(7) Bolin, D. W., *J. Agr. Research*, **48,** 657 (1934).

(8) Brown, L. C., and Viets, F. G., Jr. *Agron. J.*, **44,** 276 (1952).

(9) Coppenent, M., *Compt. rend. acad. agr. France*, **38,** 193 (1952).

(10) Cunningham, H. M., Brown, J. M., and Eddie, A. E., *Can. J. Agr. Sci.*, **33,** 254 (1953).

(11) Cunningham, I. J., *New Zealand J. Agr.*, **69,** 559 (1944).

(12) Davis, G. K., Symposium on Copper Metabolism, pp. 216–29, Johns Hopkins Press, Baltimore, 1950.

(13) De Renzo, E. C., Kaleita, E., Heytler, P. G., Oleson, J. J., Hutchings, B. L., and Williams,

J. H., *J. Am. Chem. Soc.*, **75,** 753 (1953).

(14) Evans, H. J., Purvis, E. R., and Bear, F. E., *Anal. Chem.*, **22,** 1568 (1950).

(15) Marston, H. R., *Phys. Rev.*, **32,** 66 (1952).

(16) Price, N. O., Linkous, W. N., and Hill, H. H., Va. Agr. Expt. Sta., *Bull.* 117 (1951).

(17) Richert, D. A., and Westerfeld, W. W., *J. Biol. Chem.*, **203,** 915 (1953).

(18) Rigg, T., Cawthorn Inst., Nelson, New Zealand, *Ann. Rept.* **1940,** 12–14.

(19) Rigg, T., New Zealand Dept. Sci. Ind. Research, *Ann. Rept.*, **14,** 41 (1940).

(20) Robinson, W. O., *Soil Sci.*, **66,** 317 (1948).

(21) Soil Survey of Culpeper County, Virginia, U. S. Dept. Agr., Series 1941, No. 3 (1952).

(22) Soil Survey of Orange County, Virginia, U. S. Dept. Agr., Series 1927, No. 6 (1930).

(23) Underwood, E. J., and Bennetts. H. W., "Trace Element Deficiencies in Stock in Western Australia," Brit. Commonwealth Sci. Office (Australia), Proc. Spec. Conf. Plant and Animal Nutrition, p. 266 (1949).

## CHELATE FUNGICIDES

# Fungitoxicity of the 8-Quinolinols

**S. S. BLOCK**

**Engineering and Industrial Experiment Station, University of Florida, Gainesville, Fla.**

The 8-quinolinols (oxines) and their chelates have been of commercial importance as fungicides in industry and medicine and highly effective in agricultural applications. Their fungitoxic activity was studied, employing different derivatives under different conditions of acidity. Ability to chelate and lipoid solubility were found requisite for the activity of this group. The copper chelates were, in most cases, many times more fungitoxic than the unchelated compounds. It is suggested that both the chelator and the metal function in producing the unusually high antifungal activity of these chelates.

THE COPPER CHELATE of 8-quinolinol (oxine, 8-hydroxyquinoline, *22*) is one of the most highly rated fungicides developed in recent years. Its effectiveness in the preservation of textiles, paint, and miscellaneous industrial products has been the subject of numerous papers (*10–12, 14, 17, 18, 24, 28, 30, 38–40*). A method of rendering the compound soluble in many common organic solvents (*25–27*) has extended its usefulness. Its merit as an agricultural fungicide has been recognized (*8, 15, 16, 35, 45, 48, 49*), but economic considerations have limited this application. Recent results, however, indicate that the cost factor may be minimized by combining this copper chelate with low-cost fungicides, without losing its protective properties against certain plant diseases (*36*).

Unlike the copper chelate, the parent compound, oxine, is no newcomer to the field of antimicrobial chemicals. As the active agent of Chinosol, it has been in use as an antiseptic and disinfectant since about 1895 (*5*), but only in recent years have its antibacterial and antifungal properties been re-examined in some detail.

Analytical chemists have shown great interest in oxine and its relatives because of the ability of these compounds to chelate with trace quantities of metals and form precipitates and colored solutions. Indeed, the ability to chelate with and precipitate metals essential for cell metabolism has been proposed as the mechanism for the antimicrobial activity of oxine (*1, 50, 51*). Zentmyer (*51*) demonstrated that its toxicity to fungi could be overcome by the addition of excess zinc to the medium. Albert and coworkers (*6*) found that structural modifications preventing chelation resulted in markedly decreased toxicity to bacteria. Other workers (*31, 43, 46, 47*) found that an excess of certain metals in the medium could eliminate the toxicity of oxine and copper oxinate.

On the other hand, the fact that the copper, nickel, cadmium, and silver salts of oxine, which are saturated with respect to metal, had high fungistatic activity indicated to Sexton (*44*) that chelation was not the basis for the toxicity. He suggested, as had Hata (*23*), that oxine owes its toxicity to its phenolic properties. Mason (*32*) and Manten and coworkers (*31*) shared this view. The examination of many chelating compounds for toxicity to fungi and bacteria (*6, 7, 42*) has clearly demon-

strated that ability to chelate is not necessarily synonymous with toxicity.

In more recent work, Rubbo and coworkers (*41*) and Albert and coworkers (*3*) demonstrated the startling fact that oxine owes its toxic effect on bacteria to the metal chelate alone, and if a medium is depleted of iron and copper, oxine is no longer inhibitory. Their theory of the mechanism of action (*3*) is that the chelate enters the cell as the 2 to 1 (oxine to divalent metal) complex and that
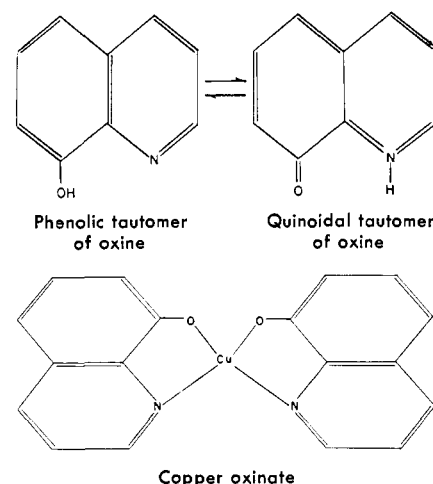


| Phenolic tautomer of oxine | Quinoidal tautomer of oxine |



Copper oxinate

within the cell this form is in equilibrium with 1 to 1 complex, which is said to be the true toxic agent. The reversals in the presence of excess metals are explained by the inability of the ionically charged 1 to 1 chelate, which is produced in the presence of excess metal ions, to penetrate the cell membrane. Chelation, therefore, is suggested as important only in providing a means for the toxicant to enter the cell; but metal poisoning is the ultimate mode of action.
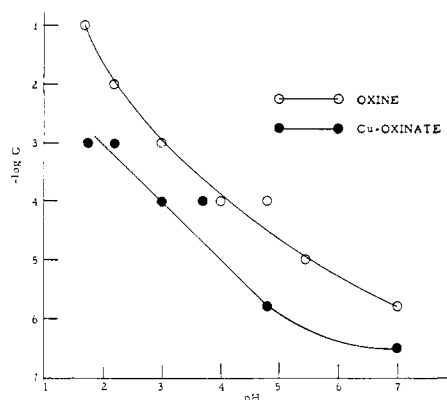


**Figure 1. Effect of pH on toxicity**

Ordinate is negative logarithm of molar concentration for complete inhibition

The high fungitoxicity of oxine and its chelates, as well as their industrial importance, suggested the desirability of further investigation of the phenomenon of chelation as related to the antimicrobial activity of these materials.

### Oxine and Chelates

Fleck and Ward (19) have shown that the ability to chelate metals decreases with increasing acidity. According to Goto (21), who studied the precipitation of 15 metals with oxine, none started to precipitate below pH 2.2. It was of interest, therefore, to determine the fungitoxicity of oxine and copper oxinate over a range of acidities for the purpose of determining how closely toxicity followed chelation. In order to obtain results at acidities below pH 2.2, the fungus Aspergillus niger was adopted as the test organism because of its known ability to tolerate high acidities. It was used in a standard toxicity test (37), in

agar which the toxicant was incorporated in tenfold stepwise concentrations from $10^{-6}$ to $10^{-3}M$. The agar was inoculated with fungus spores at the center of Petri plates and the radial growth of the fungus colony was compared with that of a control containing no toxicant. The results are given in Figure 1 with pH as the abscissa and, as ordinate, the negative logarithm of the minimum concentration of toxicant for total inhibition of mold growth. As the pH decreases, the toxicity of oxine and of its copper chelate likewise decreases. In the case of oxine prevention of fungal growth requires at pH 1.7 a concentration about 50,000 times that needed at pH 7.0. At all pH values the copper chelate was more toxic than oxine, but its activity was similarly reduced as the pH was lowered.

Being amphoteric, oxine exists in solution as three different entities: the un-ionized molecules, the cations, and the anions. Taking the data for oxine just given, the concentration of ions and un-ionized molecules was calculated and the data were plotted according to the method described by Albert (2). The curves in Figure 2, plotted on the same basis as those in Figure 1, present each entity as if it were individually responsible for the toxicity of the compound. If the toxicity of each entity is independent of pH, its concentration which will prevent growth of the fungus should remain constant as the pH is varied. From Figure 2, it can be observed that both the anions and the un-ionized molecules have a substantially constant inhibitory concentration over the greater portion of the pH range tested. Further extension of the pH range of the tests was prevented by the growth limitations of the fungus.

According to Phillips (34), 4-methyloxine precipitates copper and iron in the presence of oxine, as proved by analysis of the precipitates. This demonstrates that 4-methyloxine is a more efficient chelator than oxine and raises the question whether it will be more toxic than oxine. At pH 4.8 the reverse is true; oxine is more toxic than 4-methyloxine. Computation, however, discloses that at pH 4.8 there are about 3.5 times as many un-ionized molecules of oxine as of 4-methyloxine (Table I). At pH 5.45 the ratio of neutral molecules is less than

two to one in favor of oxine, and the superiority of oxine is less marked. At pH 6.7 both compounds are principally in the un-ionized condition, and the order of toxicity is reversed in favor of the methyl derivative.

### Toxicity of Copper Chelates

Table II compares the fungitoxicity of a number of oxine derivatives and their copper chelates. In all but one case the copper chelates were 25 or more times as toxic as the unchelated compounds. The one exception was the dichlorooxine; the lower activity of the copper chelates at the higher pH values is typical of the dihalogenated oxines.
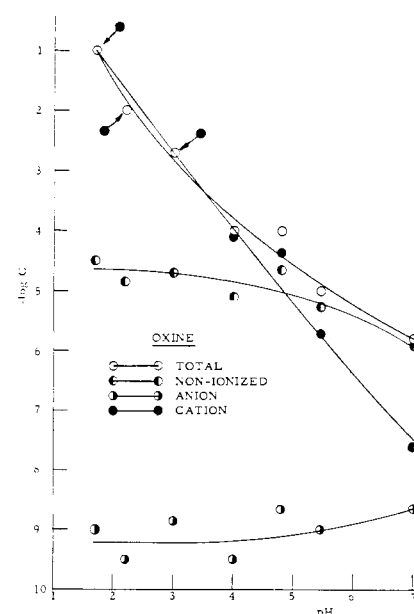


**Figure 2. Effect of pH on toxicity of oxine and its ions**

Ordinate is negative logarithm of molar concentration of each entity when oxine gives complete inhibition

Copper is a well-known fungicide, but it is relatively ineffective against Aspergillus niger, the organism used in these tests. [At pH 4.8 a concentration of $10^{-1}M$ copper acetate is required to prevent growth of A. niger (Table III).] The work of Maley and Mellor (29) has shown that in stability copper oxinate tops the list of a series of metal oxinates. Chelated oxine is less ionized than oxine, and the data suggest a correlation between concentration of un-ionized molecules and fungitoxicity. It is possible, then, that copper serves not as a fungicide but merely to reduce ionization. If the latter hypothesis is correct, the metals that chelate less strongly should form less toxic oxinates than copper oxinate.

Zinc, ion, and magnesium also react with oxine, giving chelates with the following order of stability (29): copper> zinc>iron>magnesium. Figure 3 shows that the toxicity of these chelates at pH 4.8 is in the same order. These

**Table I. Comparative Fungitoxicity and Ionization of Oxine and 4-Methyloxine**

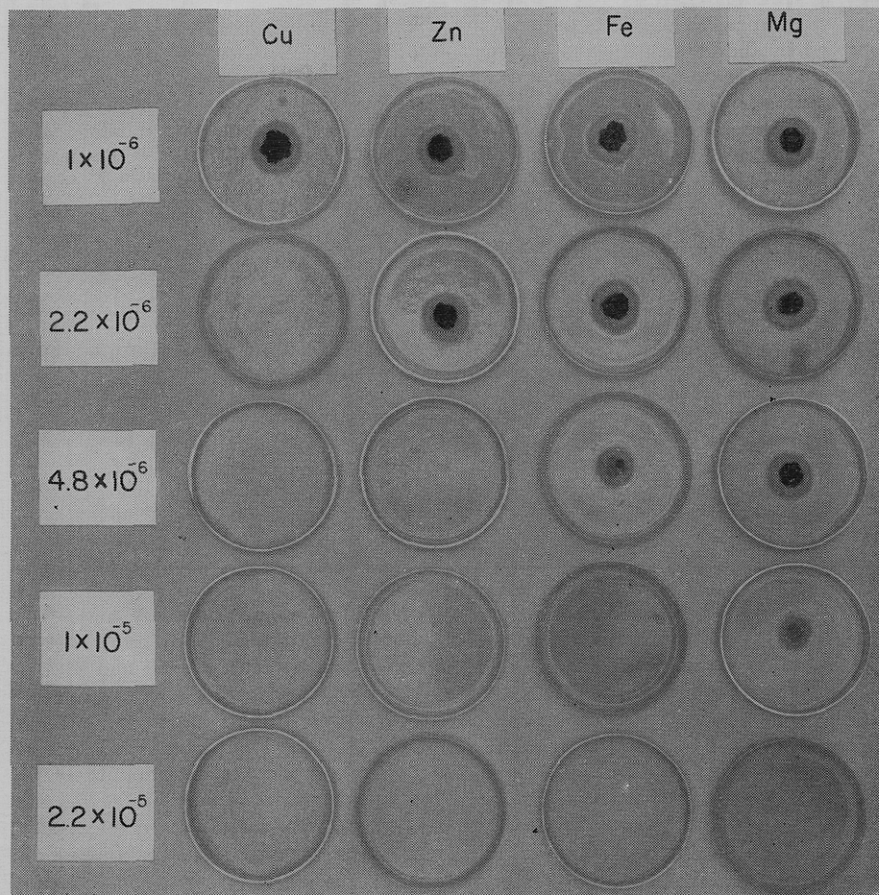| pH | Compound | % of Compound as | | | % Inhibition of Growth | |
| | | Anions | Cations | Un-ionized molecules | Concn., M | % Inhibition |
|---|---|---|---|---|---|---|
| 4.8 | Oxine | 0.001 | 61.3 | 38.7 | $7 \times 10^{-5}$ | 59 |
| | 4-Methyloxine | 0.0007 | 88.8 | 11.2 | $7 \times 10^{-5}$ | 17 |
| 5.45 | Oxine | 0.008 | 22.0 | 78.0 | $2 \times 10^{-6}$ | 55 |
| | 4-Methyloxine | 0.005 | 58.0 | 42.0 | $2 \times 10^{-6}$ | 29 |
| 6.7 | Oxine | 0.100 | 1.56 | 98.3 | $10^{-6}$ | 11 |
| | 4-Methyloxine | 0.050 | 7.36 | 92.6 | $10^{-6}$ | 31 |

data suggest that a chelate more stable than the copper chelate should be more toxic. While the stability constant for palladium oxinate is not available, in other complex combinations palladium forms a more stable chelate than does copper. As the order of stability of the metal chelates with different chelating agents is generally the same (29), it may be assumed that palladium forms a more stable chelate with oxine than does copper. Furthermore, palladium oxinate is dissolved at a pH much lower than that required to dissolve other metal oxinates of the Maley-Mellor series, and the lower the pH of chelate formation within a series, the greater the stability (33). The toxicity data for copper oxinate and palladium oxinate (Table III) show that the palladium chelate is less rather than more fungitoxic than the copper chelate, a fact which makes it apparent that chelate stability is not the only property influencing toxicity.

### Halogenated Oxines

In this attempt to ascertain the relationship between chelation and toxicity in the oxine series of compounds, derivatives of oxine that would be capable of forming metal chelates in acid solutions where oxine does not chelate would be extremely useful. Such a group of compounds were found in the 5,7-dihalogenated oxines (13). In Table IV are presented the comparative data for the fungitoxicity of oxine and its dihalogenated derivatives as well as for the copper chelates of these compounds at three acidities. The surprising revelation of these data is the high toxicity of the dihalogenated oxines and their copper chelates at pH 1.5. These compounds are approximately as toxic at pH 1.5 as oxine and copper oxinate at pH 7 (Figure 4). Mason (32) has reported the dichloro-, dibromo-, and dinitro-oxines to be inactive. His tests, however, were conducted in a comparatively neutral solution. The dihalogenated series differs from the unsubstituted oxine in that the toxicity of the former decreases rather than increases with increasing pH. A comparison within the dihalogenated series shows



**Figure 3.** Comparative toxicity of metal chelates of oxine at pH 4.8

Molar concentrations given at left

that there is a pattern of decreasing fungitoxicity: chlorine>bromine>iodine. The copper chelates of the dihalogenated oxines exhibit the same general pattern as the unchelated oxines. At pH 1.5 the copper chelates are of the same order of toxicity as the related unchelated oxines, but at pH 4.0 and 7.0 they are decidedly less active.

For dichloro-oxine, further toxicity data were obtained at intermediate pH values and, as is shown in Figure 5, curves were plotted for the total compound, its un-ionized molecules, and its ions. Examination of Figure 5 shows that dichloro-oxine behaves differently from oxine. With dichloro-oxine, the toxicity is related to no single entity as in the case of oxine.

One might wonder whether the mono-chloro-oxines would resemble either oxine or the dichloro compound, or would be hybrids. From Table V it is possible to observe the effect of progressive substitution of chlorine in oxine on the ionization and the fungitoxicity. The nature of the activity of 7-chloro-oxine was related more to that of oxine, while 5-chloro-oxine (and the 5-nitro derivative) appeared to behave more like the dichloro derivative, although both showed hybrid characteristics. A thousandfold increase in the un-ionized oxine molecules from pH 2.0

to pH 5.7 was accompanied by a thousandfold increase in toxicity, while a hundredfold increase in the neutral molecules of 7-chloro-oxine brought about a hundredfold increase in toxicity. At pH 2.0, 7-chloro-oxine has ten times the number of neutral molecules as oxine and is ten times as toxic. As pH 5.7, both compounds are ionized to about the same degree and have about the same activity. These relationships did not hold for the 5-chloro- or the 5,7-dichloro-oxines, which were less affected by the change in acidity of the medium. That the "ring substituent effect" is not limited to the halogens is shown by the fact that the behavior of the 5-nitro- and 5,7-dinitro-oxines is similar to that of their chlorine analogs. Barratt and Horsfall (9) and later Mason (32) noted with the oxinates the unusual "double peaked" dosage-response curve characteristic of the dithiocarbamate fungicides. The only compound that gave this unusual response in the present work was the copper chelate of 5,7-diiodo-oxine at pH 1.5. This is illustrated in Figure 6.

### Discussion

The data with oxine show that as the pH is progressively lower from 7 to 1.7 the toxicity is decreased until it prac-

### Table II. Comparative Fungitoxicities of Oxines and Their Copper Chelates at pH 4.8

| Compound | Concn. Required for Complete Inhibition, M | |
| | Unreacted compound | Copper chelate |
| --- | --- | --- |
| Oxine | $10^{-4}$ | $2 \times 10^{-6}$ |
| 4-Methyloxine | $>10^{-4}$ | $4 \times 10^{-6}$ |
| 5-Methyloxine | $10^{-4}$ | $4 \times 10^{-6}$ |
| 7-Chloro-oxine | $>10^{-4}$ | $4 \times 10^{-6}$ |
| 5-Chloro-oxine | $10^{-3}$ | $4 \times 10^{-5}$ |
| 5,7-Dichloro-oxine | $10^{-4}$ | $>10^{-4}$ |

tically vanishes. The same pattern is followed by the copper chelate. Oxine is known to chelate with many metals at pH 7, but the number decreases as the pH is lowered and at pH 1.7 is reported to be incapable of chelation with any of the common metals. The dihalogenated oxines, on the contrary, chelate readily at pH 1.5 and are highly toxic to fungi at this acidity.

lipoid solubility is indeed important to the toxicity of oxine and related chelators.

This basis of explanation may then be examined in the case of the dihalogenated oxines. At pH 1.7 there is a considerably greater concentration of neutral molecules of dichloro-oxine in equimolar solutions than of oxine. Nevertheless, like oxine, dichloro-oxine exists essen-

also demonstrated that in each case where high toxicity was manifested the compound was highly soluble in the oil, whereas, where it was relatively nontoxic, it was preferentially soluble in the aqueous phase.

Although the data were gathered to test the chelation theory of toxicity, they can be equally well explained on the basis of lipoid solubility. In earlier work (6), Albert and coworkers have shown that the 5-carboxylic acid derivative of oxine, which is hydrophilic but capable of chelation, is nontoxic, but if this compound is esterified, making it hydrophobic, toxicity is restored. It would thus appear that lipoid solubility is definitely necessary for toxicity. In order to examine the other possibility—where oil solubility is retained but ability to chelate is lost—the methoxy derivative of 5,7-dichloro-oxine was prepared and tested. This compound was nontoxic regardless of the pH of the medium. It would appear, therefore, that both lipoid solubility and the ability to chelate are required for toxic activity by members of this group of compounds based upon oxine.

### Table III. Comparative Fungitoxicities of Palladium and Copper Salts and Oxinates at pH 4.8

| Compound | % Inhibition of Growth at Molar Concns. of | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $5 \times 10^{-6}$ | $2.5 \times 10^{-6}$ | $10^{-6}$ |
| Copper oxinate | ... | ... | ... | ... | 100 | 100 | 100 | 0 |
| Palladium oxinate | ... | ... | ... | ... | 100 | 32 | 18 | −5 |
| Cupric acetate | 100 | 72 | 47 | 0 | ... | ... | ... | ... |
| Palladium nitrate | ... | ... | 100 | 18 | 3 | ... | ... | ... |

In addition to the relationships between pH and toxicity and pH and chelation, a third relationship that has been considered is that of pH and ionization. Calculation shows that whereas oxine is almost completely unionized at pH 7, it is almost completely in the form of cations at pH 1.7. According to Overton's concept of cell permeability, the lipoid membrane that surrounds the cell favors the passage through that membrane of lipoid-soluble materials but restricts the passage of electrolytes. Oxine and copper oxinate would come under the former classification, whereas the oxine cation and the dissociated copper oxinate, as they exist at pH 1.7, would come under the latter. If it is assumed that lipoid solubility is important to the function of oxine, as differentiated from those fungitoxic compounds which are electrolytes, another basis for explaining the effect of pH on the toxicity of oxine is suggested. Recent work (4) has demonstrated that

tially as cations at pH 1.5 and if the same quantitative relationship existed between neutral molecules and toxicity as with oxine, dichloro-oxine would be approximately 100 times as toxic at pH 5 as at pH 1.5. This is not the case. To explain the nonconformance of the dichloro-oxine, the possibility that the dichloro-oxine cation might be capable of membrane penetration despite its charge, owing to the lipophilic halogens, was considered. This possibility was put to the test.

Oxine, dichloro-oxine, methyloxine, and their copper chelates were dissolved in chloroform and extracted twice with an equal volume of aqueous phosphate buffer at pH 6.0 and 1.5. The extracted chloroform solutions were read in the spectrophotometer at 262 m$\mu$ and the percentage transmittance was recorded in Table VI. Also in Table VI are given the ratios of each compound remaining in the chloroform after extraction at pH 6 and pH 1.5. Oxine, as would be expected, remained in the oil layer at pH 6 but transferred to the aqueous layer at pH 1.5. Dichloro-oxine, on the other hand, remained in the oil layer despite the pH. Thus the toxicity of dichloro-oxine and the lack of toxicity of oxine at the low pH are a function of their oil solubilities. The methyl derivative, which acts like oxine in regard to toxicity with change in pH, was similar to oxine in solubility at the high and low pH. The copper chelates

### Table IV. Fungitoxicity of Oxine, Dihalogenated Derivatives, and Copper Salts

[Concentrations expressed as negative logarithm of minimum concentration (molar) to effect total inhibition]

| Compound | pH | | |
|---|---|---|---|
| | 1.5 | 4.0 | 7.0 |
| Oxine | 1 | 3 | 6 |
| 5,7-Dichloro-oxine | 6 | 5 | 4 |
| 5,7-Dibromo-oxine | 5 | 4 | 4 |
| 5,7-Diiodo-oxine | 5 | <3 | <3 |
| Copper oxinate | 3 | 4 | 6 |
| Copper-5,7-dichloro-oxinate | 6 | <3 | <3 |
| Copper-5,7-dibromo-oxinate | 5 | <3 | <3 |
| Copper-5,7-diiodo-oxinate | 5 | 3 | <3 |

In view of the cited reports (41) that oxine is not toxic in the absence of chelating metals, it should be indicated that the cited work was done with bacteria. Considerable work with oxine in a metal-deficient medium, to be published later, has indicated that oxine is inhibitory to fungi.

If oxine in its own right is toxic to fungi, the copper chelate is much more toxic. To explain the greater toxicity of the chelate, it has been suggested (7) that the copper and iron oxinates are much more soluble in organic solvents than in water, whereas with oxine this partition is not so marked. This does not appear to explain the greater toxicity of the copper chelate, because oxine, owing to its internal hydrogen bonding, possesses a high partition coefficient

### Figure 4. Comparative toxicity of oxine and dichloro-oxine at pH 1.7

Left. Untreated control
Top. $10^{-2}$ and $10^{-1}$M oxine
Bottom. $10^{-6}$ and $10^{-5}$M dichloro-oxine

| Compound | pH | Concn. for Complete Inhibition, M | % of Compound as | | |
|---|---|---|---|---|---|
| | | | Anions | Cations | Un-ionized molecules |
| Oxine | 2.0 | $10^{-2}$ | 0.000005 | 99.9 | 0.09 |
| | 5.7 | $10^{-5}$ | 0.016 | 9.0 | 91.0 |
| 7-Chloro-oxine | 2.0 | $10^{-3}$ | 0.0003 | 99.0 | 0.99 |
| | 5.7 | $10^{-5}$ | 1.96 | 00.99 | 97.0 |
| 5-Chloro-oxine | 2.0 | $10^{-4}$ | 0.00003 | 98.4 | 1.56 |
| | 5.7 | $10^{-4}$ | 0.20 | 0.63 | 99.2 |
| 5,7-Dichloro-oxine | 2.0 | $10^{-5}$ | 0.003 | 97.6 | 2.45 |
| | 5.7 | $10^{-4}$ | 9.09 | 0.79 | 90.1 |
| 5-Nitro-oxine | 2.0 | $10^{-4}$ | ... | ... | ... |
| | 5.7 | $10^{-4}$ | ... | ... | ... |
| | 7.0 | $10^{-4}$ | ... | ... | ... |
| 5,7-Dinitro-oxine | 2.0 | $10^{-5}$ | ... | ... | ... |
| | 5.7 | $>10^{-4}$ | ... | ... | ... |
| | 7.0 | $>10^{-4}$ | ... | ... | ... |

favoring the oil (4). The explanation that ionization is minimized by chelation was bolstered by the finding that toxicity of the chelates was in the same order as the chelates in the stability series. Contrary evidence, however, is observed in the greater activity of the copper chelate over unchelated oxine near the isoelectric point. If stability of chelation were the criterion of activity, the palladium chelate should be expected to be greater than that of the copper chelate, but it was less active.

Furthermore, the toxicity of ionic salts of copper, zinc, iron, and magnesium, although much less than their chelates, fell in the same order of toxicity. It is likely, then, that the metals are toxic but are better transported to their site of action in the form of the chelate. The fact that other chelates are not similarly toxic suggests that oxine contributes special properties to the chelate which enhance the toxicity.

Albert has proposed (2) that oxine removes a "guardian metal," such as cobalt, which protects cell thiol groups, and, then, the iron or copper of the chelate promotes oxidation of the essential thiol. Gerber and Block have shown (20) that oxine is a strong inhibitor of the cresolase (polyphenoloxidase) enzyme. This is a copper-containing enzyme which occurs widely in the fungi. Oxine was, in fact, the strongest inhibitor of all the compounds, chelators and nonchelators, tested against this enzyme. Dichloro-oxine was also inhibitory, but copper oxinate, which is already chelated, was not. It is possible that within the cell the copper of the chelate is transferred to another system with strong complexing power, such as the porphyrins or thiols, and the oxine remaining may combine with cresolase or other metal enzymes.

## Summary

A study of the relationship of chelation to fungitoxicity of the oxines showed that oxine, which loses its ability to chelate as

the pH is lowered, also loses its toxicity. The 5,7-dihalogenated and dinitro-oxines, which retain their ability to chelate, retain their toxicity as the pH is lowered. Of the monohalogen and mononitro-oxines, the derivatives with substituents in the 5 position more closely resemble the disubstituted derivatives in activity, whereas that substituted in the 7 position of the ring more closely resembles oxine. Methoxy-dichloro-oxine, which is unable to chelate because the hydroxyl group in the 8 position is reacted, is inactive at both high and low pH.
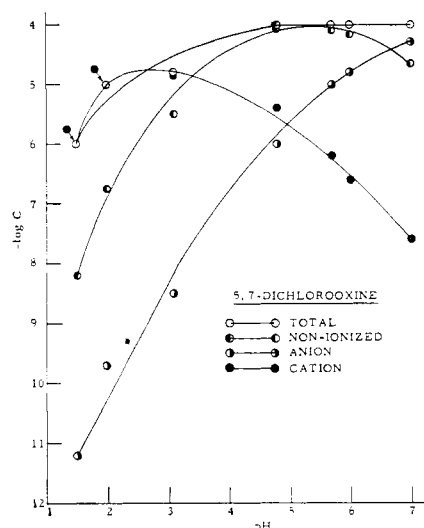


**Figure 5. Effect of pH on toxicity of dichloro-oxine and its ions**

Ordinate is negative logarithm of molar concentration of each entity when oxine gives complete inhibition

By computing the degree of ionization of oxine across the pH range, it was found that its loss of activity to A. niger as the pH was lowered to below 2 was quantitatively related to its increase in ionization. This relationship between activity and ionization was found also for 4-methyloxine and 7-chloro-oxine, but did not hold for 5-chloro- and 5,7-dichloro-oxine. A more fundamental

relationship superseding ionization was found in the lipoid solubility of the compounds, which could explain the difference in activity between oxine and the dihalogenated oxine with reference to pH. As oxine becomes more highly ionized it becomes less soluble in oil solvents, whereas dichloro-oxine retains its lipoid solubility even at pH 1.5. Both lipoid solubility and chelation are essential for the fungitoxic activity of the oxines.

The copper chelates of all but the dihalogenated oxines were many times more fungitoxic than the chelators. Oxine itself, however, in metal-deficient medium was definitely inhibitory. The order of toxicity to A. niger of the oxine chelates was copper>zinc>iron>magnesium. The palladium chelate, although more stable than the copper chelate, was not so toxic. Both the chelator and the metal are believed to contribute to the high fungitoxicity of the metal chelates of the oxines.

## Literature Cited

(1) Albert, A., Med. J. Australia, 1, 245-8 (1944).
(2) Albert, A., "Selective Toxicity," John Wiley & Sons, New York, 1951.
(3) Albert, A., Gibson, M. I., and Rubbo, S. D., Brit. J. Exptl. Pathol., 34, 119 (1953).
(4) Albert, A., Hampton, A., Selbie, F. R., and Simon, R. D., Ibid., 35, 75-84 (1954).
(5) Albert, A., and McGrath, D., Biochem. J., 41, 534-5 (1947).
(6) Albert, A., Rubbo, S. D., Goldacre, R. J., and Balfour, B. G., Brit. J. Exptl. Pathol., 28, 69-87 (1947).
(7) Anderson, B. I., and Swaby, R. J., Australian J. Sci. Research, B-4, 275-82 (1951).
(8) Atkins, J. G., Jr., and Horn, N. L., Plant Disease Reptr., 36, 270-2 (1952).
(9) Barratt, R. W., and Horsfall, J. G., Conn. Agr. Expt. Sta., Bull. 508 (June 1947).
(10) Benignus, P. G., Ind. Eng. Chem., 40, 1426-9 (1948).
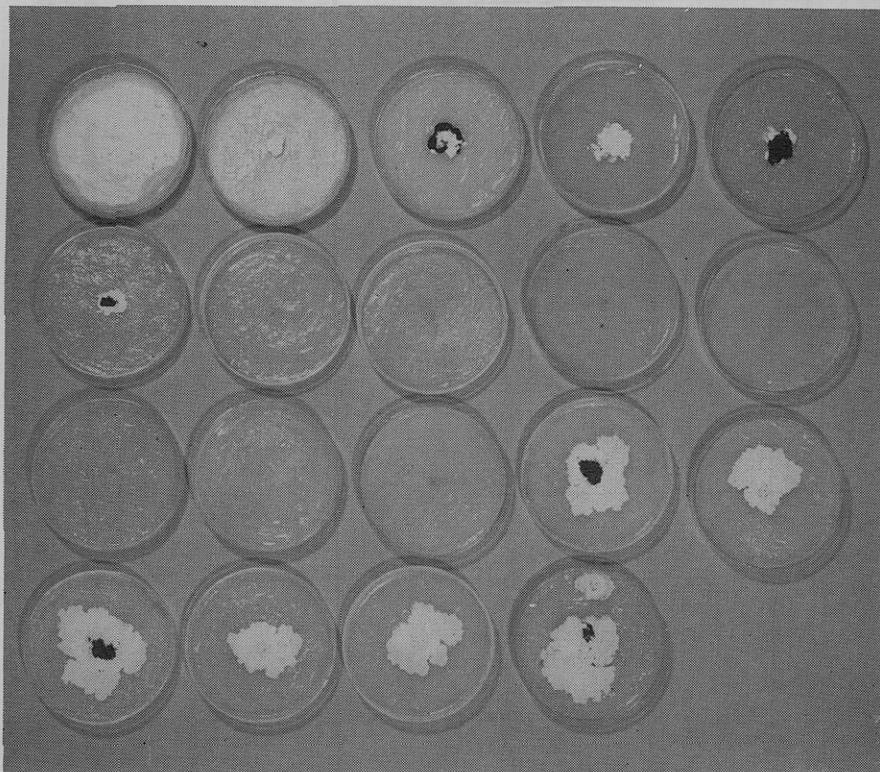(11) Benignus, P. G., U. S. Patent 2,457,025 (Dec. 21, 1948).

**Figure 6.** Inversion-type dosage response of *A. niger* to copper diiodo-oxinate at pH 1.5

Molar concentrations (left to right). *Top.* $10^{-2}$, $4.8 \times 10^{-3}$, $2.2 \times 10^{-3}$, $10^{-3}$, $4.8 \times 10^{-4}$
*Second.* $2.2 \times 10^{-4}$, $10^{-4}$, $4.8 \times 10^{-5}$, $2.2 \times 10^{-5}$, $10^{-5}$
*Third.* $4.8 \times 10^{-6}$, $2.2 \times 10^{-6}$, $10^{-6}$, $4.8 \times 10^{-7}$
*Fourth.* $10^{-7}$, $4.8 \times 10^{-8}$, $2.2 \times 10^{-8}$, $10^{-8}$
No growth at $10^{-2}$M; what appears to be growth is a light reflection in photograph

(12) *Ibid.*, **2,476,235** (July 12, 1949).
(13) Berg, R., *Z. anorg. allgem. Chem.*, **204**, 208–14 (1932).
(14) Berk, S., *Am. Dyestuff Reptr.*, **36**, 541–3 (1947).
(15) Clayton, E. E., Goldsworthy, M. C., Haskell, R. J., Heuberger, J. W., Leukel, R. W., McClellan, W. D., and Miller, P. R., *Plant Disease Reptr.*, Suppl. **181**, 87 (1949).
(16) Dunegan, J. C., Goldsworthy, M. C., and Wilson, R. A., *Ibid.*, **32**, 135–6 (1948).
(17) Eisenschiml, O., and Kalberg, V. N., *Paint, Oil Chem. Rev.*, **111**, No. 24, 17–19, 29–30 (1948).
(18) Field, W. E., U. S. Patent **2,567,905** (Sept. 11, 1951).
(19) Fleck, H. R., and Ward, A. M., *Analyst*, **58**, 388 (1933).
(20) Gerber, D., and Block, S. S., "Inhibition of Cresolase Activity by Fungicidal Chemicals," Florida Section, AMERICAN CHEMICAL SOCIETY, Meeting-in-Miniature, May 1953.
(21) Goto, H., *Science Repts. Tohoku Imp. Univ.*, First Ser., **26**, 391–413 (1937).
(22) Hahn, F. L., and Vieweg, K., *Z. anal. Chem.*, **71**, 122 (1927).
(23) Hata, S., *Kitasato Arch. Exptl. Med.*, **9**, 1 (1932).
(24) Illman, W. I., Semeniuk, B., Neish, A. C., and Ledingham, G. A., *Can. J. Research*, **F-26**, 311–17 (1948).
(25) Kalberg, V. N., U. S. Patent **2,561,379** (July 24, 1951).
(26) *Ibid.*, **2,561,380** (July 24, 1951).
(27) *Ibid.*, **2,608,556** (Aug. 26, 1952).
(28) Lee, S., *Am. Dyestuff Reptr.*, **39**, 145–51, 156 (1950).
(29) Maley, L. E., and Mellor, D. P., *Australian J. Sci. Research*, **2a**, 92–110 (1949).
(30) Malone, R. W., Jr., U. S. Patent **2,567,910** (Sept. 11, 1951).
(31) Manten, A., Klöpping, H. L., and Van der Kerk, G. J. M., *Antonie van Leeuwenhoek J. Microbiol. Serol.*, **17**, 58–68 (1951).
(32) Mason, C. L., *Phytopathology*, **38**, 740 (1948).
(33) Merritt, L. L., Jr., *Record of Chem. Prog.*, **10**, 59–70 (1949).
(34) Phillips, J. P., "Chelate Formation by 8-quinolinol and Derivatives," doctoral dissertation in chemistry, Indiana University, 1949.
(35) Powell, D., *Phytopathology*, **36**, 572–3 (1946).
(36) Powell, D., *Plant Disease Reptr.*, **38**, 76 (1954).
(37) Prevention of Deterioration Center, Natl. Research Council, "Official Screening Test Method," 1948.
(38) Reed, C. L., and Herman, L. G., *Food Eng.*, **24**, 71 (February 1952).
(39) Richardson, J. H., and Del Giudice, V. J., *Modern Sanitation*, **4**, 32–7 (March 1952).
(40) *Ibid.*, **4**, 32–5 (April 1952).
(41) Rubbo, S. D., Albert, A., and Gibson, M. I., *Brit. J. Exptl. Pathol.*, **31**, 425–41 (1950).
(42) Schraufstätter, E., *Z. Naturforsch.*, **5G**, 190–5 (1950).
(43) Schuler, W., and Meier, R., *Schweiz Z. Path. u. Bakt.*, **13**, 463–9 (1950).
(44) Sexton, W. A., "Chemical Constitution and Biological Activity," Van Nostrand, New York, 1952.
(45) Strong, M. C., Mich. Agr. Expt. Sta., *Quart. Bull.* **30**, 407–12 (1948).
(46) Teitell, L., unpublished data.
(47) Vicklund, R. E., and Manowitz, M., *CADO Tech. Data Dig.*, **15**, No. 5, 18–21 (1950).
(48) Wilson, J. D., *Plant Disease Reptr.* Suppl. **174**, 41–86 (1948).
(49) Wilson, J. D., and coworkers, *Ibid.*, **183**, 111–77 (1948).
(50) Zentmyer, G. A., *Phytopathology*, **33**, 1121 (1943).
(51) Zentmyer, G. A., *Science*, **100**, 294 (1944).

**Table VI. Relative Solubility of Oxines and Copper Chelates between Oil (Chloroform) and Water**

(At pH 1.5 and pH 6.0 as determined by absorption spectra at wave length of 262 m$\mu$)

| Compound | Molar Concn. | % Transmittance of CHCl₃ Layer | | Ratio of Concn. in CHCl₃ at pH 6.0 to 1.5 |
| --- | --- | --- | --- | --- |
| | | pH 1.5 | pH 6.0 | |
| Oxine | $10^{-4}$ | 92.7 | 24.0 | 16.4 |
| 4-Methyloxine | $10^{-4}$ | 95.0 | 50.7 | 12.9 |
| 5,7-Dichloro-oxine | $10^{-4}$ | 15.8 | 16.0 | 0.995 |
| Copper oxinate | $10^{-5}$ | 98.7 | 19.0 | 103 |
| Copper-4-methyloxinate | $10^{-5}$ | 98.8 | 25.6 | 84.7 |
| Copper-5,7-dichloro-oxinate | $10^{-5}$ | 83.1 | 59.0 | 2.87 |