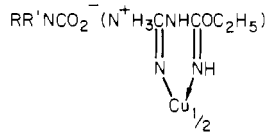
Fungicidal Bis(1-amidino-*O*-ethylisourea)copper(II) Carbamates

Eight title compounds (I; R = H; R′ = H, Me, Et, *i*-Pr; R = R′ = Me, Et; R and R′ = 2-oxydiethyl,



I

pentamethylene), in which the ligand can serve as a copper carrier to a copper-susceptible site in a fungus, are synthesized. Minimum concentrations causing complete growth inhibition of *Curvularia*, *Alternaria solani*, *Fusarium*, and *Aspergillus niger* are determined for these compounds along with those of copper acetate, 1-amidino-*O*-ethylisourea sulfate, and bis(1-amidino-*O*-ethylisourea)copper(II) acetate, chloride, and sulfate. I (R = H, CH_3; R′ = CH_3) was found to be the most effective. Each part of the molecule, viz., copper, ligand, and anion, seems to play a role in determining the resultant activity. Moderate solubility of carbamates, in contrast to high solubility of acetate and insolubility of sulfate, facilitates their transport to the susceptible site and also retards their detoxification by the acidic metabolites of fungi.

Dithiocarbamates constitute an important group of fungicides. Investigations regarding the mode of action of ferbam and ziram have established that the fungitoxicity of these compounds arises neither from the dimethyl dithiocarbamate moiety nor from iron or zinc; rather, it seems to be associated with the ability of the dithiocarbamate anion to complex copper which is present, in traces, in all natural waters. The chelate is transported to the copper-susceptible intracellular system which is the dithiol compound lipoic acid or the dithiol system lipoic acid hydrogenase (Van der Kerk, 1977). Various ligands which can carry copper to a copper-susceptible site are likely to be fungitoxic. Therefore, bis(1-amidino-*O*-ethylisourea)copper(II) acetate (Dutta and Ray, 1959; Diana et al., 1965) and chloride were evaluated for their fungicidal activity. They were, however, found to be inactive even up to a concentration of 2000 μg/mL. Their inactivity could plausibly be attributed to their high aqueous solubility which makes them more susceptible to decomplexation by acidic metabolites. Bis(1-amidino-*O*-ethylisourea)copper(II) sulfate (loc. cit.), which has very little solubility, also proved to be inactive, which may be attributed presumably to the difficulty in its transport to the active site. Compounds with intermediate solubility were, therefore, sought for. This was accomplished by exchanging acetate with carbamates. The carbamate anion and the ligand, being a substituted guanidine (Kiselev et al., 1972; Gaetzi, 1973), were also expected to contribute to pesticidal activity.

METHODS AND MATERIALS

Preparation of Bis(1-amidino-*O*-ethylisourea)copper(II) Acetate, Chloride, and Sulfate. The acetate is prepared by the method of Dutta and Ray (1959) by reacting dicyandiamide with ethanol and copper acetate. Even if one-third the quantity of ethanol, as recommended by them, is used, the yield is unaffected. It is recrystallized from ethanol. Dutta and Ray (1959) assumed an erroneous structure of this compound; the correct structure was confirmed by Diana et al. (1965). The chloride and sulfate are precipitated from the aqueous solution of the acetate by the addition of sodium chloride and ammonium sulfate, respectively.

Preparation of Alkylammonium Carbamates (Lindahl and Hennig, 1960; Fernelius et al., 1946). An amine liberated by warming its aqueous solution and dried by passing through a column packed with KOH pellets is reacted with dry carbon dioxide in a chilled flask fitted with a long condenser (1.2 m) under moisture exclusion. In the case of liquid amines, dry carbon dioxide is slowly bubbled through the chilled solution of a dry amine in dry benzene. The solid which deposits is collected. In some cases (cf. Table I) the amine is reacted with carbon dioxide in absolute ethanol and the resulting solution of alkylammonium carbamate used as such.

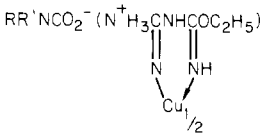
Preparation of Bis(1-amidino-*O*-ethylisourea)copper(II) Carbamates. Bis(1-amidino-*O*-ethylisourea)copper(II) acetate (0.01 mol) is dissolved in a minimum quantity of water at ~50 °C, and the solution is allowed to cool to room temperature. It is added dropwise into the freshly prepared, moderately saturated, aqueous solution of an alkylammonium carbamate (0.04 mol). Often, the product separates immediately. It is filtered, rinsed with a little water, and dried at room temperature. For analysis of copper, ~0.2 g of the product, thus obtained, is accurately weighed into an iodine flask and decomplexed with 5 mL of 50% aqueous acetic acid; 5 mL of a 10% solution of potassium iodide is then added, and the iodine, thus liberated, is titrated against 0.04 N sodium thiosulfate solution. Results are summarized in Table I. In some cases, as mentioned in Table I, products of higher purity were obtained by carrying out the reaction in ethanolic instead of aqueous medium. Similar reaction with phenylhydrazine gives an unsatisfactory fleshy product which is not filterable. All these compounds are stable up to ~150 °C, above which they undergo slow decomposition.

Attempts to synthesize bis(1-amidino-*O*-ethylisourea)copper(II) dithiocarbamates were rendered unfructuous because of decomplexation and formation of copper dithiocarbamates.

Evaluation of Fungicidal Activity. Minimum concentrations causing complete growth inhibition were determined for compounds I–XIII against four fungi by employing the method of Lindenfelser (1967). The fungal cultures are grown in Petri dishes containing peptone–glucose–agar medium (Cruickshank, 1968), pH 6.4, and examined visually for their purity and growth after 48 h. Results are summarized in Table I.

0021-8561/81/1429-0421$01.25/0

Table I. Bis(1-amidino-*O*-ethylisourea)copper(II) Carbamates



	R, R′	yield, %	Cu, % calcd	Cu, % found	min concn, μg/mL, causing complete growth inhibition of *Curvularia*	*Alternaria solani*	*Fusarium*	*Aspergillus niger*
I	R = R′ = H-[a]	81.8	14.33	13.58	>2000	>2000	>3000	>2000
II	R = H-; R′ = CH_3-[b]	51.3	13.48	12.94	1500	900	1500	900
III	R = R′ = CH_3-[b]	48.2	12.73	12.73	900	900	>2000	900
IV	R = H-; R′ = C_2H_5-[a]	67.0	12.73	13.24	2000	2000	2000	2000
V	R = R′ = C_2H_5-[b]	49.0	11.44	11.33	1750	1750	2000	2000
VI	R = H-; R′ = $(CH_3)_2CH$-[a]	72.5	12.05	12.27	2000	1500	1500	1500
VII	R and R′ = $O(CH_2CH_2-)_2$[b]	45.0	10.89	11.00	1750	1750	2000	1500
VIII	R and R′ = $CH_2(CH_2CH_2-)_2$[a]	55.0	10.96	11.59	>2000	>2000	>2000	2000
IX	bis(1-amidino-*O*-ethylisourea)copper(II) acetate				>2000	>2000	>2000	>2000
X	bis(1-amidino-*O*-ethylisourea)copper(II) chloride				>2000	>2000	>2000	>2000
XI	bis(1-amidino-*O*-ethylisourea)copper(II) sulfate				>2000	>2000	>2000	>2000
XII	1-amidino-*O*-ethylisourea sulfate				>2000	>2000	>2000	>2000
XIII	copper acetate monohydrate				>1000	>1000	>1000	>1000

[a] Prepared in aqueous medium. [b] Prepared in ethanolic medium.

RESULTS AND DISCUSSION

Compounds II and III are the most active, and IV–VII are completely inhibitive in the concentration range 1500–2000 μg/mL. I and VIII–XII are inactive even at 2000 μg/mL except VIII which is active at 2000 μg/mL against *Aspergillus niger*; XIII is inactive up to 1000 μg/mL which is more than the concentration of copper attainable in the medium after complete decomplexation of active substances. It is, therefore, evident that neither Cu(II) nor the ligand (XII) are separately responsible for activity. Each part of the molecule, viz., copper, ligand, and anion, apparently plays a specific role in determining the resultant activity. Copper is the active fungicidal entity (Van der Kerk, 1977), the ligand serves as its carrier to the copper-susceptible site, and the anion modulates solubility to an optimum level, thereby facilitating its transport and at the same time retarding its detoxification by decomplexation. The decomplexation is further retarded by the interaction of the acidic metabolites with the carbamate anion. IX and X, being more soluble, are susceptible to rapid detoxification as compared to less-soluble carbamates, and XI being highly insoluble seems to be incapable of adequate transport to the active site.

ACKNOWLEDGMENT

The authors are grateful to K. Suresh for his assistance in evaluation of fungicidal activity.

LITERATURE CITED

Cruickshank, R. "Medical Microbiology", 11th ed.; ELBS: London, 1968; p 771.
Diana, G. D.; Zalay, E. S.; Cutler, R. A. *J. Org. Chem.* **1965**, *30*, 298.
Dutta, R. L.; Ray, P. *J. Indian Chem. Soc.* **1959**, *36*, 499.
Fernelius, W. C.; Audreith, L. F.; Bailor, J. C.; Booth, H. S.; Johnson, W. C.; Kirk, R. C.; Schumb, W. C.; Scott, J. D. *Inorg. Synth.* **1946**, *2*, 294.
Gaetzi, K. German Offen. 2212827, 1973; *Chem. Abstr.* **1973**, *78*, 15857u.
Kiselev, L. A.; Titova, E. B.; Ryabova, A. N.; Shvetsova-Shilovskaya, K. D.; Mel'nikov, N. N. *Khim. Sredstva Zashch. Rast.* **1972**, *2*, 299.
Lindahl, H. A.; Hennig H. U.S. Patent 2927128, 1960; *Chem. Abstr.* **1960**, *54*, 12000*h*.
Lindenfelser, L. A. In "Sourcebook of Laboratory Exercises in Plant Pathology"; Kelman, A., Ed.; W. H. Freeman: London, 1967; p 301.
Van der Kerk, G. J. M. *ACS Symp. Ser.* **1977**, *No. 37*, 125.

Sharad Medhekar
K. S. Boparai*

School of Studies in Chemistry
Vikram University
Ujjain 456 010, India

Received for review April 21, 1980. Accepted November 12, 1980. S.M. is grateful to C.S.I.R. for a research fellowship.