Catalytic Hydrolysis of Some Organic Phosphate Pesticides by Copper(II)

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Cu(II) catalyzes the hydrolysis of some organic phosphate pesticides in a pH range (5 to 6) where they are normally considered relatively stable. The catalytic properties of Cu(II) are compared with a number of other metal ions. Its ability to catalyze the reaction was in proportion to its activity. The mechanism is suggested to be bidentate chelation through nitrogen in the ring structure and sulfur on the phosphate side chain in Dursban and Diazinon. This forms a closed cyclic resonance system; electron shifts in the proposed ring structure weaken the bonding of the side chain to pyridine or pyrimidine, promoting hydrolysis. Organic phosphate pesticides (Ronnel and Zytron) which have chlorinated benzene rings also undergo hydrolysis catalyzed by Cu(II) but more slowly. Kinetics and possible mechanisms of the reactions are discussed.

Metallic ions have great influence in the enzymatic hydrolysis of organic phosphates. In addition, certain metallic ions themselves catalyze the nonenzymic hydrolysis of some organic phosphates (1, 7). Cu(II) was particularly active in such reactions, especially when present as chelates with dipyridyl, histidine, etc. Decomposition of the insecticide (O,O-dimethyl-O-2, 4,5-trichlorophenyl) phosphorothioate (Ronnel) on clays treated at high temperatures has been suggested to be a rearrangement reaction rather than a hydrolysis type of reaction (5).

In studying the adsorption of O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate (Dursban) on clay minerals with various exchangeable cations, it was observed that the compound was completely hydrolyzed in a short time when Cu(II) was the saturating ion on the exchange complex of the mineral. This conclusion was reached when 3,5,6-trichloro-2-pyridinol was produced as a hydrolysis product. The following study was undertaken to clarify the nature of this hydrolysis, since Dursban is normally stable at pH 6.0. Dursban and compounds of similar structure which were included in this study are as follows:

Dursban

Diazinon

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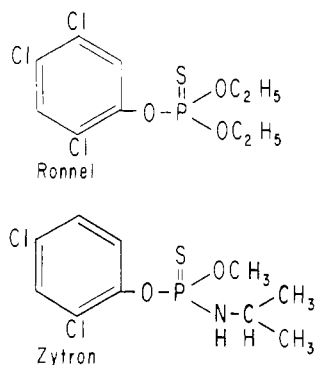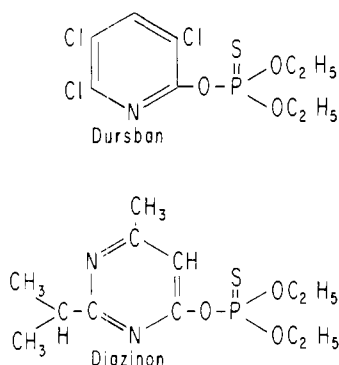
Ronnel

Zytron

Dursban and Diazinon have nitrogen in the ring and possess pyridyl and pyrimidinyl ring structures, respectively. Ronnel and Zytron, on the other hand, are substituted tri- and dichlorophenols. All four compounds have phosphorus, sulfur, and ethoxy or methoxy groups in the side chains, while Zytron also has a nitrogen.

Experimental

Hydrolysis of the organic phosphate pesticides was followed by observing the appearance of the product with ultraviolet absorption. The product compound containing the ring structure had variable absorption in the ultraviolet region, depending upon whether or not Cu(II) was present. In the presence of Cu(II), Dursban absorbs at 287 mμ and its pyridinol product at 320 mμ. Diazinon absorbs at 247 mμ and its product at 262 mμ. Zytron absorbs at 275 and 283 mμ and the hydrolysis product, dichlorophenol, has a band at 294 mμ. Ronnel absorbs at 280 and 290 mμ and the trichlorophenol product has a band at 297 mμ. These compounds have limited solubility in water; consequently, in order to have large enough concentrations in solution for convenient study, a 1 to 1 mixture of water and methanol was used as a solvent in the reactions studied. However, pilot experiments were made on the compounds in equilibrium with water where CuCl$_2$ was added to the solution phase and the hydrolysis established.

The effect of various salts and ion exchangers on the

hydrolysis of these organic phosphates was determined by placing 0.004 and 0.008 mmole of the compound in 50 ml. of a 1 to 1 mixture of water and methanol together with 0.1 mmole of the salt or 100 mg. of ion exchanger. The samples were mixed and incubated at 20° C. for 24 hours. The solutions were then scanned on a Beckman DK2A spectrophotometer from 360 to 190 mμ using a hydrogen lamp as a source and measuring the absorbance. The spectra were recorded automatically as this wavelength range was scanned. Matched silica cells of high quality were used, and the reference beam cell contained the same solvent, and in the case of the salts the concentration was the same as in the sample cell containing the organic phosphate.

Kinetic studies of the rate of hydrolysis were made by recording spectra of the systems at various intervals of time, with the time of mixing the compounds as the starting point. Effect of temperature on the decomposition rates was obtained by incubating the samples at the appropriate temperature and removing samples from time to time for analysis.

In the presence of the clay minerals the reaction was allowed to proceed, then the clay was centrifuged down and the absorption spectra of the clear supernatant solution were recorded in the spectrophotometer. The clay minerals were obtained from Wards Natural Science Establishment, except beidellite which was from the A$_2$ horizon of a northern Michigan soil (6). The clay minerals and ion exchange resins were saturated with the appropriate cation by repeated treatment with the chloride salts, and washing with distilled water until free of salt.

Discussion of Results

The effect of various ions as salts, and as the exchangeable cations in some clay minerals and resins, on the hydrolysis of Dursban, Diazinon, Ronnel, and Zytron is shown in Table I. The almost complete specificity of Cu(II) on the reaction is shown by the fact that Cu(II) in the form of the chloride salt or when present as the exchangeable cation in montmorillonite, brings about a complete hydrolysis of the Dursban, Diazinon, and Ronnel in 24 hours or less. This effect of Cu(II) is apparently a function of its activity, since some exchangers which bind metal ions more tightly than montmorillonite did not hydrolyze Dursban and Diazinon. The Cu(II) would have its maximum activity as the chloride salt.

The effects of the clay minerals used are in accord with their known properties. The three montmorillonites (H-24, H-25, and H-28) have their electrical charge arising primarily from isomorphous substitutions in the octahedral layer, while the nontronite, vermiculite, and beidellite have their source of charge in the tetrahedral layer, which places the electrical charge nearer the mineral surface and thus closer to the exchangeable cation. The result is that generally tetrahedrally substituted minerals tend to bind metal cations more tightly than the octahedrally substituted montmorillonites, thus giving the cation, Cu(II) in this case, a lowered activity.

Table I. Hydrolysis of Four Organic Phosphates after 24-Hour Contact with Various Salts and Ion Exchangers

Metallic Ion System	% Hydrolysis after 24 Hours (20°)			
	Dursban	Diazinon	Zytron	Ronnel
CuCl$_2$[a]	100	100	30	100
CoCl$_2$	0	0	0	0
ZnSO$_4$	0	0	0	0
NiSO$_4$	0	0	0	0
AlCl$_3$	0	10	0	0
CaCl$_2$	0	0	0	0
MgCl$_2$	10	0	0	0
Cu-nontronite[b]	10	10	0	5
Cu-vermiculite	0	10	0	0
Cu-IR-120	0	0	0	0
Cu-IRC-50	0	10	0	0
Cu-montmorillonite H-24	100	100	. . .	. . .
Cu-montmorillonite H-28	100	100	0	100
Cu-montmorillonite H-25	100	100	. . .	100
Al-montmorillonite H-25	0	0	0	0
Zn-montmorillonite H-25	0	0	0	0
Ca-montmorillonite H-25	0	0	0	0
Mg-montmorillonite H-25	10	0	0	0
Ca-vermiculite	0	0	. . .	. . .
Al-vermiculite	0	0	. . .	. . .
Cu-beidellite	20	40	. . .	. . .
Cu-organic soil	0	0	0	0

[a] 0.008 mmole of Dursban, Diazinon, Ronnel, or Zytron plus 0.1 mmole of salts in 50 ml. of a 1:1 solution of methanol and water.
[b] 0.008 mmole of Dursban, Diazinon, Ronnel, or Zytron plus 100 mg. of ion exchanger in 50 ml. of a 1:1 solution of methanol and water.

The experiments with Cu-saturated exchange resins, IR-120 and IRC-50, further illustrate that the activity of copper is a limiting factor for the catalytic hydrolysis of the organic phosphates. IR-120 is an exchange resin capable of binding the metal ions rather tenaciously; thus Cu-saturated IR-120 does not hydrolyze either Dursban or Diazinon. However, Cu-saturated IRC-50, an exchange resin which does not bind Cu ions as energetically as IR-120, causes a small but detectable amount of hydrolysis in the Diazinon. Organic soils also bind Cu(II) tightly enough to prevent rapid catalytic hydrolysis.

Kinetic Studies

The rates of hydrolysis of the organic phosphates were studied (Table II). In the case of Diazinon, the rate studies were made by observing the decrease in absorbance of the band at 247 mμ as a function of time, while for the Dursban studies, the increase in absorbance of the pyridinol product at 320 mμ was measured with

Table II. Kinetics of Diazinon, Dursban, Ronnel, and Zytron Hydrolysis in Systems Swamped with Cu(II)

Compound	Reaction Order	Activation Energy, Kcal./Mole	Half Time, Hours, for Reactions (20° C.)
Diazinon	2nd	11–13	4.0 (0.008 mmole Diazinon)
Dursban	1st	6–9	0.9
Ronnel	1st	14–16	6.0
Zytron	1st	17–19	48

Figure 1. First-order plots of hydrolysis of 0.008 mmole of Dursban in presence of 0.05 mmole of $CuCl_2$ at four temperatures

time. The production of tri- and dichlorophenol from Ronnel and Zytron, respectively, was monitored. The rate data were tested for first-, second-, and third-order kinetics with the results as presented.

An example of the kinetic results appears in Figure 1, where data on Dursban hydrolysis are plotted according to the first-order rate law, $\log C = A - kt$, where C is the concentration of Dursban at time t, k is the reaction rate constant, and A is the concentration at $t = 0$. Rather good linear plots result, much better than for second- or third-order reaction rate equations. In addition, the effect of temperature on the velocity of hydrolysis is shown in the different plots. The conclusion as to order of reaction is based on numerous runs at varying concentrations of compound as well as $CuCl_2$. The concentration of $CuCl_2$ did affect the rate of hydrolysis but the order of the reaction was maintained. Most of the rate studies were made in systems where the Cu(II) was in large excess [5/1 to 10/1 ratio of Cu(II) to organic phosphate], where rate of hydrolysis was pretty much independent of Cu(II) concentration. However, when a 1/1 ratio was used in Diazinon experiments, second-order kinetics were maintained but time required for half the compound to disappear (20° C.) was extended to about 35 hours compared with 4 hours (Table II) in the presence of a large excess of Cu(II). In the case of Diazinon the half time of hydrolysis was dependent upon the concentration of Diazinon, while the half time of hydrolysis of Dursban, Ronnel, and Zytron was independent of concentration, a characteristic of first-order reactions. By use of the half times of hydrolysis for the first-order reactions at different temperatures, the reaction rate constants were determined and from those, the activation energy was calculated utilizing the classical Arrhenius equation. For the Diazinon, the slopes of the second-order graphical plots were used to calculate the reaction rate constants, which were then utilized in the Arrhenius equation to calculate activation energy for hydrolysis. The range of activation energies in Table II for a given hydrolysis reaction reflects the authors' estimate of the accuracy of the activation energy calculations.

General Discussion

The specificity of Cu(II) in the hydrolysis reactions is apparent, in accord with the observations of Wagner-
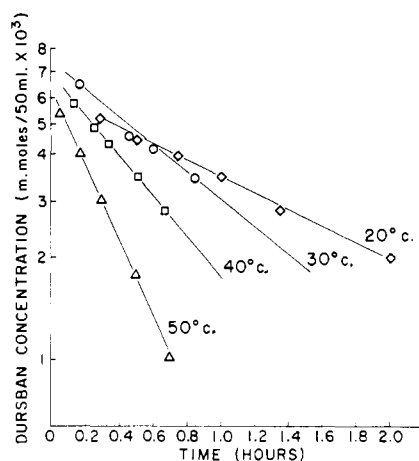
Jauregg (7) and Augustinsson and Heimburger (1), who found that of all the metal ions they studied, Cu(II) was particularly active in the nonenzymic hydrolysis of organic phosphorus compounds. Martell *et al.* (4) showed that Cu(II) was especially active in such reactions when present as a chelate.

The results obtained here on the Diazinon and Dursban decomposition in the presence of Cu(II) are suggested to be the result of coordination of molecules to the copper, followed by cleavage of the side chain containing the phosphorus as, for example, for Dursban:
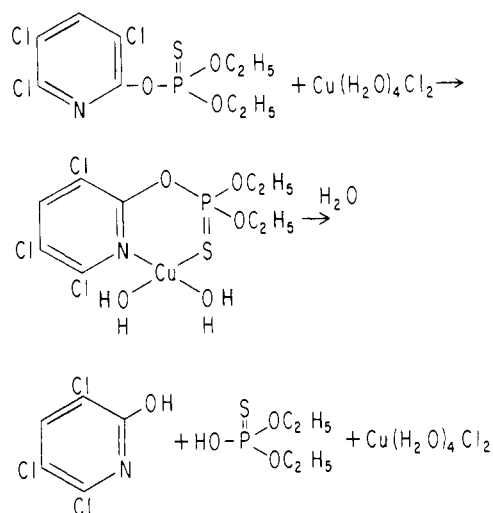
This reaction would appear to be first-order, in that the rate should be dependent upon Dursban concentration in the presence of a large excess of $CuCl_2$. In systems containing less Cu(II), rate depends on its concentration, but it is being regenerated and not consumed in the reaction. In the Diazinon system two molecules of the compound are required to coordinate with the Cu(II) ion to satisfy the second-order kinetics:

$$A + A \rightarrow B$$
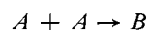
In this case the catalyst may be Cu(II) coordinated to one molecule of Diazinon and is in accord with observations of Courtney *et al.* (*3*) that incomplete chelates of Cu(II) are effective catalysts in the hydrolysis of some organic phosphorus compounds. However, it would seem that Dursban should also undergo similar interactions with resulting similarity of reaction order. Another explanation might be that two kinds of complexes of Diazinon result with Cu(II), one as indicated for Dursban and the second coordination through the other nitrogen in the ring structure. If hydrolysis resulted only from the first type of coordination and not the latter, second-order kinetics might conceivably be observed. In fact, the complex formed with the number 5 nitrogen might be an incomplete Cu(II) chelate which is active in the catalysis. The coordination of Diazinon and Dursban to Cu(II) resulting in hydrolysis is suggested to be bidentate through nitrogen in ring structure and sulfur (or oxygen if the sulfur has been replaced) on the side chain. The result of this is to form a benzenoid or Kekulé-type ring which is especially effective in some catalytic reactions in that it forms a closed cyclic resonance system through the orbitals of the metal ion. The fact that the Cu(II) ion catalyzes these reactions, while ions like Mg(II) are not effective, points to a catalytic function involving electronic changes rather than entropy or steric effects. Cu(II) has a strong affinity for the nitrogen in organic compounds, since the nitrogen has two electrons for donation to the metal ion. The demand for electrons may result in electron shifts in the proposed ring structure which weaken the bonding of the side chain to the pyridine or pyrimidine structure, thus promoting hydrolysis. As Calvin (*2*) points out, Cu(II) is more effective than any other ion in certain nonenzymic transformations and he suggests that this may be due to the unique position of Cu(II) with respect to the energy level of the unoccupied *dsp*-orbitals. The magnitudes of the energies of activation for hydrolysis in Table II are relatively low, suggesting that the catalytic effect has lowered the activation for hydrolysis from higher values which might be expected in ordinary hydrolysis.

Evidence that the compounds coordinate with Cu(II) is shown in Figure 2, where the ultraviolet spectra of Diazinon in the presence of several salts, including CuCl₂, are presented. Spectrum 1 of Diazinon with just the solvent shows an absorbance peak at 247 mμ and another much more intense peak near 197 mμ where the capabilities of the balancing system of the spectrophotometer are exceeded and an abrupt drop in absorbance then is observed as it proceeds toward lower wavelengths. Spectra 3 and 4 of Diazinon in the presence of ZnSO₄ and CoCl₂, respectively, show no differences from that of the Diazinon alone in spectrum 1. Spectrum 2 (circles) where CuCl₂ is present, however, shows a marked difference, in that while the first absorbance peak at 247 mμ remains unchanged, the second has been drastically reduced in intensity and shifted to a higher wavelength at about 214 mμ. This spectrum (*2*) was recorded immediately after mixing with CuCl₂ and thus represents the complexed Diazinon. Very quickly changes appear in the spectrum as the
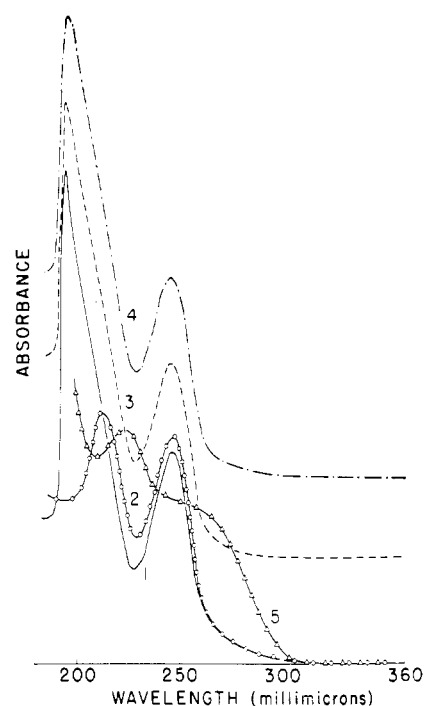

Figure 2. Ultraviolet spectra

1. 0.004 mmole of Diazinon in 50% water and methanol solvent in 50 ml.
2. Same plus 0.1 mmole CuCl₂
3. Same plus 0.1 mmole ZnSO₄
4. Same plus 0.1 mmole CoCl₂
5. Same as 2, except 24 hours later

compound hydrolyzes. In the case of spectra 3 and 4, no changes appear after 24 hours at 20° C. The authors suggest that spectrum 2 is that of Diazinon complexed with Cu(II) immediately prior to hydrolysis. Spectrum 5 (triangles) is the hydrolyzed product after 24 hours in the presence of Cu(II). As noted before, the ultraviolet spectra of the products containing the ring structures were different in some details in the presence or absence of Cu(II). This suggests that these products were also complexed with Cu(II).

The much reduced effect of Cu(II) on the velocity of Zytron hydrolysis may be the result of differences in its interaction, since the nitrogen is located far out on the side chain. Coordination through the nitrogen and sulfur (or oxygen) on the phosphorus would not result in a Kekulé-type ring, but in a four-ring structure. It may be that catalysis occurs through the four-ring bidentate chelation or possibly unidentate coordination.

Experiments were also conducted with Ronnel and hydrolysis observed in the presence of Cu(II), as indicated by the production of trichlorophenol. No changes in the spectrum of Ronnel occurred with any other salt. Kinetic studies showed this to be first-order in nature with a relatively high activation energy (14 to 16 kcal. per mole) and a much longer life than Dursban at 20° C. The mechanism of catalysis seems less clear for the Ronnel than for the Dursban and Diazinon. The chloride atoms are much poorer electron donors than nitrogen, and it is not likely that bidentate chelation

may take place between the Cu(II) and the number 2 chlorine and the sulfur on the side chain. The higher activation energy and the slower rate of hydrolysis suggest that this reaction is not as easy as in the cases of Dursban and Diazinon. It does, however, indicate that nitrogen in the ring structure is not absolutely necessary for catalysis of this group of compounds by Cu(II).

The fact that Ronnel is hydrolyzed much more rapidly than Zytron by Cu(II) may result from some influence of the nitrogen in the side chain of Zytron. The Cu(II) may prefer coordination through this nitrogen atom of the Zytron which might impose relatively little strain on the bond between the side chain and the ring structure. In the Ronnel, the interaction may be between the sulfonyl on the phosphorus with the Cu(II) which results in the observed hydrolysis. This latter interaction may be only secondarily preferred by the Zytron, which has a much longer half life and a higher activation energy for hydrolysis (Table II).

Conclusions

Cu(II) catalyzes the rapid hydrolysis of Dursban, Diazinon, and Ronnel, and the much slower hydrolysis of Zytron. This hydrolysis proceeds in a pH range (5 to 6) which distinguishes it sharply from ordinary base-catalyzed hydrolysis. It is proposed that the hydrolysis reactions proceed by bidentate chelation of the Dursban and Diazinon with Cu(II) through nitrogen atoms in the ring structure and the sulfur (or oxygen) on the phosphorus of the side chain, followed by electron drain through the nitrogen to the Cu(II) with resulting weakening of the bond between the side chain and ring structure, causing cleavage. In the cases of Ronnel and Zytron, coordination may take place through the sulfur or oxygen on the phosphorus.

The catalytic effect of Cu(II) in these reactions is dependent upon its activity; it is relatively inactive when present on ion exchangers which bind it tightly. It appears that the presence of nitrogen in the ring structure is an important factor in the catalytic hydrolysis of this group of organic phosphates by Cu(II), but is not absolutely necessary for hydrolysis to occur. It might be possible to utilize this catalysis reaction to eliminate residues of these kinds of compounds under certain situations.

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