J. Pesticide Sci. **17**, 39–46 (1992)

Original Article

Mechanism of Action and Selectivity of a Fungicide, Mepronil*

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(Received June 21, 1991; Accepted October 15, 1991)

A fungicide, mepronil (3′-isopropoxy-2-methylbenzanilide, trade name: Basitac) inhibited potently the oxidation of succinate in mitochondria isolated from mycelia of *Rhizoctonia solani*, which was measured by oxygen consumption, while it did not affect the oxidation of NADH of mitochondria from the fungus. It inhibited the following enzymes in the isolated mitochondria from *R. solani* which are responsible for the oxidation of succinate; succinate-cytochrome *c* reductase (I_{50}: 0.25 μM), succinate-coenzyme Q_{10} reductase, succinate dehydrogenase assayed using DCPIP as an electron acceptor (I_{50}: 0.24 μM, K_i: 0.082 μM K_i': 0.23 μM) and succinate dehydrogenase assayed using both PMS and DCPIP as electron acceptors. It, however, did not inhibit succinate dehydrogenase which was solubilized from the mitochondria. It had no significant effect on succinate dehydrogenases in mitochondria isolated from other sources such as *Pyricuralia oryzae*, *Botrytis cinerea*, rat liver, mouse liver, etiolated pea seedling and sweet potato root. These results indicated that mepronil selectively inhibits succinate dehydrogenase in mitochondria of *R. solani*.

INTRODUCTION

Mepronil, 3′-isopropoxy-2-methylbenzanilide, (trade name: Basitac) is a fungicide which is highly effective against *Rhizoctonia solani* IA[1,2] a rice sheath blight fungus. It is less phytotoxicity than other benzanilide fungicides such as mebenil.[2]

Suda *et al.*[3] have reported that mepronil inhibited glucose, pyruvate and oxalacetate from being oxidized by mycelia of *R. solani* at concentrations high enough to inhibit the mycelial growth. This observation suggests that it inhibits some energy-linked metabolism of the fungus such as the citric acid cycle or electron transport system.

Carboxin, which is structurally related to mepronil and has a fungicidal spectrum similar to that of mepronil, has been reported to have inhibited the succinoxidase system in isolated mitochondria from *Ustilago maydis* and rat liver.[4] Subsequently, its site of action has been shown to be in the complex II region of the respiratory chain.[5-7] A recent report has also demonstrated that substituted benzanilides including mepronil inhibited succinate dehydrogenase (succinate-DCPIP-reductase) in isolated mitochondria of *U. maydis*[8] and *R. solani*.[9]

In the present work, we studied not only the effect of mepronil on succinate oxidation in mitochondria isolated from *R. solani* and other organisms including fungi, animals and plants but also the kinetics of the inhibition of succinate dehydrogenase of *R. solani*. From results we found the action mechanism, selective toxicity and phytotoxicity of mepronil.

MATERIALS AND METHODS

1. *Materials*

R. solani IA, *Pyricuralia oryzae* and *Botrytis cinerea* were maintained on potato-sucrose or

* Part of this work was reported at the Meeting of Pesticide Science Society of Japan, Kawasaki, March 1987.

potato-dextrose agar slants in our laboratory. Wister rats and B6C3F1 mice were obtained from Japan SLC Co., Ltd. Etiolated pea seedlings (*Pisum sativum*, cv. Akabana-tsurunashi) were grown in the dark for 5 days at 30°C. Sweet potato tuberous roots (*Ipomea batatas*, cv. Koukei) were obtained in a local market.

2. Chemicals

The following chemicals were purchased from commercial sources: MOPS and EDTA from Doujin Chemical Institute, cytochrome *c* from horse heart, antimycin A and coenzyme Q_{10} from Sigma Chemical Company, NADH from Oriental Yeast Co., Ltd., rotenone from Aldrich Chemical Company, agar and yeast extract from DIFCO.

Mepronil, carboxin and oxycarboxin were provided as reagent-grade products from KI Chemical Research Institute Co., Ltd.

3. Preparation of Mitochondrial Fraction

Mitochondria were prepared from rats and mice by the method of Johnson & Lardy,[10] and those from etiolated pea seedlings and sweet potato tuberous roots by the methods of Nawa *et al.*[11] and Asahi,[12] respectively. Mitochondria were isolated from *R. solani*, *P. oryzae* and *B. cinerea* as follows: mycelial fragments of *R. solani* from the colonies on potato-sucrose agar slants were inoculated into a Czapeck-yeast extract liquid medium containing 2% glucose, 0.2% ammonium sulfate, 0.2% sodium nitrate, 0.1% potassium dihydrogenphosphate, 0.1% sodium citrate, 0.1% yeast extract, 0.01% calcium chloride, 0.01% magnesium sulfate and 0.005% ferric(II) sulfate, and precultured at 28°C on a rotary shaker (200 oscillations/min) for 2 days. Two hundred milliliters of the precultured medium in a 500-ml Erlenmeyer flask containing some masses of mycelia was homogenized in a sterilized Waring blender and 10 ml each of the aliquots was again cultured in 200 ml of the Czapeck-yeast extract liquid medium for 38 hr under the same condition as for the preculture. The mycelia were collected by vacuum filtration and then washed three times with distilled water and twice with 0.6 M of mannitol solution. Ten grams of mycelia thus obtained was ground with 30 ml of 20 mM MOPS-KOH buffer, pH 7.1, containing 0.3 M mannitol, 0.1% (w/v) BSA, 1 mM EDTA and 20 g of sea sand (buffer A) in an ice-cold mortar. The homogenate was squeezed through one layer of nylon gauze and centrifuged at $700\times g$ for 7 min and $2000\times g$ for 10 min. The supernatant was centrifuged at $27{,}000\times g$ for 20 min. The resultant aggregate was collected with a Pasteur pipette and washed three times with approximately 20 ml of 10 mM MOPS-KOH buffer, pH 7.1, containing 0.25 M sucrose and 1 mM EDTA (buffer B). The aggregate was finally recovered as a pellet by centrifugation at $15{,}000\times g$ for 20 min and suspended in 1.5 ml of buffer B. This suspension was freshly prepared for each experiment. The mitochondrial preparation thus obtained showed electron-transport activity, but no oxidative-phosphorylation activity.

In the case of *P. oryzae*, 10 ml of each spore suspension was inoculated into a glucose-yeast extract medium consisting of 2% glucose and 0.4% yeast extract. The inoculated medium was cultured for 3 days under the conditions described for the culture of *R. solani*. The mycelia were collected, washed and homogenized with buffer A in an ice-cold mortar. The homogenate was passed through a nylon gauze and centrifuged at $2000\times g$ for 7 min. The supernatant was then centrifuged at $15{,}000\times g$ for 20 min. The resultant precipitate was washed twice with buffer B and recovered by centrifugation at $15{,}000\times g$ for 20 min. Finally the pellet was suspended in buffer B and used for assays.

B. cinerea was cultured in the conditions described for *R. solani* except the temperature of 20°C and the culture medium of glucose-yeast extract. Mitochondria from *B. cinerea* were prepared by the method described for *P. oryzae*.

All operations described above were carried out at 0–4°C.

4. Measurement of Oxygen Consumption

Oxygen consumption was measured polarographically with a recording oxygen electrode (Yellow Springs Instrument Co.) in 3 ml of 10 mM sodium phosphate buffer, pH 7.0, containing 0.3 M mannitol, 5 mM magnesium

chloride, 10 mM potassium chloride and 0.1% (w/v) BSA at 25°C. Oxygen uptake was calculated on the basis of 240 μM O_2 in the aerated medium[13] and expressed as nmol O_2 per minute per mg protein. An inhibitor was dissolved in ethanol and added to a reaction mixture (final ethanol concentration: 0.3–1% (v/v)).

5. Enzyme Assays

Succinate- and NADH-cytochrome *c* reductases were assayed by measuring the increase in the absorbancy at 550 nm resulting from the reduction of cytochrome *c* in a double-beam spectrophotometer with a sipper cell (Hitachi 220). An extinction coefficient of 1.92×10^4 M^{-1} cm^{-1} was used to calculate the amount of reduced cytochrome *c*.[14] The reaction mixtures contained mitochondria (5–25 μg protein), 50 mM potassium phosphate (pH 7.2), 1 mM KCN, 27 μM cytochrome *c*, and 20 mM sodium succinate or 0.2 mM NADH in a final volume of 3 ml.

Succinate- and NADH-coenzyme Q_{10} reductases were assayed by measuring the decrease in the absorbancy at 275 nm resulting from the reduction of coenzyme Q_{10} after extraction into hexane layers.[15] The reaction mixtures were composed of mitochondria (approximately 200 μg protein), 33 mM potassium phosphate (pH 7.2), 0.25 M sucrose, 1.5 mM KCN, 0.232 μM coenzyme Q_{10}, and 20 mM succinate or 1.5 mM NADH in a final volume of 3 ml.

Succinate dehydrogenases were assayed by both directly reducing 2,6-dichlorophenolindophenol (succinate-DCPIP reductase) and reducing DCPIP or cytochrome *c* from phenazine methosulfate (succinate-PMS-DCPIP reductase and succinate-PMS-cytochrome *c* reductase).[8,16,17] DCPIP reduction was measured by the decrease in the absorbancy at 600 nm and enzyme activity was calculated by using an extinction coefficient of 2.1×10^4 M^{-1} cm^{-1}. Maximum velocity (V_{max}) was obtained from double reciprocal plots of velocity against dye concentration. Solubilized succinate dehydrogenase from mitochondria of *R. solani* was prepared by sonication and centrifugation at $100{,}000 \times g$ for 30 min. The standard reaction mixture for succinate-DCPIP reductase contained mitochondria (approximately 100 μg protein), 50 mM potassium phosphate (pH 7.2), 0.25 M sucrose, 1 mM KCN, 50 μM DCPIP and 16.7 mM sodium succinate in a final volume of 3 ml. PMS was also included in the succinate-PMS-DCPIP reductase assay mixture. Mitochondria (approximately 50 μg of protein) and solubilized dehydrogenase (approximately 2 μg of protein) were used for each assay. The standard reaction mixtures of succinate-PMS-cytochrome *c* reductase contained mitochondria (approximately 50 μg of protein), 50 mM potassium phosphate (pH 7.2), 0.25 M sucrose, 1 mM KCN, 2.5 μM antimycin A, 0.54 mM PMS, 54 μM cytochrome *c* and 16.7 mM sodium succinate in a final volume of 3 ml.

All reactions were initiated by adding mitochondria or solubilized succinate dehydrogenase and carried out at 28°C. Each inhibitor to be tested was dissolved in ethanol and added to a reaction mixture (final ethanol concentration: 1% (v/v)).

6. Protein Determination

Soluble proteins were determined by the method of Bradford[18] using bovine serum albumin as a standard. Mitochondrial proteins were determined by adding 400 μl of 6%(w/v) TCA to each mitochondrial solution. The solutions were left standing for 1 hr at 4°C and then centrifuged. The resultant precipitates were dissolved in 100 μl of 1 N NaOH and assayed for protein content by the method of Bradford.

RESULTS AND DISCUSSION

1. Effect of Mepronil on the Oxidation of Succinate and NADH by Mitochondria Isolated from R. solani

Mepronil strongly inhibited succinate from being oxidized by isolated mitochondria from *R. solani*, while it hardly inhibited NADH even at as high as 100 μM (Table 1). This result suggested that mepronil selectively inhibits the complex II region but not complex I, III and IV regions in the electron trnasport system of *R. solani*.

Table 1 Effect of mepronil on the oxidation of succinate and NADH by mitochondria isolated from *Rhizoctonia solani* IA.

Chemicals	Inhibition (%)	
	Succinate[a]	NADH[a]
Mepronil (μM)		
0.3	41	—[b]
3.3	57	0
33	86	4.1
100	—	14
Rotenone (μM)		
1.6	14	95
Antimycin A (μM)		
1.6	—	92

[a] The respiratory rates of succinate oxidation and NADH oxidation without inhibitors were 4.4 and 52 nmol O_2/min/mg protein respectively.

[b] Not determined.

2. *Effect of the Mepronil on Cytochrome c Reductase and Coenzyme Q Reductase of R. solani*

The activity of succinate-cytochrome *c* reductase was inhibited to nearly the same extent as the inhibition of succinate oxidation which was measured polarographically (Table 2). The I_{50} value of this enzyme inhibition was 0.25 μM. Succinate-coenzyme Q_{10} reductase was inhibited 95% by 5 μM mepronil. On the other hand, neither NADH-cytochrome *c* reductase nor NADH-coenzyme Q_{10} reductase was affected by mepronil as significantly as succinate oxidation. These results supported that the action site of mepronil is in the complex II region in the electron transport system of *R. solani*.

3. *Properties of Succinate Dehydrogenase of R. solani*

When DCPIP was used as a direct electron acceptor, the V_{max} of succinate dehydrogenase in the mitochondria isolated from *R. solani* was 0.214±0.042 μmol DCPIP reduced per minute per mg protein. On the other hand, the V_{max} was 0.519±0.098 μmol reduced DCPIP per minute per mg protein when PMS was employed as an intermediate electron acceptor. The *Km* value was 0.084±0.016 mM for succi-

Table 2 Effect of mepronil, carboxin and oxycarboxin on succinate-, NADH-cytochrome *c* reductase and succinate-, NADH-coenzyme Q_{10} reductase from *R. solani* IA.

Reductase Reaction[a]	Compounds (μM)	Inhibition (%)
Succinate-cytochrome *c*	Mepronil	
	0.10	32
	0.25	49
	0.50	67
	1.0	79
	10	99
Succinate-cytochrome *c*	Carboxin	
	0.10	39
	0.30	53
	0.60	62
	1.0	69
	3.0	79
Succinate-cytochrome *c*	Oxycarboxin	
	1.0	8.3
	5.0	29
	10	58
	25	83
	100	97
NADH-cytochrome *c*	Mepronil	
	10	9.7
	100	18
NADH-cytochrome *c*	Carboxin	
	10	5.8
	100	23
NADH-cytochrome *c*	Oxycarboxin	
	10	0
	100	0
Succinate-Coenzyme Q_{10}	Mepronil	
	5.0	95
NADH-coenzyme Q_{10}	Mepronil	
	5.0	4.5

[a] The activities of succinate- and NADH-cytochrome *c* reductases of control runs were 0.435 and 2.15 μmol of cytochrome *c* reduced per minute per mg protein, respectively. The activities of succinate- and NADH-coenzyme Q_{10} reductases in control runs were 0.604 and 0.578 μmol of coenzyme Q_{10} reduced per 25 min per mg protein.

nate and 0.053±0.023 mM for DCPIP in direct DCPIP reduction assay, and 0.420±0.023 mM for PMS in succinate-PMS-cytochrome *c* reductase assay by Lineweaver-Burk plots. The specific activity of succinate dehydrogenase of *R. solani* determined above was considerably lower than those of mammals,[19] and slightly higher than that of *U. maydis*.[5] The *Km* values for substrate and electron acceptors of the enzyme from *R. solani* were comparable with those of *U. maydis*.

Wowery *et al.*[7] have shown that the turnover numbers of succinate dehydrogenase in a typical preparation of electron-transporting particles (ETP) and complex II from beef heart were 22,000 and 10,000, respectively, in an assay system using PMS as a first electron acceptor and DCPIP as a second acceptor. When the activity of the enzyme was assayed by direct reduction of DCPIP, however, the turnover numbers of the enzyme were 6000 with both preparation. Thus, the ratio of the turnover numbers by PMS reduction to those by direct DCPIP reduction was over 3 (22,000/6000) in ETP and below 2 (10,000/6000) in complex II. Our preparation of mitochondria from *R. solani* seemed to be composed of a mixture of ETP and complex II, since the ratio of enzyme activity determined by PMS reduction to that determined by DCPIP reduction was approximately 2.4 (0.519/0.214).

4. *Effect of Mepronil on the Succinate Dehydrogenase of R. solani*

Mepronil markedly inhibited the activity of succinate-DCPIP reductase of mitochondria from *R. solani* with its I_{50} value being 0.24 μM (Fig. 1). This I_{50} value was almost the same as that obtained with succinate-cytochrome *c* reductase described above (0.25 μM) and approximately half of those obtained based on the mycelial growth and the glucose oxidation by mycelia of *R. solani* in the previous papers.[2,3] The results suggested that succinate dehydrogenase inhibition is a primary action of mepronil.

As shown in Fig. 2, the inhibition of succinate-DCPIP reductase by mepronil was completely, showing the nature of simple saturation kinetic, while the inhibition of succinate-PMS-cytochrome *c* reductase was never completed. The maximum inhibition was about 60% when 0.54 mM PMS was employed in the assay.

On the other hand, the solubilized succinate dehydrogenase, which did not show direct

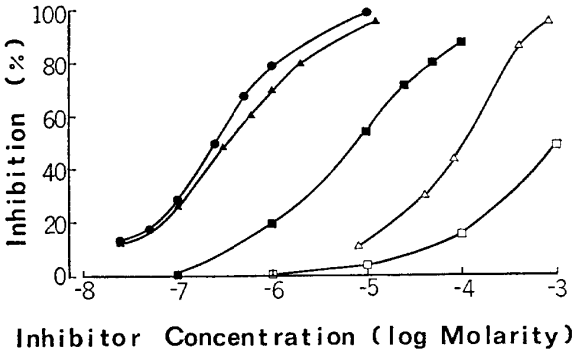

Fig. 1 Relative potency of mepronil, carboxin, oxycarboxin, thenoyltrifluoroacetone (TTA) and oxalacetate as inhibitors of succinate-DCPIP reductase of *Rhizoctonia solani* mitochondria.

Assay condition and enzyme activity of the control are described in the text.

●, Mepronil; ▲, Carboxin; ■, Oxycarboxin; △, Oxalacetate; □, TTA.

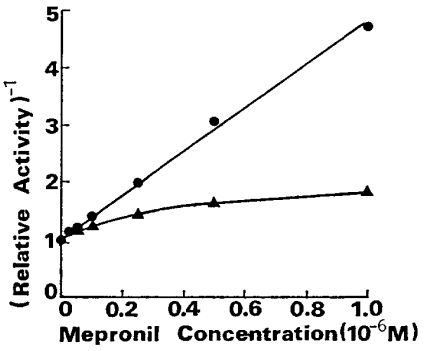

Fig. 2 Dixon plots of the inhibition of succinate-DCPIP reductase and succinate-PMS-cytochrome *c* reductase of *Rhizoctonia solani* mitochondria by mepronil.

The concentration of DCPIP, PMS and cytochrome *c* employed in this assay were 50 μM, 0.54 mM and 54 μM, respectively. The activities of succinate-PMS-cytochrome *c* reductase and succinate-DCPIP reductase without mepronil was 0.310 μmol of cytochrome *c* reduced per minute per mg protein and 0.214 μmol of DCPIP reduced per minute per mg protein, respectively under saturated condition of succinate.

●, Succinate-DCPIP reductase; ▲, Succinate-PMS-cytochrome *c* reductase.

Table 3 Effect of mepronil, carboxin, oxycarboxin and oxalacetate on succinate dehydrogenase from *Rhizoctonia solani* IA.
Enzyme activity was assayed using phenazine methosulfate (0.54 mM) as an intermediate electron acceptor. Each compound was tested at 100 μM of concentration.

Enzyme form	Chemicals	Inhibition(%)
Mitochondria[a)]	Mepronil	60
	Carboxin	59
Solubilized	Mepronil	3.3
Succinate	Carboxin	0.8
Dehydrogenase[b)]	Oxycarboxin	3.3
	Oxalacetate	36

a) The activity of mitochondrial succinate dehydrogenase of the control run was 0.311 μmol of cytochrome *c* reduced per minute per mg protein.
b) The activity of solubilized succinate dehydrogenase of the control run was 0.488 μmol DCPIP reduced per minute per mg protein.

DCPIP reduction but showed PMS-reducing activity, was not inhibited by mepronil, nor carboxin, nor oxycarboxin (Table 3). It is known that PMS reacts with succinate dehydrogenase at two sites on it, one of which loses reactivity with PMS by solubilization.[20] Our results indicated that mepronil reacts with one of the PMS reaction sites which has been inactivated by solubilization and that it never reacts with the other site which remains reactive with PMS irrespective of solubilization.

Motoba *et al.*[9] have studied the mode of action of flutolanil and reported that 1 μM and 10 μM mepronil inhibited succinate-DCPIP reductase of *R. solani* by 29% and 47% respectively, and that the I_{50} value was 1.4 μM. The I_{50} value of succinate-DCPIP reductase of *R. solani* obtained in this study is considerably lower than that obtained by Motoba *et al.* In the course of our study the sensitivity of the inhibition of succinate-DCPIP reductase of *R. solani* by mepronil changed depending on the growth stage of *R. solani*. Longer culture period and faster oscillation than those described in the method lowered the sensitivity of the inhibition and the inhibition was turned from saturated to unsaturated by unknown factors. Since mepronil inhibited the glucose oxidation of mycelia of *R. solani* by approximately 40% and 80% at concentrations of 0.37 μM and 3.7 μM, respectively,[2] the I_{50} value obtained in our study seems to be consistent with mycelial growth inhibition, suggesting that the culture condition of *R. solani* employed in this experiment is suitable to clarify the true potency of enzyme inhibition by mepronil.

5. *Kinetics of the Inhibition of Succinate Dehydrogenase from R. solani by Mepronil*

Inhibition of mitochondrial DCPIP reduction by mepronil was analyzed by double reciprocal plots. The results demonstrated a mixed-type inhibition with respect to succinate (Fig. 3). The inhibition constant was

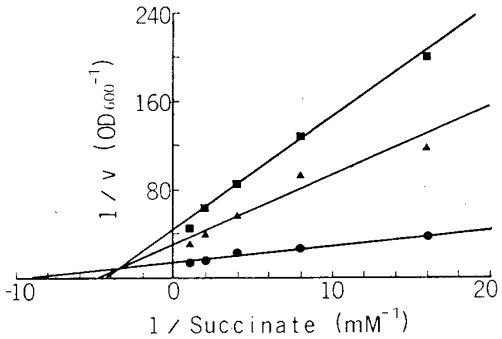

Fig. 3 Lineweaver-Burk plots of the inhibition of succinate-DCPIP reductase of *Rhizoctonia solani* mitochondria by mepronil at various succinate concentrations.
●, Control; ▲, 0.25 μM mepronil; ■, 0.5 μM mepronil.

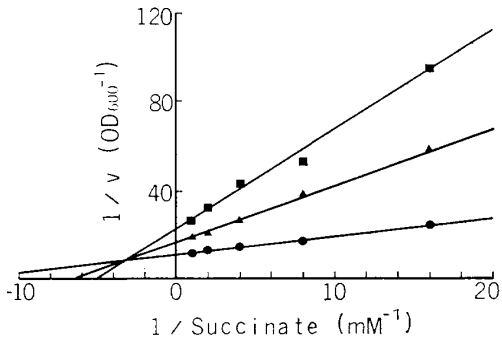

Fig. 4 Lineweaver-Burk plots of the inhibition of succinate-DCPIP reductase of *Rhizoctonia solani* mitochondria by carboxin at various concentrations of succinate.
●, Control; ▲, 0.3 μM carboxin; ■, 0.6 μM carboxin.

Table 4 I_{50}-values of various compounds to succinate-DCPIP reductase in mitochondria isolated from various sources.

Mitochondria isolated from	I_{50}[a] ($\times 10^{-6}$ M)				
	Mep	Car	Oxy	TTA	Oxa
R. solani	0.24	0.32	6.8	>1000	94
P. oryzae	>100	4.2	100	710	202
B. cinerea	86	1.4	—[b]	—	—
Rat liver	99	5.3	99	3.9	395
Mouse liver	61	4.6	51	4.6	—
Pea	>100	>100	>100	>1000	146
Sweet potato	>100	>100	>100	>1000	428

[a] Test compounds: mepronil (Mep), carboxin (Car), oxycarboxin (Oxy), thenoyltrifluoroacetone (TTA) and oxalacetate (Oxa).

[b] Not determined.

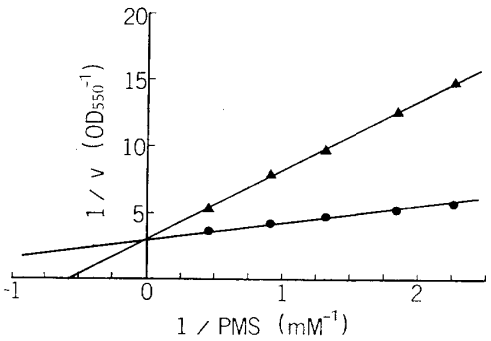

Fig. 5 Lineweaver-Burk plots of the inhibition of succinate-PMS-cytochrome *c* reductase by mepronil with respect to phenazine methosulfate.
●, Control; ▲, 100 μM mepronil.

calculated to be 0.082 μM for K_i and 0.23 μM for K_i' from regression curves. The similar inhibition curves were also obtained for carboxin (Fig. 4) and oxycarboxin, from which the values of K_i and K_i' were calculated to be 0.14 μM and 0.55 μM for carboxin, 4.1 μM and 17 μM for oxycarboxin, respectively. On the other hand, the inhibition was uncompetitive, contained a small noncompetitive component (K_i: 4.1 μM, K_i': 0.18 μM) with respect to DCPIP. When cytochrome *c* was used as the electron acceptor from PMS, the inhibition by mepronil was partially competitive with respect to PMS (Fig. 5). The inhibition pattern of carboxin was similar (data not shown).

From these kinetic data, it seems likely that mepronil binds to the enzyme at a different site with succinate and DCPIP. Further study is necessary before drawing a conclusion on the binding site of PMS since a report[7] says that 3′-methylcarboxin, an analog of carboxin, changed the inhibition pattern depending upon mitochondrial preparation used for assay.

6. *Effect of Mepronil on Succinate Dehydrogenase in Mitochondria Isolated from Various Organisms*

As given in Table 4, succinate-DCPIP reductase of *R. solani* was inhibited potently by mepronil, while DCPIP reduction by mitochondria from other sources was not markedly affected by mepronil. The inhibitory potency of mepronil to succinate-DCPIP reductase from rat liver and mouse liver was nearly identical with that to oxygen uptake, which showed a good correlation of the inhibition between succinate-DCPIP reductase and oxygen consumption (data not shown).

On the other hand, carboxin inhibited not only succinate-DCPIP reductase of *R. solani* but also those of *P. oryzae, B. cinerea,* rat liver and mouse liver at relatively low concentrations. TTA, which is known to be a potent inhibitor of mammalian succinate dehydrogenase, inhibited only succinate-DCPIP reductase from rat and mouse livers and did not inhibit succinate-DCPIP reductase of *R. solani.*

As seen above, there were great differences in the sensitivity to these inhibitors among succinate dehydrogenases isolated from various sources. Mepronil was a fairly selective inhibitor to succinate dehydrogenase of *R. solani.*

ACKNOWLEDGMENTS

The authors are indebted to Kumiai Chemical Industry Co., Ltd., for permission to publish this work.

REFERENCES

1) S. Kawada, Y. Suda, N. Muramatsu & I. Chiyomaru: *Ann. Phytopathol. Soc. Jpn.* **45** (4), 547 (1979)
2) S. Kawada, A. Sakamoto & I. Shimazaki: *J. Pesticide Sci.* **10**, 315 (1985)
3) Y. Suda, N. Muramatsu, I. Chiyomaru & S. Kawada: 5th ICPC, IVc-2, 1982
4) D. E. Mathre: *Pestic. Biochem. Physiol.* **1**, 216 (1971)
5) G. A. White: *Biochem. Biophys. Res. Commun.* **44** (5), 1212 (1971)
6) J. T. Ulrich & D. E. Mathre: *J. Bacteriol.* **110** (2), 628 (1972)
7) P. C. Wowery, D. J. Steenkamp, B. A. C. Ackrell, T. P. Singer & G. A. White: *Arch. Biochem. Biophys.* **178**, 495 (1977)
8) G. A. White: *Pestic. Biochem. Physiol.* **27**, 249 (1987)
9) K. Motoba, M. Uchida & E. Tada: *Agric. Biol. Chem.* **52** (6), 1445 (1988)
10) D. Johnson & H. Lardy: "Methods in Enzymol," ed. by R. W. Estabrook & M. E. Pullman, Vol. X, Academic Press, New York and London, pp. 94–96, 1967
11) Y. Nawa & T. Asahi: *Plant Physiol.* **48**, 671 (1971)
12) T. Asahi: "Methods in Pesticide Science," ed. by J. Fukami, Y. Uesugi, K. Ishizuka, C. Tomizawa, Vol. 3, Soft Science, Inc., Tokyo, pp. 250–259, 1981 (in Japanese)
13) J. B. Chappell: *Biochem. J.* **90**, 225 (1964)
14) T. E. King: "Methods in Enzymol," ed. by R. W. Estabrook & M. E. Pullman, Vol. X, Academic Press, New York and London, pp. 216–225, 1967
15) T. Ramasarma & R. L. Lester: *J. Biol. Chem.* **235** (11), 3309 (1960)
16) T. E. King: "Methods in Enzymol," ed. by R. W. Estabrook & M. E. Pullman, Vol. X, Academic Press, New York and London, pp. 322–331, 1967
17) H. Haraguchi, T. Tanaka, M. Taniguchi, S. Oi & K. Hashimoto: *Agric. Biol. Chem.* **51** (5), 1453 (1987)
18) M. Bradford: *Anal. Biochem.* **72**, 248 (1976)
19) W. G. Hanstein, K. A. Davis, M. A. Ghalambor & Y. Hatefi: *Biochemistry* **10** (13), 2517 (1971)
20) A. Giuditta & T. P. Singer: *J. Biol. Chem.* **234**, 666 (1959)

要　　約

殺菌剤メプロニルの作用機作と選択性

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メプロニルは，イネ紋枯病菌ミトコンドリアのコハク酸を基質とする酸素消費を低濃度で阻害したが，NADH を基質とする酸素消費にはほとんど影響を与えなかった．そこでコハク酸酸化に関与する酵素に対する影響を検討したところ，本剤は低濃度でイネ紋枯病菌ミトコンドリアのコハク酸–チトクロム *c* 還元酵素 (I_{50}; 0.25 μM)，コハク酸–コエンザイム Q_{10} 還元酵素ならびにコハク酸–DCPIP 還元酵素 (I_{50}; 0.24 μM, K_i; 0.082 μM, K_i'; 0.23 μM) を阻害することが明らかとなった．以上の結果から，メプロニルの作用点は呼吸鎖複合体Ⅱのコハク酸脱水素酵素であると考えられる．一方，イネいもち病菌，灰色かび病菌，ラット肝臓，マウス肝臓，エンドウ黄化幼植物ならびにサツマイモのコハク酸–DCPIP 還元酵素は弱く阻害されたに留まったことから，本剤は高い選択性を有した薬剤であると考えられる．