Fungitoxicity of Some Substituted Pyridines and Quinolines Related to 8-Quinolinol (Oxine)

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Some heterocyclic nitrogen compounds structurally related to 8-quinolinol were synthesized or purified from commercially available chemicals and assessed for toxicity against *Monilinia fructicola* by a standard bioassay. Oxine and its 2-methyl derivative, together with their respective copper salts, were highly fungitoxic. Two other quinoline derivatives were slightly toxic, but all the pyridine derivatives were inactive against this organism. A combination of lipoid solubility and chelation ability was related to toxicity, but definite evidence was obtained that chelation with copper to form water-insoluble salts was no assurance that either the compound itself or its copper salt would be toxic.

THE USE of 8-quinolinol (oxine) and some of its derivatives in the control of scab and blotch of apples has been reported as promising (*19*). Workers at the Connecticut Experiment Station used oxine in the chemotherapy of Dutch Elm disease and found it to be effective (*23*). Unfortunately, the cost of material has been and still is a matter of extreme importance in developing these derivatives on a commercial basis.

Because of the large number of possible derivatives of oxine, many workers have studied this group in an effort to explain the mechanism of action (*4*, *6*, *16*, *22*).

Zentmeyer (*22*), the first to propose a hypothesis for the mechanism of action of oxine, suggested that oxine, acting through its ability to chelate heavy metals, removed essential nutrients from the growth medium by precipitation. About the same time, Albert and coworkers (*4*) found that of the seven possible isomers only 8-quinolinol was toxic to bacteria and only this isomer was capable of chelating heavy metals. After Powell (*19*) reported that copper-8-quinolinolate was also highly fungitoxic and more efficient in field tests than 8-quinolinol itself, other workers (*15*, *16*, *20*) questioned the chelation theory.

The more recent investigations of Albert and associates (*3*, *4*) and Block (*6*, *7*) support the chelation theory, but these workers state that the site of action is inside the cell rather than in the medium. The toxicity of copper-8-quinolinolate is explained by equilibrium phenomena. Albert and coworkers (*3*) found that 8-quinolinol was no longer toxic to *Staphylococcus aureus*, if the medium were depleted of trace amounts of copper or iron. They propose then that the metal oxinate, because of its lipophilic nature, enters the cell and dissociates yielding the 1 to 1 ionic complex which is the toxic entity. Block (*6*) working with *Aspergillus niger* showed that the toxicity of substituted 8-quinolinols was a function of both their ability to chelate with metals and their lipoid solubility. He later demonstrated the presence of the 1 to 1 chelate and its formation from the stable 2 to 1 chelate in the presence of excess metal ions (*7*).

The toxicity of 8-quinolinol appears to be due to the formation of a metal chelate complex, and the site of action is inside the cell.

A study of the chelation ability and lipophilic properties of some heterocyclic nitrogen compounds related to 8-quinolinol and of the relationship these properties have to toxicity is reported herein.

Material and Methods

Quinoline Derivatives. 8-Quinolinol (oxine) was purchased from Eimer and Amend and purified by recrystallization from an alcohol-water mixture. It melted at 74–5° C. (uncorrected).

2-Quinolinol (Eastman), melting point 198–9° C. (uncorrected) was not further purified.

Quinaldic acid was prepared by bromination in the side chain of quinaldine, according to Hammick (*12*), and subsequent acid hydrolysis of the tribromoquinaldine, according to Campbell, Helbig, and Kerwin (*9*). The yield was 53%.

2-Methyl-8-quinolinol was synthesized from crotonaldehyde and *o*-aminophenol by the method of Merritt and Walker (*17*). After three recrystallizations from ethyl alcohol–water (2 to 1), it melted sharply at 74° C.

8-Quinolinecarboxylic acid was prepared from anthranilic acid and glycerol through a modified Skraup reaction in a somewhat better yield (62%) than that reported by Campbell and coworkers (*10*), who obtained, at best, a 53% yield. Two recrystallizations from 95% ethyl alcohol yielded a yellow solid, melting point 186–8° C. which agreed with the literature values for 8-quinolinecarboxylic acid.

A new four-step synthesis yielded light yellow crystals of 8-hydroxyquinaldic acid according to the method of Irving and Pinnington (*14*). The yields for each step were nearly quantitative, and the final product melted at 218–19° C. (uncorrected) after two recrystallizations from hot dioxane. Irving and Pinnington reported a melting point of 211° C. for 8-hydroxyquinaldic acid. (Analysis: $C_{10}H_7O_3N$ requires 7.40% of nitrogen; found, 7.34% of nitrogen.) The chemical and physical properties agreed with those found by Irving and Pinnington.

Pyridine Derivatives. The method of Adams and Jones (*1*) was used to prepare both 2-hydroxypyridine (2-pyridone) and 5-methyl-2-hydroxypyridine from the corresponding aminopyridines in 60% yield. After recrystallization from acetone, 2-hydroxypyridine melted at 107–8° C. (uncorrected) and 5-methyl-2-hydroxypyridine at 180–2° C. (uncorrected) after recrystallization from acetone containing a little water.

Picolinic acid was prepared as the hydrochloride by oxidation of 2-picoline with potassium permanganate (*21*).

2-Pyridine-methanol was synthesized in low yield by conversion of picolinic acid to the ethyl ester (*8*) and reduction of the ester with lithium aluminum hydride (*18*). The product was a light yellow viscous oil, boiling point 90–5° C. at 4 mm. (Literature values: boiling point 103–5° C. at 11 mm. and 112–13° C. at 16 mm.)

Copper Chelate Complexes. The water-insoluble complexes with copper

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were prepared essentially as follows:

An alcoholic or aqueous solution of the compound in excess was added slowly to a solution of copper sulfate, reagent grade, in 0.1*N* acetic acid. The colored complex which precipitated was coagulated by heating for a few minutes on the hot plate. The solid was collected on a Büchner funnel, and purification was carried out by first washing with hot water to remove excess salts and then with 95% ethyl alcohol to remove any unreacted organic compound. The salts were allowed to air-dry and were crushed to a fine powder. 8-Quinolinol, 2-methyl-8-quinolinol, quinaldic acid, 8-quinolinecarboxylic acid, and picolinic acid all formed water-insoluble salts with copper under these conditions.

Aqueous Solubilities. The aqueous solubilities of most of the compounds were determined at 25° ± 0.1° C. using a spectrophotometric method of analysis. The absorbances were measured in the ultraviolet region of the spectrum. The solubilities of the very water-soluble derivatives were determined gravimetrically.

Relative Distribution between Chloroform and Water (Lipoid Solubility). The relative distribution between chloroform and water was found by measuring the change in absorbance of the chloroform layer before and after equilibration with an equal volume of aqueous phosphate buffers at pH 8.0 and pH 3.0. The solutions—diluted if necessary—were examined with the Beckman DU spectrophotometer at suitable wave lengths for the various compounds in the ultraviolet region (see Table I).

Bioassay. The organic compounds and the five copper salts were subjected to bioassay, using the spore-drop germination method (*5*). *M. fructicola* was the test organism. The index of toxicity was the ED_{50} (effective dosage inhibiting the germination of 50% of the spores). This value was obtained by plotting the dosage in parts per million against the per cent inhibition on logarithmic probability paper and using the best straight line drawn through the points.

Results and Discussion

The toxicities and solubility data for these derivatives are summarized in Tables I and II. The current literature has reported mainly on homologs of oxine or substituted oxines. In this work, pyridine and quinoline hydroxy compounds and carboxylic acids, which may be classed as analogs of oxine, have been assessed for toxicity to *M. fructicola* and for their ability to form water-insoluble chelate complexes with copper.

Most of the derivatives gave good dosage-response curves. The copper salt of 8-quinolinecarboxylic acid gave a poor curve, and no confidence can be placed in the ED_{50} value for this salt as the curve was nearly horizontal. Five of the compounds were capable of complex formation with copper. All attempts to prepare a copper salt of 8-hydroxyquinaldic acid failed, but it is possible that other ions, such as Fe^{+++}, may form precipitates under the above conditions, as this acid is a possible tridentate chelator. The ED_{50} values for the copper salts of 8-quinolinol and its 2-methyl derivative are slightly higher than for the parent compounds. However, no significance can be attached to this difference as no attempt was made to free the media of traces of heavy metals.

Table I. Toxicity and Solubility of Oxine and Related Compounds

Compound	ED_{50}, P.P.M.	Max. Abs. Mμ, ($CHCl_3$)	$CHCl_3$ to H_2O Ratios pH 3.0	$CHCl_3$ to H_2O Ratios pH 8.0	H_2O, G./100 Ml.
8-Quinolinol (oxine)	0.3	310	4.60	16.5	0.054
2-Quinolinol	805	330	5.14	5.14	0.087
2-Methyl-8-quinolinol	11.4	304	2.15	9.50	0.044
Quinaldic acid	137	290	1.68	0.00	1.600
8-Quinolinecarboxylic acid	700	241	10.4	0.68	0.350
8-Hydroxyquinaldic acid	178	258	0.30	0.00[a]	0.028
Picolinic acid	500–1000	264	0.00	0.00	45.00
2-Hydroxypyridine	1000	300	0.00	0.00	80.00
5-Methyl-2-hydroxypyridine	1000	241	0.30	0.07	20.00
2-Pyridinemethanol	300–400	260	0.00	0.75	∞

[a] Ratios of zero indicate that compound was exclusively in water layer.

Table II. Toxicity and Solubility of Copper Chelates

Copper Salt	ED_{50}, P.P.M.	$CHCl_3$ to H_2O Ratios pH 3.0	$CHCl_3$ to H_2O Ratios pH 8.0	Aqueous Solubility, P.P.M.
8-Quinolinol (oxine)	0.72	0.22	15.4	6.1
2-Methyl-8-quinolinol	20.00	0.00[a]	∞	3.9
Quinaldic acid	1000	...	...	8.0
Picolinic acid	1000	...	...	152
8-Quinolinecarboxylic acid	62 (?)	...	...	6.6

[a] See Table I.

Toxicities of the Quinoline Derivatives. Only 8-quinolinol and 2-methyl-8-quinolinol and their respective copper salts possessed a satisfactory fungitoxic action against this organism and may be classed as suitable fungicides. Quinaldic acid and 8-hydroxyquinaldic acid were very weakly active in these tests. 8-Quinolinecarboxylic acid and 2-quinolinol were for all intents and purposes completely inactive against *M. fructicola*.

The toxic 8-quinolinols were capable of chelation with copper to form lipoid-soluble complexes and both these compounds and their salts had relatively high chloroform-water partition ratios at pH 8.0. Quinaldic acid and 8-quinolinecarboxylic acid formed water-insoluble complexes with copper but these complexes were only very slightly soluble in chloroform and hence non-lipophilic. The partition ratios of these acids at pH 8.0 were low, and, as the copper salts were so slightly soluble in both water and chloroform, their ratios were not determined. Quinaldic acid complexes are known to be hydrated (*11*), and it is probable that 8-quinolinecarboxylic acid salts are also hydrated to some extent. The weak activity of quinaldic acid and 8-hydroxyquinaldic acid is not easily explained, but may be due to the common presence of a 2-carboxyl grouping.

Toxicities of the Pyridine Derivatives. All of the hydroxypyridines and picolinic acid were inactive against *M. fructicola*. Picolinic acid was capable of chelate formation with copper, but the complex so formed was like those of the quinaldic acids and was nonlipophilic. The hydroxypyridines were nonchelators and all the pyridines had an appreciable solubility in water and extremely low partition ratios at both pH values. The hydroxypyridines or pyridones, like 2-quinolinol, are keto-enol tautomers and very weak phenols. Hoffman, Schweitzer, and Dalby (*13*) tested the three isomeric pyridine carboxylic acids and found that picolinic acid was active at pH 2 to 8 which is in the chelation range. However, in these tests, picolinic acid and its copper salt failed to have an appreciable effect on *M. fructicola*.

Influence of Structure, Lipoid Solubility, and Chelation

As all the weakly phenolic hydroxypyridines, 2-pyridinemethanol, 2-quinolinol, and the quinoline carboxylic acids were relatively nontoxic, the phe-

nolic hydroxy group appears to be a factor influencing the toxicity. In this series of compounds, only those having the properties of aromatic phenols were satisfactory fungicides. This is in agreement with Sexton (*20*), who attributed the activity of oxine to its phenolic nature. The hydroxyl group in the 8-position on quinoline is an important toxiphore. Only those chelating agents which readily form stable lipophilic complexes with copper are strongly toxic. This finding is in agreement with Albert and associates (*2*, *3*) and Block (*6*, *7*), who suggest that the 2 to 1 chelate is necessary for penetration, and that the site of action is inside the cell. Zentmeyer (*22*), assuming that 8-quinolinol precipitated trace metals from the medium, found that the toxicity could be reversed by adding excess metal ions. If the mechanism were simply one of precipitation, any strong chelating agent would be toxic. Zentmeyer did find that ammonium nitrosophenylhydroxylamine (cupferron) had considerable fungistatic value. Quinaldic acid and 8-quinolinecarboxylic acid are well known for their ability to precipitate heavy metals through chelation and have been used by analytical chemists for this purpose, but these tests with *M. fructicola* show that these chelators were unsatisfactory toxicants regardless of their complexing ability.

From the limited number of derivatives tested, a combination of lipoid solubility and chelation ability appears to be necessary for toxicity, perhaps for penetration of the cell wall only. The phenolic 8-hydroxyl group on quinoline must play an important role in spore inhibition at a site of action inside the cell.

Acknowledgment

The author wishes to thank Hubert Martin, director of the Science Service Laboratory, London, Ontario, for the provision of laboratory facilities and for suggesting the project; and E. Y. Spencer and R. A. Ludwig, of the same laboratories for their direction, helpful suggestions, and encouragement during the course of this research.

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Received for review May 25, 1957. Accepted August 19, 1957. Work based on a thesis submitted to the graduate school at the University of Western Ontario in partial fulfilment of the requirements for the M.Sc. degree. Contribution No. 108, Science Service Laboratory, Department of Agriculture, London, Canada.

INSECTICIDE RESIDUES

Determination of Endrin in Agricultural Products and Animal Tissues

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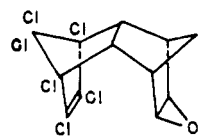
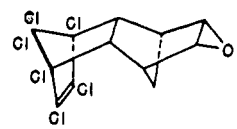
ENDRIN (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4,-*endo*-*endo*-5,8-dimethanonaphthalene) is an insecticide having the structure:



It is a diastereoisomer of dieldrin (1,-2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene):



but differs from dieldrin in its insecticidal activity, chemical properties, and residual persistence. Endrin is being used in ever-increasing amounts in agriculture because of its ability to control a wide variety of economically important insects. Its use on crops that subsequently may be consumed by man or domestic animals necessitated the development of methods capable of accurately determining residues of this material in concentrations in excess of 0.1 p.p.m. in crops and animal products.

Bioassay techniques that employ insects have been used extensively for assaying insecticide residues and for screening compounds for insecticidal properties. Techniques such as those of Sun and Sun (*7*), Laug (*4*), and Klein and coworkers (*3*), have been applied successfully to the determination of endrin residues in crops. Davidow and Schwartzman (*2*) also have found a method using goldfish capable of determining endrin at the 5-γ level. While bioassay methods have been useful in laboratories possessing the necessary facilities, chemical methods were desired because of their relative specificity.

Combustion chlorine methods (*1*, *5*, *6*),