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Stability Constants of the Copper(II) and Zinc(II) Chelates of 3,4-Dihydroxybenzaldehyde in Aqueous Solution

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The conditional stability constants of the chelates of 3,4-dihydroxybenzaldehyde (LH_2) with Cu(II) and Zn(II) (M^{2+}), defined as $K_1 = [ML] / ([M^{2+}][LH_2])$ and $K_2 = [ML_2^{2-}] / ([ML][LH_2])$, in 0.2 M MES buffer (pH, 5.6) at various temperatures were calculated from the absorption at 350 nm of the solution containing varying concentrations of the ligand and the metal ions. From the method of least squares, we obtained $\log K_1 = 4.38$ (21 °C), 4.51 (30 °C) and 4.44 (37 °C) for the Cu(II) chelate, and $\log K_1 = 1.53$ (37 °C) for Zn(II) chelate. The value of K_2 for the Cu(II) chelate at 37 °C was $\log K_2 = 0.442 \pm 0.5$ and that of the Zn(II) chelate was too small to obtain reliable results by the present method.

Catechol-*O*-methyltransferase (COMT, EC 2.1.1.6) catalyzes the transfer of the methyl group of *S*-adenosyl-*L*-methionine to phenolic groups of catechol or substituted catechols. The reaction is an important step in the metabolism of catecholamines. The enzyme occurs widely in mammalian tissues and requires divalent metal ions such as Mg^{2+} for the catalysis. The role of metal ion and the mechanism of *O*-methylation is not fully understood at present.¹⁾

To shed light on these problems, a non-enzymatic *O*-methylation of a catechol derivative (3,4-dihydroxybenzaldehyde, LH_2) catalyzed by metal ions (M^{2+}) has been studied.^{2,3)} In this chemical model of COMT, the catalytic effects of metal ions were explained in terms of the formation of metal chelates. For the full understanding of the reaction, the knowledge of the stability constants of the metal chelates is required. Those of the Cu(II) and Zn(II) chelates were calculated from the results of the spectral measurements.

Experimental

3,4-Dihydroxybenzaldehyde was obtained from commercial sources and recrystallized from toluene prior to use. Deionized and distilled water was used. Other chemicals were of reagent grade. Ultraviolet absorption spectra were measured in a

pair of 1.0 cm silica cells with a Shimadzu UV-240 spectrophotometer equipped with a thermostated cell compartment. An NEC Model PC-9801 personal computer was used for the calculation and figure preparation.

Results and Discussion

The ultraviolet absorption spectrum of 3,4-dihydroxybenzaldehyde (abbreviated as LH₂, where the H represents the dissociable hydrogen atom of the phenolic groups) in 0.2 M MES (2-(*N*-morpholino)ethane sulfonic acid-NaOH; pH, 5.6) buffer showed two peaks at 279 and 310 nm. The spectrum in alkaline solution had a band at 345 nm. The addition of divalent metal ions also resulted in the appearance of the band similar to that of the alkaline solution. The results show that the ligand is present in a neutral species, LH₂, in MES buffer. It has been well established that the dissociation and metal chelation of the phenolic hydrogen cause a considerable red shift to the $\pi-\pi^*$ band.⁴⁾

The spectra of the buffer solutions containing various concentrations of the ligand and the divalent metals, M²⁺ were measured. Fig. 1 and 2 show the spectral changes in the solutions of the ligand and the varying concentrations of Cu(II) and Zn(II) perchlorates, respectively. The spectra show that the metal chelate with the band at 350

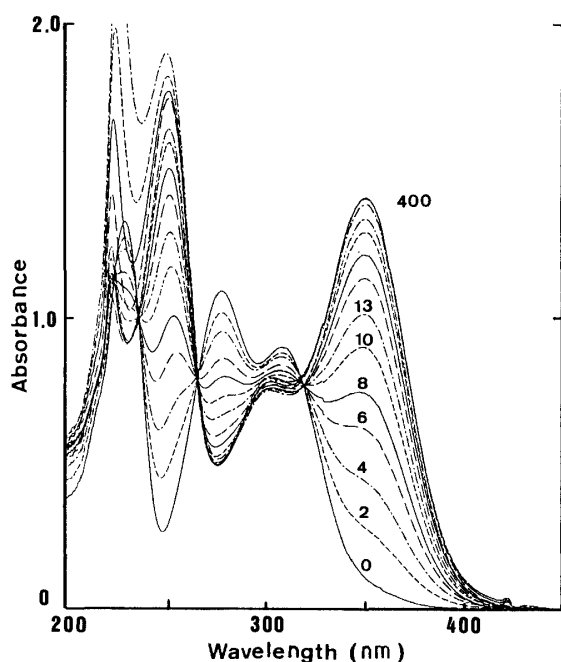


Fig. 1 Absorption spectra of 1×10^{-5} M of 3,4-dihydroxybenzaldehyde in 0.2 M MES buffer (pH, 5.6) at 37°C with varying concentration of Cu(II) perchlorate. The concentration of Cu(II) are indicated besides the curves in 10^{-5} M unit.

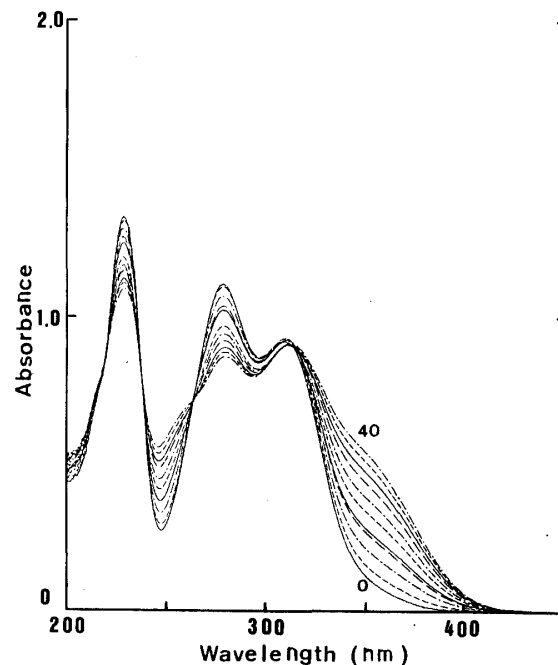


Fig. 2 Absorption spectra of 1.02×10^{-5} M of 3,4-dihydroxybenzaldehyde in 0.2 M MES buffer (pH, 5.6) at 37°C with varying concentration of Zn(II) perchlorate. The concentration of Zn(II) ($0-40 \times 10^{-3}$ M) are indicated besides the curves in 10^{-3} M unit.

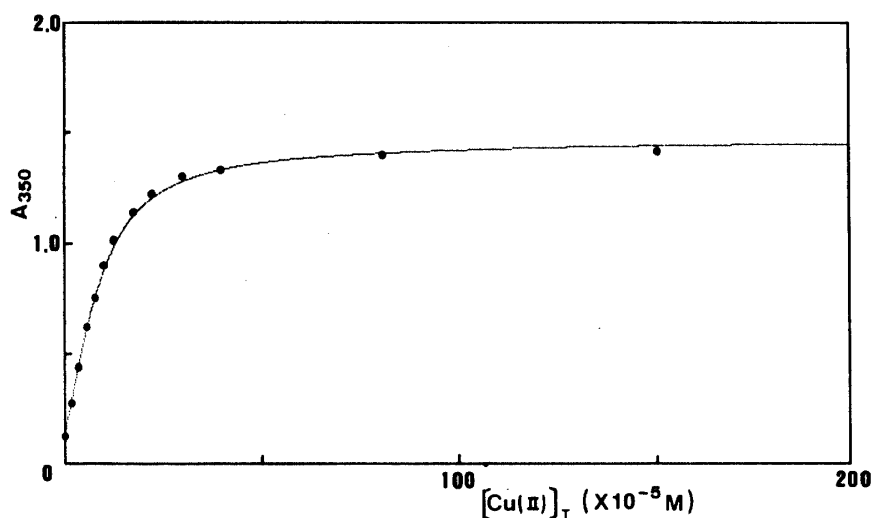


Fig. 3 Variation of the absorbance at 350 nm of 1×10^{-5} M of 3,4-dihydroxybenzaldehyde in the buffer at 37°C as a function of Cu(II) concentration. The points are the experimental data, while the line represents calculation from the following values; $\epsilon_0=2.32$, $\epsilon_1=1070$, $\epsilon_2=14680$, $\epsilon_3=29360$, $\log K_1=4.44$, $\log K_2=0.442$. The line was essentially the same for the assumption, $K_2=0$.

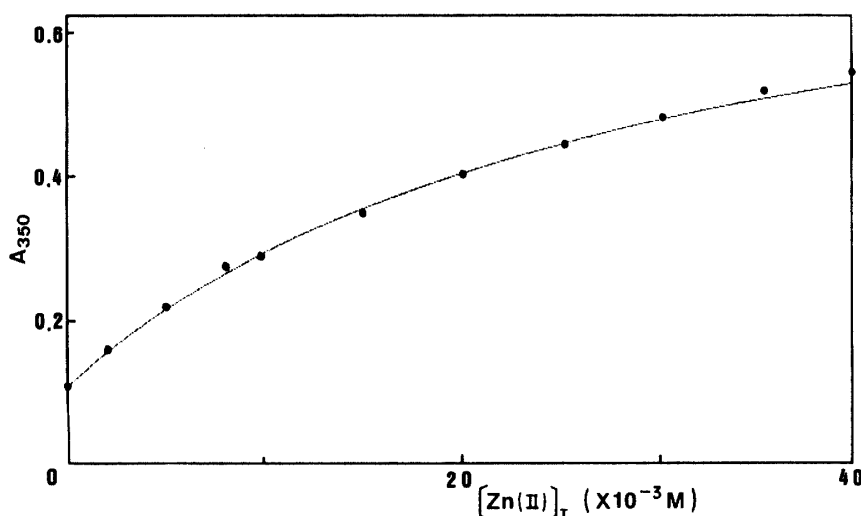
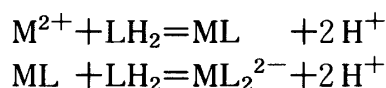


Fig. 4 Variation of the absorbance at 350 nm of 1.02×10^{-5} M of 3,4-dihydroxybenzaldehyde in the MES buffer at 37°C with varying concentration of Zn(II) perchlorate. The points are the experimental data, while the line represents calculation from the following values; $\epsilon_0=0$, $\epsilon_1=1070$, $\epsilon_2=8400$, $\log K_1=1.54$, $\log K_2=0$.

nm was formed from LH_2 with an increase of the concentration of M^{2+} . Variations of the absorbance at 350 nm (A_{350}) with the concentrations of Cu(II) and Zn(II) are shown in Fig. 3 and 4, respectively. The conditional stability constants in the buffer were calculated from the data as described below.

The equilibria between the ligand and M^{2+} are written as follows.



Under the conditions, the formations of the 1 : 3 and higher complexes, protonated complexes and polynuclear complexes are negligible. The conditional stability constants are defined as follows.

$$K_1 = [ML] / ([M^{2+}] [LH_2]) \quad \dots (1)$$

$$K_2 = [ML_2^{2-}] / ([ML] [LH_2]) \quad \dots (2)$$

Total concentration of M or LH₂ are expressed as follows.

$$[M]_T = [M^{2+}] + [ML] + [ML_2^{2-}] \quad \dots (3)$$

$$[L]_T = [LH_2] + [ML] + 2 [ML_2^{2-}] \quad \dots (4)$$

A₃₅₀ can be written as

$$A_{350} = \epsilon_0 [M^{2+}] + \epsilon_1 [LH_2] + \epsilon_2 [ML] + \epsilon_3 [ML_2^{2-}] \quad \dots (5)$$

where ϵ_0 , ϵ_1 , ϵ_2 and ϵ_3 are the molar extinction coefficients of M²⁺, LH₂, ML and ML₂²⁻, respectively. The values of ϵ_0 and ϵ_1 were calculated from the spectra of the solutions of M²⁺ and LH₂, respectively. The values of A₃₅₀ were obtained from measurements and [M]_T and [L]_T from the experimental conditions. Then there are eight unknown quantities in the five equations.

A general method of calculation of in these cases is to obtain optimal values by the treatment of experimental data by the method of least squares. The computer aided method consists of assuming of initial values and improving of these values by successive approximation. The programs such as SCOGS⁵⁾ and MINIQUAD⁶⁾ have been widely used for the calculation of complex stability constants. Since the equilibria of the present study are relatively simple, we calculated by a personal computer using the generalized method.

We assumed that K₂ is of relatively small value. By neglecting K₂, the calculation becomes very simple. There are five unknown quantities in the four equations, eq. (1), (3), (4) and (5), in which the term of [ML₂²⁻] is not present. For each assumed value of ϵ_2 , a value of K₁ was calculated from a set of experimental data. The deviation (δ) was defined as eq. (6).⁷⁾

$$\delta = \Sigma (\bar{K}_1 - K_1)^2 / (nK_1^2) \quad \dots (6)$$

\bar{K}_1 and n indicate the mean value of K₁ and a number of experimental data, respectively. The value of δ was plotted against ϵ_2 , as shown in Fig. 5 and 6. The values of ϵ_2 corresponding the minimum value of δ should be the best approximated ones from the data. For CuL, the temperature dependence of K₁ was small. The heat of the reaction (ΔH) was obtained as 7.3 kJ/mol from the van't Hoff plots, though the correlation coefficient is small.

For the calculation of K₂, the values of K₁ and ϵ_2 thus calculated were employed. The value of ϵ_3 was assumed to be twice as much as that of ϵ_2 . Then the number of the unknown quantities was reduced to six. The ratio of K₁ to K₂ was defined as m .⁸⁾

$$K_1 = mK_2 \quad \dots (7)$$

Using the experimental data for the Cu(II) chelation, the value of δ was plotted for each assumed value of m . The results showed some scatter for the $\log m$ value larger than 4 as shown in Fig. 7. The value of $\log m$ corresponding to the minimum value of δ was estimated to be $\log m = 4 \pm 0.5$. The scatter somewhat lowered the reliability of the estimation. From the value, $\log K_2 = 0.442 \pm 0.5$ was obtained at 37 °C. From the data at 21 °C and 30 °C, almost the same values of K_2 as at 37 °C were obtained. The value of K_2 for Zn (II) was too small to obtain by the present method.

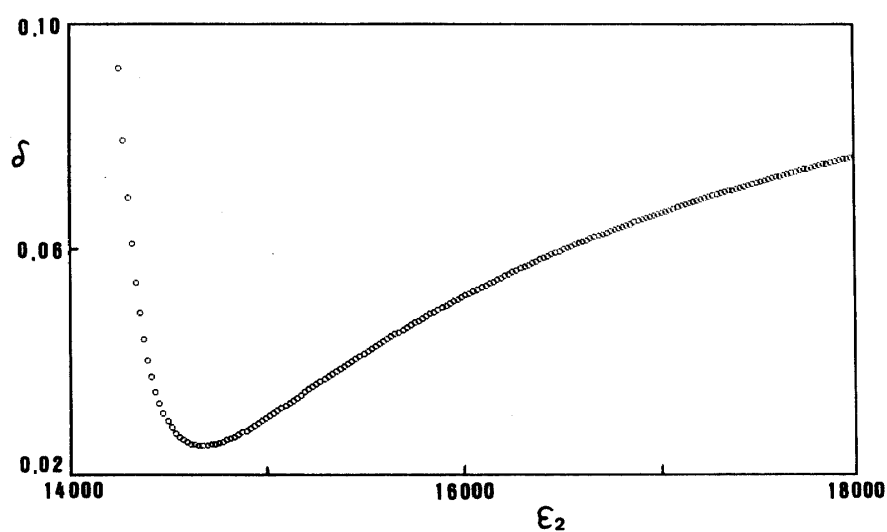


Fig. 5 Variation of the deviation (δ) as a function of ϵ_2 calculated from the data shown in Fig. 1.

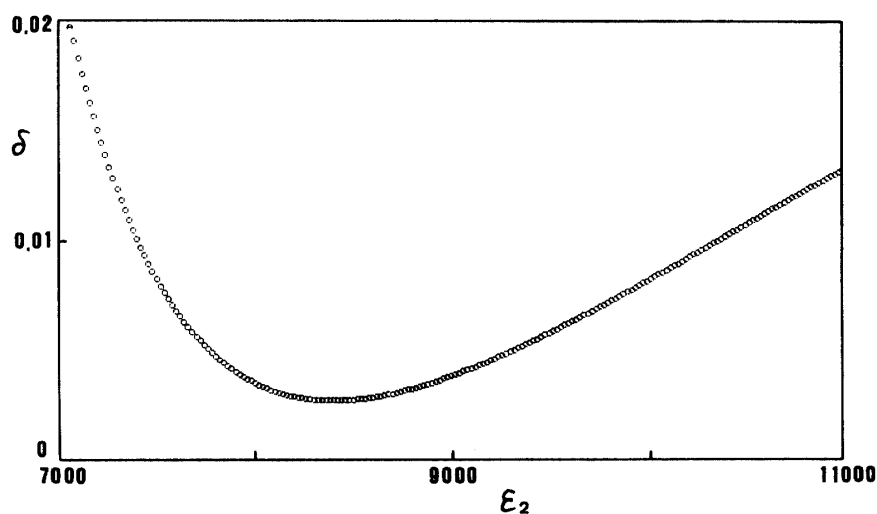


Fig. 6 Variation of the deviation (δ) as a function of ϵ_2 calculated from the data shown in Fig. 2.

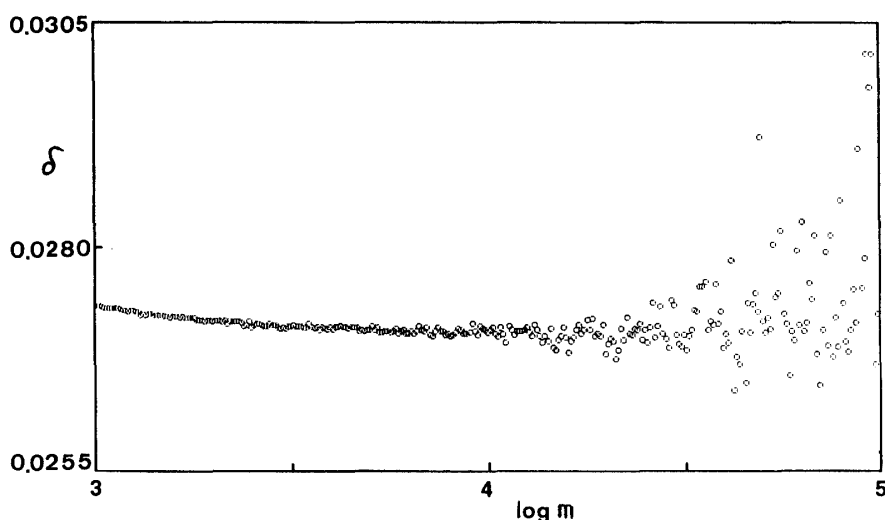


Fig. 7 Variation of the deviation (δ) as a function of m calculated from the data shown in Fig. 1.

TABLE I The stability constants of the complexes of 3,4-dihydroxybenzaldehyde with Cu(II) and Zn(II)

Metal ion	Cu(II)		Zn(II)
Temperature (°C)	21	30	37
ϵ_2^a	15990	13840	8400
$\log K_1$	4.38	4.51	4.44
$\log K_2$	0.442 ± 0.5		

a) The values of ϵ_2 were obtained from the method shown in Figs. 5 and 6.

The calculated values are tabulated in Table I. The values reported previously^{3b)} were calculated from a set of experimental data without the variance analysis and must be replaced with those reported here.

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- 8) The value of $\log m$ generally lies in the range of 1 and 5. The limited range for an assumed quantity should simplify the computation. For the present reaction, the calculation went through with K_1 or K_2 similarly without defining m .