UDC 547.831.7 : 541.486 : 545.822

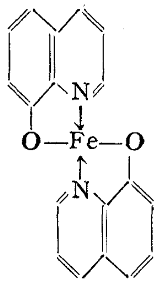
17. **Yoshinori Kidani and Naoki Chiba**: Spectrophotometric Studies on Copper Chelates of Oxine and its N-Oxide.

(*International Christian University**)

Many investigations have been reported on the complexes of oxine (8-hydroxyquinoline). Albert, *et al.*[1,2] recently proposed the hypothesis that oxine showed antibacterial action against *Staphylococcus aureus*, in the presence of iron or copper, and such action was no longer observed when these metals were removed from the medium. This action may be well explained by the formation of a complex (I) which possesses a toxic action against bacteria. In the presence of excess oxine, (II) is formed instead of (I). In formula (II) metal-avid groups are no longer able to combine, as it is well-saturated with iron or copper. Therefore, these compounds are said to be non-toxic.

(I)

(II)



Erlenmeyer, *et al.*[3,4] also experimented the effect of oxine, as well as the compounds which possess oxine-like structure, against *Mycobacterium tuberculosis* and observed a fairly strong effect. This phenomenon was also explained by the formation of metal chelate compounds.

Phenazine derivatives which possess oxine-like structure have been studied and, in order to examine the relationship between chemical properties and biological action of phenazine-copper chelates, preliminary spectrophotometric studies on the copper chelates of oxine and its N-oxide have been carried out. Though the structure was not quite the same to oxine, its N-oxide[5] also possesses a chelating functional group and a similar investigation has been carried out.

Experimental

Apparatus—(a) pH measurements were made with a Toyo-Rika glass-electrode pH meter, direct-reading type.

(b) Measurements of absorbance were made with a Shimadzu Electro-Spectrophotometer, Model QB-50, using glass and quartz cells of 10-mm. light path.

Materials—(a) Oxine: A $2\times10^{-4}M$ solution was prepared by dissolving 2.90 mg. of oxine in 100 cc. of dehyd. EtOH. (b) Oxine N-Oxide: A $2\times10^{-4}M$ solution was prepared by dissolving 3.22 mg. of oxine N-oxide in 100 cc. of dehyd. EtOH. (c) Copper acetate: $1\times10^{-2}M$ and $2\times10^{-4}M$ solutions were prepared by dissolving 0.199 g. or 3.99 mg. of $Cu(OAc)_2$ in 100 cc. of dehyd. EtOH, respectively.

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* 1500 Osawa, Mitaka-shi, Tokyo (喜谷喜徳, 千葉直樹).

1) A. Albert: Symposium, Growth Inhibition and Chemotherapy. VIth International Congress of Microbiology, 1953.

2) A. Albert, D. Magrath: Biochem. J., **41**, 534(1947).

3) E. Sorkin, W. Roth: Helv. Chim. Acta, **34**, 427(1951).

4) E. Sorkin, W. Roth, H. Erlenmeyer: *Ibid.*, **35**, 1736(1952).

5) I. Murase: Nippon Kagakkai Zasshi, **75**, 1180(1954).

Results

I. Oxine-Copper Chelate

(**1**) **Absorption Spectra**—When one drop of $1\times10^{-2}M$ EtOH solution of $Cu(OAc)_2$ was added to 5 cc. of $2\times10^{-4}M$ EtOH solution of oxine, the solution assumed a faint yellowish green color. Absorption curves of the reagent (solid line) and of the copper chelate (dotted line) solutions are shown in Fig. 1, at pH 8.0. λ_{max} of the copper chelate appeared at 290 and 400 mμ. No marked change was observed in reagent or copper absorptions when pH was varied.

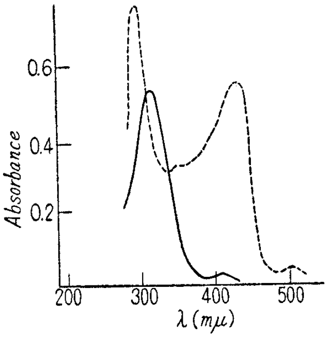

Fig. 1. Absorption Spectra of Oxine-Copper Chelate

(The solution contains $2\times10^{-4}M$ of oxine)

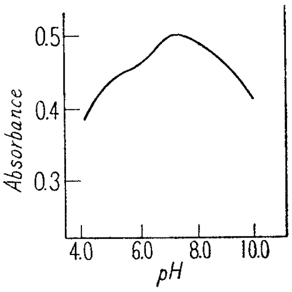

Fig. 2. Effect of pH Change on Oxine-Copper Chelate, measured at 400 mμ

(The solution contains $2\times10^{-4}M$ of oxine)

(**2**) **Effect of pH Change**— pH of the solution was varied by dissolving KOH or AcOH in EtOH in a range of pH 4.0~10.0. It showed a maximum absorption at pH 7.5~8.5, at 400 mμ. The absorption decreased if the pH increased or decreased, as shown in Fig. 2.

(**3**) **Composition of the Chelate**—Composition of a chelate was determined by Job's method of continuous variation⁶⁾ and by the molar ratio method.⁷⁾ At the wave length of 400 mμ, the ratio of copper to oxine was 1:2 at a pH between 7.0 and 10.0. The measurement at pH 5.0 gave the minimum at 290 mμ at a ratio of about 0.45, indicating the ratio to be 1 : 1.

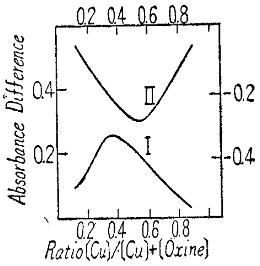

Fig. 3. Absorption Curves of Oxine-Copper Chelate (Job Method)

(Measured at 400 mμ, at pH 8.0 (I) and at 290 mμ, at pH 5.0 (II). The total concentration is $2\times10^{-4}M$)

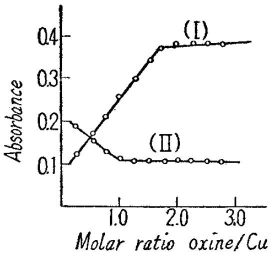

Fig. 4. Absorption Curves of Oxine-Copper Chelate (Molar Ratio Method)

(Measured at 400 mμ, at pH 8.0 (I) and at 290 mμ and pH 5.0 (II). The concentration of copper is $4\times10^{-5}M$)

In the molar ratio method, the concentration of copper was maintained constant at $4\times10^{-4}M$ and molar ratio of copper to oxine varied from 0.25 to 3.0. The results shown in Fig. 4 indicate a 2 : 1 ratio at pH 8.0, 400 mμ, and a 1 : 1 ratio at pH 5.0, 290 mμ.

(**4**) **Dissociation Constant**—The dissociation constant was calculated from the results obtained by the molar ratio method. The average $-\log K$ was 8.43, at pH 8.0, 400 mμ, in the normal complex.

(**5**) **Structure**—From the results obtained, the following structures for the copper chelate of oxine are considered, forming a five-membered ring. (III) is a normal complex and (IV) is assumed to be a cation complex.

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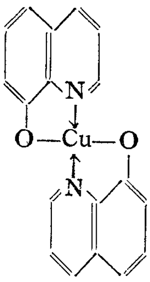
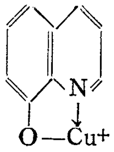
6) P. Job: Ann. Chim., [10] **9**, 113(1928).

7) J. H. Yoe, A. L. Jones: Ind. Eng. Chem., Anal. Ed., **16**, 111(1946).

(III)



(IV)



II. Oxine N-Oxide-Copper Chelate

All the procedures of the spectrophotometric measurements were carried out under the same conditions.

(1) **Absorption Spectra**—When one drop of $1\times10^{-2}M$ EtOH solution of $Cu(OAc)_2$ was added to 5 cc. of $2\times10^{-4}M$ EtOH solution of oxine N-oxide, no marked change of coloration was observed. Absorption curves of both the reagent and copper chelate solutions are shown in Fig. 5. λ_{max} of the copper chelate occurred at 360 mμ and it is close to that of the reagent, which shows maximum absorption at 350 mμ. For measurements, the wave length of 450 mμ was selected.

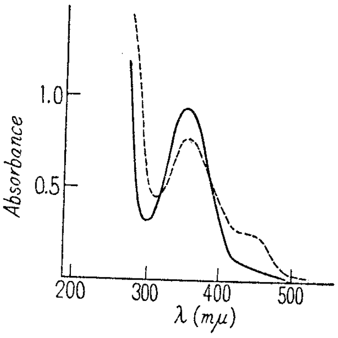


Fig. 5. Absorption Spectra of Oxine N-Oxide-Copper Chelate

(The solution contains $2\times10^{-4}M$ of oxine N-oxide)

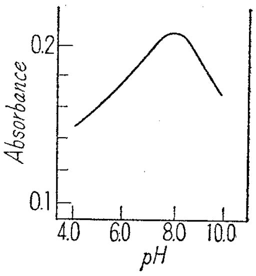


Fig. 6. Effect of pH Change on Oxine N-Oxide-Copper Chelate

(Measured at 450 mμ. The solution contains $2\times10^{-4}M$ of oxine N-oxide)

(2) **Effect of pH Change**—The maximum absorption appears at pH 8.0, when measured at 450 mμ, and the absorption decreases if the pH is increased or decreased, as shown by the curves in Fig. 6.

(3) **Composition of Chelate**—Composition of the chelate was determined by Job's method of continuous variation and by the molar ratio method. In Fig. 7 are shown curves obtained by Job's method. At pH 8.0, the ratio of copper to oxine N-oxide is 1:2, at 450 mμ.

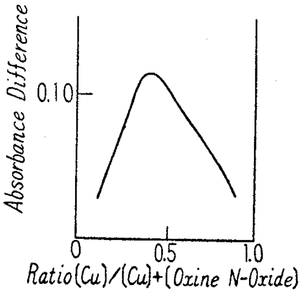


Fig. 7. Absorption Curve of Oxine N-Oxide-Copper Chelate (Job Method)

(Measured at 450 mμ and at pH 8.0. The total concentration is $2\times10^{-4}M$)

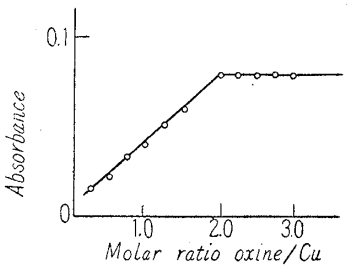


Fig. 8. Absorption Curve of Oxine N-Oxide-Copper Chelate (Molar Ratio Method)

(Measured at 450 mμ and at pH 8.0. The concentration of copper is $2\times10^{-4}M$)

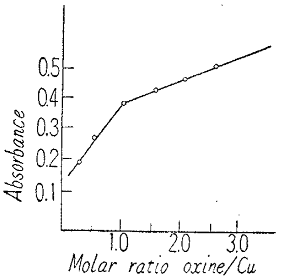


Fig. 9. Absorption Curve of Oxine N-Oxide-Copper Chelate (Molar Ratio Method)

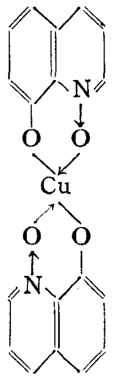
(Measured at 360 mμ and at pH 6.5. The concentration of copper is $2\times10^{-4}M$)

By the molar ratio method, at pH 8.0, the ratio of oxine N-oxide to copper at the wave length of 450 mμ, was 2:1. When a measurement was carried out in an acid medium (pH 6.5) at 360 mμ, shown in Fig. 9, a sharp break was observed at a ratio of 1 : 1, though at this wave length, absorption of reagent interferes with that of the chelate formed.

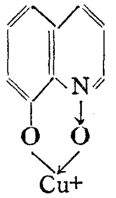
(4) **Dissociation Constant**—The value of $-\log K$, obtained from the molar ratio method, is 7.99, at pH 8.0, and at 450 mμ, in the normal complex.

(5) **Structure**—From the results obtained, the structure of copper chelate of oxine N-oxide is considered to be (V) for normal complex and (VI) for a possible cation complex, forming a six-membered ring.

(V)



(VI)



In neutral media, normal complexes of both oxine and its N-oxide were observed. When the measurements were carried out in an acid medium, the cation complex, postulated by Albert, was considered to be present.

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Summary

Spectrophotometric studies of copper complexes of oxine and its N-oxide were carried out in ethanol solutions. At pH 8.0, the chelates of both oxine and its N-oxide indicated the normal complex and, when the measurements were carried out in an acid medium, the formation of a cation complex was taken into consideration.

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