed by CD spectroscopic measurements were investigated. Thus, addition of 0.1 ml of 0.3% ethanolic solution of ethambutol to 2.9 ml of 0.01% ethanolic solution of cupric chloride was ready to form copper chelated ethambutol which gave CD ellipticity value 4.6 ± 0.3 in its CD spectrum. The present method is one of the convenient procedures to introduce a chromophoric nature to an optically active amino alcohol for a CD measurement.

Antituberculostatic chemotherapeutic agent ethambutol (*d*-2,2′-ethylenediiminodi-1-butanol) (I) was first found out by Wilkinson, *et al.*[3)] in 1961, to have an excellent antituberculostatic activity. Currently, *d*-form of ethambutol has been widely used clinically as antituberculostatic agent but *l*- or *meso* form of this compound possesses little potency and of no practical



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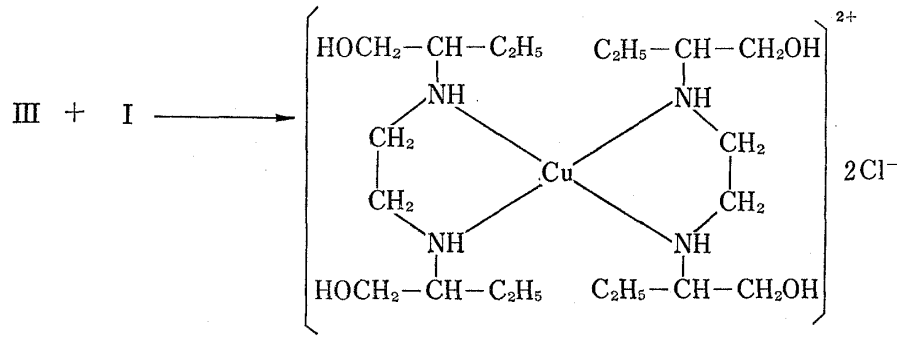

IV

Chart 1

1) Presented in part at the 93rd Meeting of Kyushu Branch, Pharmaceutical Society of Japan, held at Nagasaki, July 5th, 1975.
2) Location: *Bunkyo-machi, Nagasaki.*
3) *a*) R.G. Wilkinson, R.G. Shepherd, J.P. Thomas, and C. Baughn, *J. Amer. Chem. Soc.*, **83**, 2212 (1961); *b*) J.P. Thomas, C. Baughn, R.G. Wilkinson, and R.G. Shepherd, *Am. Rev. Resp. Dis.*, **83** 891 (1961); *c*) R.G. Wilkinson, M.B. Cantrall, and R.G. Shepherd, *J. Med. Pharm. Chem.*, **5**, 835 (1962).

use. A quantitative analysis of the *d*-form of ethambutol is practically important and its purity has been measured by means of its molar specific rotation. The value +7.6,[3c] however, is too small to determine the purity of the commercial product conveniently.

Recently, a measurement of optical rotatory dispersion (ORD) or circular dichroism (CD) has been found to have more useful applications than that of specific rotation for characterizations of optically active compounds.

Ethambutol, having both amino and hydroxyl functions in its molecule, is expected to form several metal chelates easily as suggested by Wilkinson, *et al.*[3c] Since some metal chlorides possess their own absorption maximum in ultraviolet (UV) field, the resulting chelates might possess large ellipticities and large specific rotations in their CD and ORD spectra. Thus, several metal chelated ethambutol were synthesized. Among the chelated derivatives prepared, copper chelated ethambutol which was easily formed, showed a large specific rotation in its ORD and a large ellipticity in its CD spectrum. The present paper describes a quantitative analysis of ethambutol by means of copper chelation followed by CD spectroscopic measurements.

Experimental

Materials——Hydrochlorides of *d*- and *l*-ethambutol (I) were kindly provided from Kaken Chemical Ind. Ltd. (Tokyo). Their free forms were obtained from their hydrochlorides by means of an ion exchange resin (Amberlite IRA 410, OH^- form) followed by recrystallization from acetone. Metal chlorides ($CuCl_2 \cdot 2H_2O$, $ZnCl_2$, $CoCl_2 \cdot 6H_2O$, and $NiCl_2$) used in the present experiments are reagent grade obtained from Nakarai Chemicals Ltd. (Kyoto) and the standard solution of copper and zinc ions for the atomic absorption apparatus are the products of Wako Chemicals Ltd. (Osaka).

Equipments——A Hitachi 124 type spectrophotometer, a JASCO automatic J-15 type ORD spectrophotometer with a CD attachment and a Hitachi 208 type atomic absorption spectrophotometer were used.

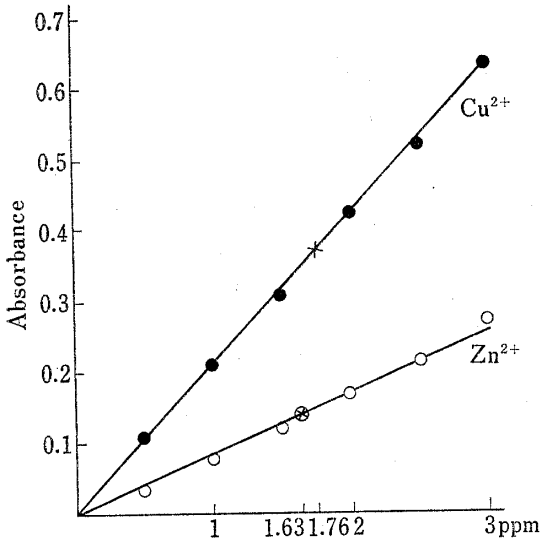

Fig. 1. Atomic Absorptions of Metal Ions
—●—: atomic absorptions of the standard Cu^{2+} solutions
The absorption of a 10 ppm solution of copper chelated I (×) correspond to that of 1.76 ppm of the standard Cu^{2+} solution.
—○—: atomic absorptions of the standard Zn^{2+} solutions
The absorption of a 10 ppm solu- tion of zinc chelated I (⊗) correspond to that of 1.63 ppm of the standard Zn^{2+} solution.

Analyses of Metal Contents——Method a: Metal contents were calculated from the weight of the residues of elemental analyses assuming that the residues are the oxides of the corresponding metals.

Method b: Atomic absorption of 10 ppm solution of copper or zinc chelated ethambutol was measured spectrophotometrically with an atomic absorption apparatus and by comparison of their absorbances with those of the corresponding standard solution (0.5 to 3 ppm), metal contents of the chelated compounds were calculated as shown in Fig. 1.

General Methods for the Preparations of Methal Chelated *d*-Ethambutol——To an ethanolic solution of 20—40% (w/v) *d*-ethambutol (*ca.* 10 ml), an equivalent molar amount of each of metal chlorides was added. To precipitate the metal chelates from the corresponding solution, acetone (10 ml) and adequate amounts of ether were added except for the copper chelate which was precipitated by an addition of dioxane (30 ml). Recrystallizations of the precipitates from ethanol–ether furnished purified metal chelates of *d*-ethambutol, respectively.

Copper Chelated *d*-Ethambutol——Blue prisms, mp 169—171°,[4] yield 43.6%. *Anal.* Calcd. for $C_{10}H_{24}O_2N_2CuCl_2$: C, 35.45; H, 7.14; N, 8.27; Cl, 20.93; Cu, 18.76. Found: C, 35.47; H, 7.29; N, 8.32; Cl, 21.20; Cu, 20.18 (method a), 17.60 (method b).

Zinc Chelated *d*-Ethambutol——While prisms, mp 147—150°, Yield 33.4%. *Anal.* Calcd. for $C_{10}H_{24}O_2N_2ZnCl_2$: C, 35.26; H, 7.10; N, 8.23; Cl, 20.82; Zn, 19.20. Found: C, 35.08; H, 7.00; N, 8.25; Cl, 20.74; Zn 16.30 (method b).

4) Melting points were uncorrected.

Nickel Chelated *d*-Ethambutol——Greenish hydroscopic prisms, mp 112—118°, yield 61.8%. *Anal.* Calcd. for $C_{10}H_{24}O_2N_2 \cdot 2/3NiCl_2$: C, 41.31; H, 8.32; N, 9.64; Cl, 16.26; Ni, 13.46. Found: C, 40.75; H, 8.35; N, 9.20; Cl, 16.62; Ni, 13.69 (method a).

Cobalt Chelated *d*-Ethambutol——A brownish dark amorphous powder, mp 102—106°, yield 33.7%; very hydroscopic and difficult to purify.

Results and Discussion

Among the metal chlorides used in this experiments, ethanolic solution of cupric chloride possesses UV absorption maximum at 283 nm with ε value 3500 and that of the solution of cobalt chloride, at 226 nm with ε value 1120, but the solution of nickel chloride or zinc chloride shows no absorption maximum in UV and visible light fields.

Despite *d*-ethambutol shows only end absorption in UV region, its metal chelated derivatives, the copper chelate shows an UV absorption maximum at 270 nm (ε=3120) and the cobalt chelate shows the maximum at 233 nm (ε=4400), but the maximum was not observed with the nickel and the zinc chelated derivatives.

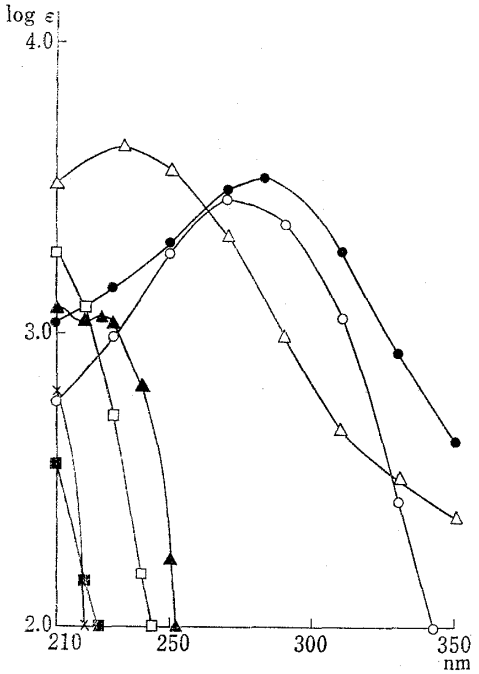

Fig. 2. UV Spectra of Ethanolic Solutions of Several Metal Chlorides and Their Chelated *d*-Ethambutol

—●—: cupric chloride; —○—: copper chelated I
—▲—: cobalt chloride; —△—: cobalt chelated I
—■—: nickel chloride; —□—: nickel chelated I
—×—: ethambutol (I)

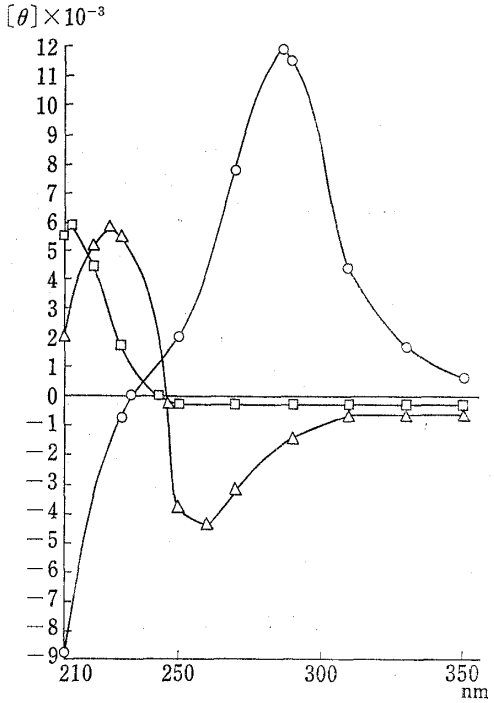

Fig. 3. CD Spectra of Ethanolic Solutions of Metal Chelated *d*-Ethambutol

—○—: copper chelated I
—△—: cobalt chelated I
—□—: nickel chelated I

As shown in Fig. 3, the largest CD ellipticities of nickel and cobalt chelated *d*-ethambutol are at 212 and 226 nm, respectively, while, that of the copper chelate at 285 nm is $[\theta]$=11900. Thus, the largest $[\theta]$ value of the copper chelate was selected to use for quantitative analysis of *d*-ethambutol.

Then, measuring conditions of the CD ellipticity of the copper chelated ethambutol were investigated.

Ethambutol possesses four polar groups, two amino and two hydroxyl groups, in its molecule. All of them may contribute to metal chelations as ligands. Ethambutol has an adequate structure to form the intramolecular chelation between these four ligands and cupric ion, shown as structure III. However, the intermolecular chelation between four

amino groups of two ethambutol molecules and cupric ion is also possible, since the chelating ability of amino group to copper is generally stronger than that of aliphatic hydroxyl group.[5]

Thus, UV and CD spectroscopic investigations were performed with different molar ratios of ethambutol and copper mixtures.

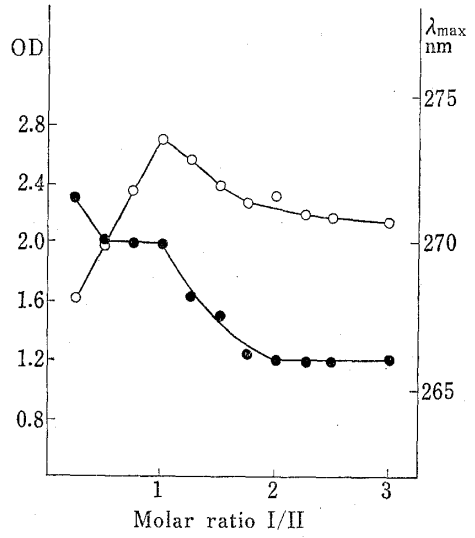

Fig. 4. The Maximum Wave Length (●) and Their Corresponding Absorbances (○) of Copper Chelated *d*-Ethambutol in UV Field, Measured at Different Molar Ratio of I/II in 5 mM Ethanolic Solution of II

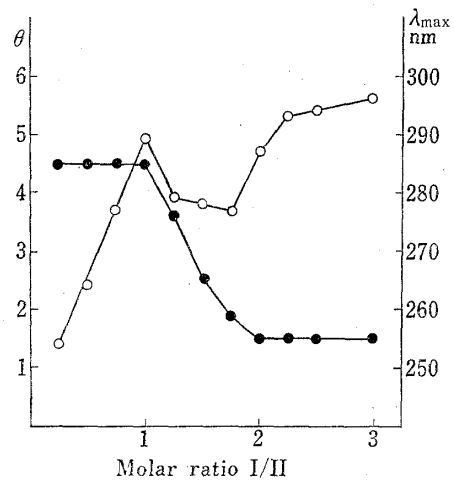

Fig. 5. The Maximum θ Values (○) and Their Corresponding Wave Lengths (●) in CD Spectra of Copper Chelated *d*-Ethambutol, measured at Different Molar Ratio of I/II in 5 mM Ethanolic Solution of II

To the 5 mM ethanolic solution of cupric chloride, different amount of ethambutol were dissolved and their UV absorption maximum wave length and absorbances were measured.

The observed maximum wave length was 270 nm and their absorbances were linearly increased until molar ratios of ethambutol and cupric chloride (II) were below 1:1. When molar ratios of I to II were between 1:1 and 2:1, both the maximum wave lengths and absorbances gradually decreased. When the molar ratios of I to II were more than 2:1, the maximum wave length was 266 nm and the absorbances were almost constant. In the lowest concentration of I in these experiments, however, some deviations of the absorption maximum wave length from 270 nm was observed owing to an absorption of free cupric chloride.

The similar relationships between the largest θ values and their corresponding wave lengths in CD spectra were observed as shown in Fig. 5. In this case, no deviation was observed at low concentration of I, since cupric chloride has no optical activity.

The structure of the isolated copper chelated ethambutol mentioned above is deduced as III, since the chelate consists of an equivalent amount of I and II, judging from its elemental analysis. As mentioned already, an ethanolic solution of the isolated copper chelated ethambutol showed UV absorption maximum at 270 nm and the largest CD ellipticity at 285 nm. Therefore, it was concluded that a mixture of I and II in ethanol is ready to form the chelate III, where molar ratios of I to II were below 1:1.

The linear relationship between the observed increasing θ values and amount of I until the ratio of I to II reached to 1:1 was further studied in detail. As shown in Fig. 6, various concentrations (0.002—0.01%) of *d*- or *l*-ethambutol in the ethanolic solution of cupric chloride (0.1 mg/ml) were prepared and their largest ellipticities were measured to give the similar linear relationship.

5) K. Ueno (ed.) "Chemistry of Chelates, 5," Nankodo Press. Ltd., Tokyo. 1975, p. 2.

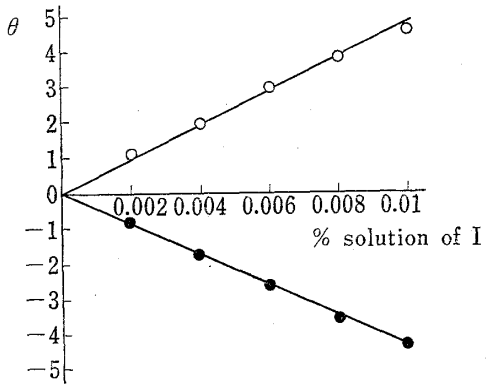

Fig. 6. The Maximum θ Values in CD Spectra of Copper Chelated *d*- (○) and *l*- (●) Ethambutol measured at Various Concentrations in 0.01 % Ethanolic Solution of II

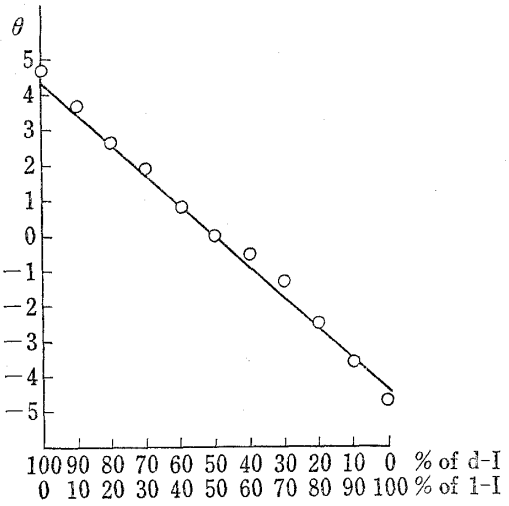

Fig. 7. The Observed θ Values in CD Spectra of *d*- and *l*-Form Mixtures of I (total 0.01%) measured in 0.01% Ethanolic Solution of II at 285 nm

Also, a linear relationship of the largest θ was obtained in ethanolic solutions containing lifferent ratios of *d*- and *l*-forms of I with constant amount of II (0.1 mg/ml), as shown in Fig. 7.

Sensitivity of the present analysis for ethambutol was limited by the observed θ value. 'his value depends on the measuring concentration of III which was limited by the starting oncentration of cupric chloride in the present method. However, cupric chloride or copper helated ethambutol possesses fairly strong UV absorption near at 285 nm which made difficult o measure CD spectrum in a concentrated solution of cupric chloride.

To find out the best concentration of the cupric chloride solution, CD spectra were deter- ıined with several concentrations of the metal containing equimolar amount of ethambutol. 'hus, 0.01% (w/v) ethanolic solution of cupric chloride dihydrate (molecular weight 170.4) 'as chosen for this purpose.

CD spectra were determined with a standard cell for the apparatus, having 1 cm of light ass length. With the cell, amount of the ethanolic solution of copper chelated ethambutol 'as needed at least 3 ml. Thus, addition of 0.1 ml of 0.3% (w/v) ethanolic solution of etham- utol (molecular weight 204.3, 0.3 mg) to the 0.01% copper solution (2.9 ml) is enough to ıeasure the practically largest CD ellipticity of the copper chelated I. The obtained value f the ellipticity at the condition was 4.6 ± 0.3. When, 0.02 ml of 0.3% ethanolic solution f I was added to 2.98 ml of the copper solution, the obtained value was 1.0 ± 0.3.

These results show that only dissolving I and II in ethanol is enogh to form the chelation. n optically active I, having no chromophoric group, are easily converted to the chelate hich possesses a chromophor and a large ellipticity in its CD spectrum. The linearity of the ırge θ value of the copper chelate made possible of quantitative analysis of *d*-ethambutol.

Several procedures to introduce chromophoric groups by chemical modifications have been :ported to measure CD spectra of optically active alcohols or amines having no chromophoric :oups.[6] The present method is a convenient procedure to introduce a chromophoric group ı a optically active amino alcohol.

) C. Djerassi, H. Wolf, and E. Bunnenberg, *J. Am. Chem. Soc.*, **84**, 4552 (1962); C. Djerassi, K. Undheim, and A.M. Weidler, *Acta Chem. Scand.*, **16**, 1147 (1962); C. Djerassi, D.A. Lightner, K. Takeda, T. Komeno and K. Kuriyama, *Tetrahedron*, **21**, 1203, 1581 (1965); J.H. Brewster and S.F. Osman, *J. Am. Chem. Soc.*, **82**, 5754 (1960); S. Yamada and K. Achiwa, *Chem. Pharm. Bull.* (Tokyo), **9**, 412 (1961); B. Sjoberg, D.J. Cram, L. Wolf, and C. Djerassi, *Acta Chem. Scand.*, **16**, 1079 (1962).

Many CD spectroscopic investigations of metal chelates are reported and these studies are reviewed recently,[7] but as far as we know, nothing has been reported about an introduction of chromophoric nature by a metal chelation.

Acknowledgements Authors wish to express their gratitude to Messrs Kaken Chemical Ind. Ltd. for generous gift of ethambutols. Thanks are also due to Mrs. Mazume of the Central Analyses Room of our Faculty for microanalyses.

7) K. Ueno (ed.) "Chemistry of Chelates, 6," Nankodo Press Ltd., Tokyo, 1975, p. 325; J. Hidaka, *Kagaku*, **22**, 10 (1967).