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The Reaction between Mercury(II) and Organic Compounds. II. The Composition of a Mercury(II)-L-Histidine Complex in an Ethanol-Water Solution

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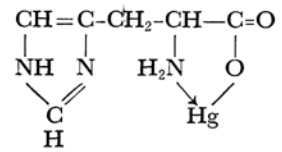
The composition of the complex formed with L-histidine and mercury(II) in a solution has been investigated spectroscopically. The complex formation in an aqueous solution was not observed, but in an alcoholic solution (1 : 1), there appeared about 274 mμ an absorption maximum which can be attributed to the complex formation. By the spectroscopic measurements of this alcoholic solution containing two components in various proportions, it was shown that only one kind of complex with a 1 : 1 composition was formed in a 50% ethanol-water solution at pH 3.25—3.75 and at 20°C. Accordingly, the composition of the complex may be considered to be as follows:



Although some complexes of L-histidine formed with such metal ions as cobalt(II), zinc(II), nickel(II), cadmium(II) and copper(II) have been studied by several workers,[1-3] the L-histidine complex formed with mercury(II) has not been studied except for the discussion in regard to the association state in an aqueous solution by Brooks *et al.*[4]

In the present author's earlier publication,[5] it was reported that the compositions of the crystalline solids of mercury(II)-histidinate complexes could be determined with elemental analytical data, and with X-ray powder diffraction and infrared patterns. In that case, the coordination number of mercury(II) was two or four in accordance with the slightly different experimental conditions.

The present investigation was carried out to determine by ultraviolet spectroscopy the composition of the complex between L-histidine and mercury(II).

Experimental

The chemicals used were purchased from the Nippon Rikagaku Yakuhin Co., Ltd., and the Wako Pure Chemical Industries, Ltd. The purity of all the chemicals used was of analytical grade or of a special grade. Ethanol was also a spectro-grade Dotite reagent, a product of the Research Laboratories of Dojindo & Co., Ltd. Solutions were prepared using redistilled water.

1) D. Burk, J. Haron, L. Caroline and A. L. Schade, *J. Biol. Chem.*, **165**, 723 (1946).
2) R. Leberman and B. R. Rabin, *Trans. Faraday Soc.*, **55**, 1660 (1959).
3) Y. Sano and H. Tanabe, *J. Inorg. Nucl. Chem.*, **25**, 11 (1963).
4) P. Brooks and N. Davidson, *J. Am. Chem. Soc.*, **82**, 2118 (1960).
5) F. Kai, *Nippon Kagaku Zasshi (J. Chem. Soc. Japan, Pure Chem. Sect.)*, **86**, 1040 (1965).

Preparation of the Mercury(II) Perchlorate Standard Solution. Mercury(II) oxide was precipitated by the addition of an excess sodium hydroxide solution to an aqueous solution containing 50 g of mercury(II) chloride. The precipitate was filtered off and washed with water until no chloride ions were detected with a silver nitrate solution; then the precipitate was dried at room temperature. 20 g of mercury(II) oxide was dissolved in 100 m*l* of a 60% perchloric acid solution in a porcelain dish, and the mixture was heated on a sand bath until white fumes were generated. After the solution had then been allowed to stand for about two hours at room temperature, white needlelike crystals of the mercury(II) perchlorate were found. These crystals of mercury(II) perchlorate were dissolved in a small amount of 60% perchloric acid and diluted to a final volume of one liter with a 50% ethanol-water solution. The mercury concentration in this solution was determined by chelatometric titration[6] or by colorimetric determination.[7,8] The mercury concentration of the solution was 10^{-1} M. The ionic strength was adjusted to 0.50 with potassium chloride.

Preparation of the L-Histidine Standard Solution. L-Histidine (15.51 g) was dissolved in a warm 50% ethanol-water solution and diluted to one liter with a 50% ethanol-water solution. The concentration of the resulting solution was 10^{-1} M. This ionic strength was also adjusted to 0.50 with potassium chloride.

Apparatus. A glass electrode (Hitachi-Horiba type M-5) was used for the pH measurements. The absorbance spectra of the solution were scanned using a recording spectrophotometer (Hitachi type EPS-2) and a photoelectric spectrophotometer (Hitachi type EPU-2) with 10-mm cells. All the measurements were carried out at 20°C and at a constant ionic strength of 0.50.

Results and Discussion

When pure water was used as a solvent for mixing the mercury(II) and the L-histidine, no complex formation was observed. On the other hand, in an ethanol-water solution, the absorption spectra of the mixture exhibited a remarkable shift of the maxima from those for each component. This shift may be attributed to the complex formation. The results obtained are summarized in Table 1. Because the data in the table suggest that the optimum concentration of an ethanol-water solution for the complex formation was 50%, the discussion below assumes the use of a 50% ethanol-water solution as the solvent. The solvent effect on the formation of the mercury(II)-histidinate complex will be studied in further detail with regard to a more fractional ethanol concentration and other solvents.

6) K. Ueno, "Kireito Tekitei-ho" (Chelatmetric Titration) (in Japanese), Nankodo, Tokyo (1964), p. 268.
7) E. B. Sandell, "Colorimetric Determination of Traces of Metals," Interscience Publ., New York (1959), p. 621.
8) F. D. Snell and C. T. Snell, "Colorimetric Method of Analysis," C. Van Nostrand Co., Philadelphia (1959), p. 63.

Table 1. Solvent effect to the complex formation with respect to various concentrations of ethanol solution by the spectroscopical measurements

1 : 1 molar ratio; total concentration 3×10^{-2} M
20 ± 1°C; $\mu=0.50$ (KCl); pH=3.50

Concn. of ethanol %	λ_{max} mμ	Molar extinction coefficient (log ε)
10	Featureless	—
20	Featureless	—
30	Featureless	—
40	274 (Slightly)	0.97
50	274	1.24
60	274	0.74
70	Featureless	—
80	Featureless	—

Spectra of Various Solutions. The absorption spectra of mercury(II) perchlorate (a), 3×10^{-2} M, L-histidine (b), 3×10^{-2} M and a mixture of mercury(II) perchlorate and L-histidine (c) (total concentration of 4.5×10^{-2} M) in a 1 : 2 molar ratio are shown in Fig. 1.

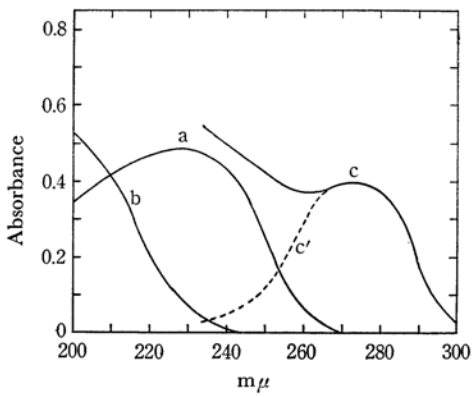


Fig. 1. Absorption curves for the 50% ethanol solutions at pH 3.50.
Curve a, Hg^{2+}: 3.0×10^{-2} M
b, L-Histidine: 3.0×10^{-2} M
c, Mixed solution of Hg^{2+} and L-histidine at 1 : 2 molar ratio, total concentration of 4.5×10^{-2} M
c', Differences of absorbance between those of curves c and a at each wavelength

The absorption curve of (a) displayed its maximum at about 230 mμ, but the curve of (b) showed that it has an absorption but no maximum in the 200—240 mμ region. This fact is in agreement with the data reported by Bhatia *et al.*[9]

On the other hand, in the curve of (c), an absorption maximum which showed a remarkable

9) D. S. Bhatia and B. E. Proctor, *Biochem. J.*, **50**, 535 (1952).

shift from the maximum of mercury(II), at about 230 mμ, appeared at about 274 mμ. This absorption maximum is probably due to some complex formations. Since the increased absorption below 255 mμ of the curve of (c) was considered to be that of mercury(II), a dotted line, (c'), was plotted as the difference between the absorptions of (a) and of (c) at each wavelength.

The spectra of buffered solutions were similarly measured in order to investigate any effect of the buffer. The resulting spectra of buffered solutions, however, did not differ appreciably from those of non-buffered solutions at the same pH.

Figure 2 shows the absorption spectra of the mixtures of L-histidine and mercury(II) in such various molar ratios as 1 : 2, 2 : 1 and 1 : 1. From the fact that maximum absorption bands of several solutions remain practically constant at about 274 mμ at different molar ratios, it can be seen that only one kind of complex is formed, or that at least one is very predominant under these conditions.

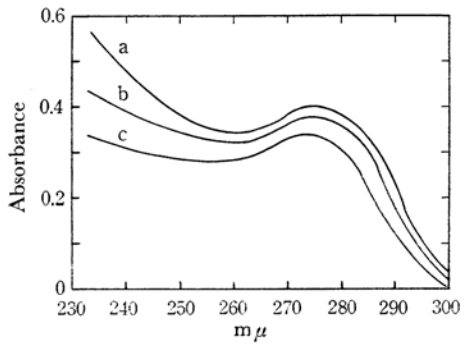


Fig. 2. Absorption curves for the solutions of Hg^{2+} and L-histidine in the 50% ethanol solutions at molar ratio: a, 2 : 1; b, 1 : 1; c, 1 : 2 at pH 3.50.

Composition of The Complex in 50% Ethanol-Water Solution. Job's continuous variation method[10,11] was adopted in order to determine the composition of the complex. In order to protect against the hydrolytic behavior of mercury, particular care was taken not to use too high pH values.*1 Therefore, as will be mentioned below with respect to the influence of the pH upon this complex formation, all the measurements were carried out at pH 3.50. The wavelengths were fixed at 274 and 280 mμ. At these wavelengths, it is sufficient, as may be seen in Fig. 1, to measure the absorbances of the complex alone, since mercury(II) and L-histidine do not absorb distinctly in these concentrations.

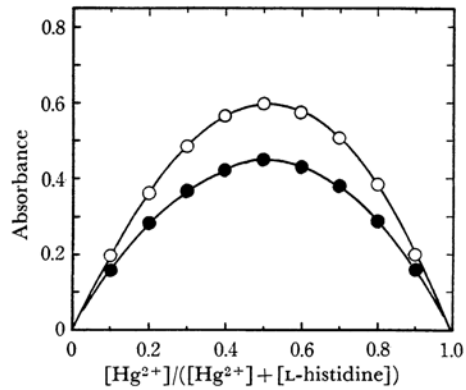


Fig. 3. Curves obtained by the continuous variation method for the 50% ethanol solutions of Hg^{2+} and L-histidine at total constant concentration 3.0×10^{-2} M at pH 3.50.
○, at 274 mμ ●, at 280 mμ

Figure 3 gives the plots obtained by this method. It can be concluded that mercury(II) forms a complex with L-histidine in a 50% ethanol-water solution in a 1 : 1 molar ratio. These results were confirmed by further experiments using the molar-ratio method.[12]

Formation of Complexes as a Function of the pH. The absorption spectra of solutions at various pH's obtained by mixing a 3×10^{-2} M solution of L-histidine and mercury(II) in a 1 : 1 molar ratio, are illustrated in Fig. 4. The absorption spectra obtained were practically identical between pH 3.25 and 3.75, but the intensity gradually decreased, while, at the other pH's, below 3.00, no maximum absorption bands were observed except for a very weak maximum at 3.00. It is,

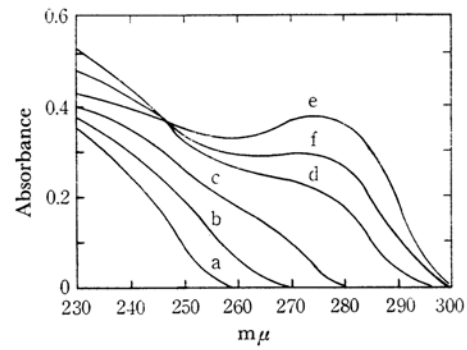
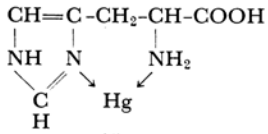


Fig. 4. Absorption curves for the 50% ethanol solutions of the mixture of Hg^{2+} and L-histidine at 1 : 1 molar ratio, total concentration of 3.0×10^{-2} M in relation to varying pH's.
Curve a pH 2.50 Curve d pH 3.25
b pH 2.75 e pH 3.50
c pH 3.00 f pH 3.75

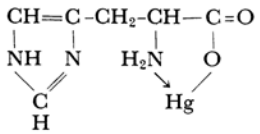
10) P. Job, *Ann. Chim.*, **9**, 113 (1928).
11) F. J. C. Rossoti and H. Rossoti, "The Determination of Stability Constants," McGraw-Hill, New York (1961).
*1 At higher pH values (beyond a pH of about 4.5) some precipitation due to the hydrolytic behavior caused trouble.
12) J. H. Yoe and A. L. Jones, *Ind. Eng. Chem.*, **16**, 11 (1944).

thus, reasonable to assume that the complex formation is influenced almost not at all by changes in pH between 3.25 and 3.75.

Judging from a previous study[13] containing several considerations of the structures of L-histidine complexes formed with metal ions, the probable structures of the complex formed with mercury(II) may be expressed by the following two forms:



(I)



(II)

However, since the acid dissociation constants of L-histidine are pK_1(COOH); 1.82, pK_2(Im); 6.00—6.08 and pK_3(NH_3^+); 9.17—9.20,[14,15] the most reasonable structure of the complex formed in a 50% ethanol-water solution is, in view of the above data, considered to be that of Type II, especially on the basis of the optimum pH values for this complex formation.

The author wishes to express his deep gratitude to Professor Dr. Daisei Yamamoto of Kumamoto University for his kind direction and encouragement.

13) A. J. Woiwood, *Biochem. J.*, **45**, 412 (1949).
14) E. J. Coh and J. T. Edsall, "Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions," Reinhold, New York (1943), p. 84.
15) J. T. Spence and J. Y. Lee, *Inorg. Chem.*, **4**, 385 (1965).