

Original Article

Intermediates Accumulation and Inhibition Sites of Carpropamid in the Melanin Biosynthesis Pathway of *Pyricularia oryzae*

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Carpropamid {(1*RS*,3*SR*)-2,2-dichloro-*N*-[1-(4-chlorophenyl)ethyl]-1-ethyl-3-methylcyclopropanecarboxamide} strongly inhibited pigmentation in shaking culture as well as in agar plate culture of *Pyricularia oryzae* and *Verticillium dahliae*. White crystals were isolated from the extract of the cultures of wild type strains of *P. oryzae* and *V. dahliae* treated with carpropamid. Analytical data of the crystals obtained from a spot at *R_f* 0.36 on the TLC plates coincided with those of scytalone. A large amount of scytalone and a small amount of vermelone accumulated in the culture treated with 10 µg/ml of carpropamid, while 2-hydroxyjuglone was not detected in these analyses. When scytalone was added to the shaking culture of albino mutants of *P. oryzae* (*P₂-alb*), it promptly disappeared from the culture and the culture was pigmented to light brown. But scytalone remained in the culture if treated with carpropamid. Scytalone and vermelone administered beside the colony of *P₂-alb* on the agar plates were converted to black pigments but the pigmentation was inhibited by carpropamid. These results suggest that carpropamid inhibits dehydration of scytalone and vermelone in the fungal melanin biosynthesis.

INTRODUCTION

Carpropamid {(1*RS*,3*SR*)-2,2-dichloro-*N*-[1-(4-chlorophenyl)ethyl]-1-ethyl-3-methylcyclopropanecarboxamide} is a potent protectant of rice plants with systemic and long lasting properties for the control of rice blast disease. Its effect is exhibited not only against leaf but also panicle blast by one shot application of nursery box treatment at a considerably low rate.^{1,2)}

Carpropamid affects pigmentation of various plant pathogenic fungi in the plate culture and their colony colors turn brown from black. Hyaline appressoria of *Pyricularia oryzae* are formed when germinated in the solution with carpropamid at more than 0.1 µg/ml, whereas the chemical does not substantially inhibit vegetative growth, spore germination, germ tube elongation and appressorium formation of *P. oryzae*.³⁾ These actions of carpropamid closely resemble those of conven-

tional melanin biosynthesis inhibitors (MBIs) such as PCBA,⁴⁾ tricyclazole,⁵⁾ pyroquilon⁶⁾ and fthalide.⁷⁾

From the fact that a fungicidal activity *in vitro* is scarcely observed in spite of excellent blast controlling efficacy *in vivo*, it could be argued that carpropamid is converted to active metabolites in the treated plants or enhances resistant reactions in plants. However, no active metabolites have been found in the metabolic study in plants.⁸⁾ On the other hand, carpropamid has a common moiety in its chemical structure with 2,2-dichloro-3,3-dimethylcyclopropane carboxylic acid (DDCP), which has been shown to exert a rice blast controlling activity by accentuating a host defense mechanism.⁹⁻¹¹⁾ Carpropamid may, therefore, be expected to induce systemic acquired resistance in plants as its mode of action. But our previous studies indicated that carpropamid had a similar property to MBIs and interferes with melanin biosynthesis in susceptible fungi.³⁾ Since melanin biosynthesis is essential for the blast fungus to penetrate into the host epidermis and to exhibit pathogenicity to rice plants,¹²⁻¹⁵⁾ carpropamid

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was suggested to exert its efficacy on the disease primarily through inhibition of the melanin biosynthesis.

Known MBI fungicides have some similarity in their structures as well as to substrates of reductase in the melanin biosynthesis pathway. However, carpropamid has little resemblance in chemical structure to these MBIs or the precursors of melanin biosynthesis. Thus carpropamid was supposed to have a novel mechanism of action in melanin biosynthesis inhibition, possibly different from that of the conventional MBIs.

This paper deals with action sites of carpropamid in the melanin biosynthesis pathway and we present here the accumulation of intermediates being different from those induced by the known MBIs.

MATERIALS AND METHODS

1. Chemicals

Carpropamid was synthesized by Bayer and Nihon Bayer Agrochem. Tricyclazole was purchased from the market. Scytalone, an intermediate in melanin biosynthesis, was prepared from the culture of *Verticillium dahliae* brm-1 mutant.¹⁶⁾ Another intermediate, 1,8-dihydroxynaphthalene (1,8-DHN), was kindly supplied by Mr. Wada at the Yuki Research Centre. Those compounds were dissolved in methanol and diluted with distilled water. A small amount of Tween 20 was added before dilution when necessary for homogeneous dissolution of the chemicals.

2. Fungi and Culture Methods

P. oryzae P₂ (wild type) and P₂-alb (albino mutant: 60-2 and IW), *V. dahliae* brm-1 (brown mutant), and *V. dahliae* (wild type strain Chr 208, kindly provided by Mr. Hoshi, Tokyo Agricultural Experimental Station) were used in this study.

Rice blast fungus P₂ was cultured with or without carpropamid on potato dextrose agar (PDA) at 25°C for 10 days or in 100 ml of liquid medium of potato dextrose (PD) by reciprocal shaking culture at 25°C for 5 to 7 days. To the liquid culture, 1 ml of carpropamid methanol solution at 1000 µg/ml was added after 0, 1, 2, 3, 4 or 5 days of culturing. For the cultivation, 1 ml of spore suspension (10⁶/ml) was mixed with 20 ml of PDA at 43°C and the inoculated medium was poured into 9 cm petri dishes or 1 ml of spore suspension at 10⁵ spores/ml was added to the PD (100 ml) culture flask. P₂-alb 60-2 and IW were inoculated with the 4 mm disc for plate culture. For liquid culture of P₂-alb, 5 pieces of the 4 mm agar disc were inoculated into the liquid medium.

V. dahliae brm-1 and Chr 208 were inoculated with the 4 mm agar disc from their preculture on PDA plates and cultured at 25°C for 28 days in the dark.

3. Characterization of Intermediates

3.1 Extraction of metabolites from agar plate culture

In order to determine the accumulation of melanin intermediates in the culture, 10 agar plates of *P. oryzae* P₂ or *V. dahliae* Chr 208 cultured with carpropamid were homogenized and extracted with 200 ml of 80% acetone overnight at room temperature. The filtered residue was re-extracted twice with 200 ml of 80% fresh acetone. The acetone in the combined extracts was evaporated *in vacuo*, and the aqueous solution was adjusted to pH 2 with HCl and saturated with NaCl. This was extracted 3 times with 200 ml of ethyl acetate and fractionated. Then the organic layer was concentrated *in vacuo* to dryness after dehydration with anhydrous Na₂SO₄. It was dissolved in a small amount of acetone and applied onto a silica gel column (25 mm i.d. × 40 cm, silica gel 60; Merck), which was eluted with mixed solvent of acetone: *n*-hexane: ethyl acetate (10:30:60). The eluate was dried *in vacuo* and filled up with 2 ml of methanol. An aliquot of the solution was applied on a TLC plate (silica gel F-254; Merck) and developed with the solvent described above. After development, the spots of metabolites were observed under UV at 254 and 366 nm. For the quantitative analysis of the intermediates, the methanol solution was applied to HPLC (HP-1090) on LiChrospher 100 RP-18 (4 mm i.d. × 25 cm) at 40°C and eluted with acetonitrile-H₂O (1 ml/min: gradient from 10–50% of acetonitrile/30 min).

3.2 Extraction of metabolites from PD liquid culture

The filtrate of the liquid culture of *P. oryzae* was extracted three times with an equivalent volume of ethyl acetate after the pH was adjusted to 2 with HCl. The organic layer was dehydrated with anhydrous Na₂SO₄ and dried *in vacuo*. Subsequent procedures for the analysis were the same as those for the PDA culture.

4. Crystallization and Identification of the Intermediates

A large spot on the silica gel TLC was scraped off from the TLC plate and extracted with 20 ml of diethyl ether. An equal volume of *n*-hexane was added to the ether solution and the mixture in the flask was kept in a draft chamber without a cap. With gentle evaporation of ether, a white substance was crystallized in the solution. The crystals were analyzed for melting point, IR, MS, NMR and optical rotation.

5. Scytalone Metabolism by P₂-alb and Melanization Recovery by Addition of Intermediates

One milliliter of scytalone methanol solution at 1000 µg/ml with or without carpropamid (1000 µg/ml) was added to 100 ml of P₂-alb culture. The P₂-alb 60-2 and IW were cultured in PD for 3 days prior to scytalone and carpropamid treatment. The remaining scytalone in the

culture was quantitatively measured at different times.

P₂-alb 60-2 was cultured for 4 days on PDA plates amended with carpropamid or tricyclazole at 10 µg/ml. Four millimeter filter paper containing 10–30 µg of scytalone, vermeline or 1,8-DHN was put beside the albino colony. After 2 days culturing, pigmentation between the colony and the filter paper was observed and assessed.

RESULTS

1. TLC Study

A large clear spot and a small spot were respectively observed at *R_f* 0.36 and 0.54 on the TLC plate under UV irradiation at 254 and 366 nm in the extracts of agar plate culture of rice blast fungus P₂ and *V. dahliae* Chr-208 treated with carpropamid. The *R_f* values coincided well with those of authentic scytalone and vermeline, respectively. The spots were hardly seen in the extracts of untreated P₂ blast culture. In the culture with tricyclazole, pink and yellow-red spots were observed at *R_f* 0.05 and 0.02, which were assumed to be those of flaviolin and 2-hydroxyjuglone (2-HJ), respectively. But these spots were not observed in the culture treated with carpropamid.

In the PD liquid culture of P₂ amended with carpropamid, the spot at *R_f* 0.36 was also evidently seen on TLC. Another spot, which is thought to be vermeline, was also observed at *R_f* 0.54 in the extract of liquid medium treated with carpropamid especially at the later stage of the culture. Thus scytalone was supposed to be abundantly accumulated in the cultures of rice blast fungus and *V. dahliae* treated with carpropamid as in the

culture of the brown mutant of *V. dahliae*. On the other hand, 2-HJ, a shunt product related to melanin biosynthesis, was not detected in the culture treated with carpropamid.

2. Analysis of the Crystals

Physical and chemical properties of the white crystals isolated from the culture broth were compared with those of scytalone isolated from *V. dahliae* brm-1.¹⁷⁾ The results of the analyses are summarized in Table 1. The melting point, IR and MS data of the crystals were very similar to those of scytalone reported by Bell *et al.*¹⁷⁾ Optical rotation, however, did not coincide with each other; optical rotation of scytalone isolated from brm-1 was reported to be (+)32,¹⁷⁾ but the crystals isolated from wild types of *P. oryzae* P₂ and *V. dahliae* Chr 208 as well as the crystals from *V. dahliae* brm-1 showed the optical rotation at (+)2-4 in our measurement. This result suggests that carpropamid did not influence the optical rotation of the crystals. However, the reason why the data of optical rotation were not identical is not clear at present.

3. Quantitative Study of Scytalone and Vermeline Accumulation

When carpropamid was added to the liquid culture of *P. oryzae* after 3-day preculture, fresh weight of the mycelia in 5-day culture was hardly influenced by application of carpropamid at 2.5 to 50 µg/ml and each weight was around 750 mg in 100 ml of the culture broth.

Table 1 Identification of isolated crystals.

	Scytalone ¹⁸⁾	Isolated crystals
m.p.	164–168°C (95% EtOH)	159–161°C (n-Hex/Et-Ether)
[α] _D	+32	+2–4
IR	(KBr) 1635, 1592	(KBr) 1639, 1593
ν _{max}	1275, 1175	1273, 1174
MS	(probe 80°C)	(probe 80°C)
<i>m/z</i> (%)	194 (M ⁺ ; 89%) 176 (M–H ₂ O; 85%) 15 (M–CH ₂ =CHOH; 100) 122 (M–CH ₂ =CHOH, CO; 26) 69 (35)	194 (92%) 176 (81) 150 (100) 122 (17) 69 (33)
H-NMR	(Acetone-D ₆) 2.6–3.2 (4H, m) 4.2 (1H, m)	(Acetone-D ₆) 2.6–3.2 (4H, m) 4.2 (1H, m, D ₂ O Ex) 4.3 (1H, m)
	6.04 (1H, d, <i>J</i> =2.5 Hz) 6.15 (1H, dd, <i>J</i> =2.5 Hz)	6.2 (1H, d, <i>J</i> =2.2 Hz) 6.3 (1H, m)
	12.45 (1H, s, D ₂ O Ex)	9.4 (1H, br. s, D ₂ O Ex) 12.8 (1H, s, D ₂ O Ex)

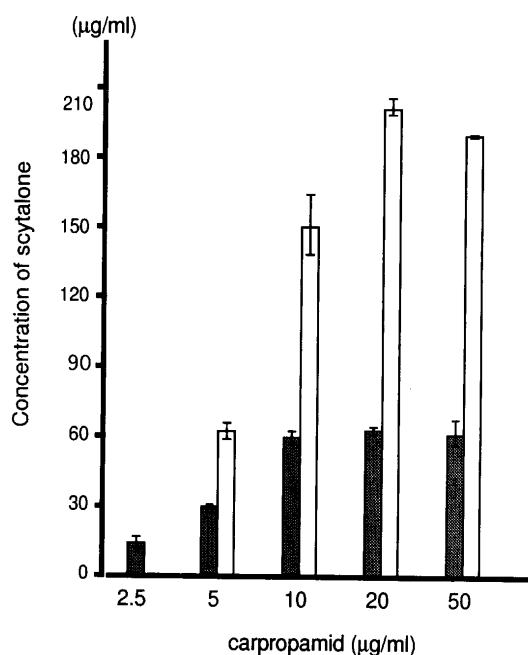


Fig. 1 Accumulation of scytalone in the culture broth treated with carpropamid at 2.5–50 µg/ml.

■: 5-day culture treated at 3-day preculture, □: 6-day culture treated at 4-day preculture.

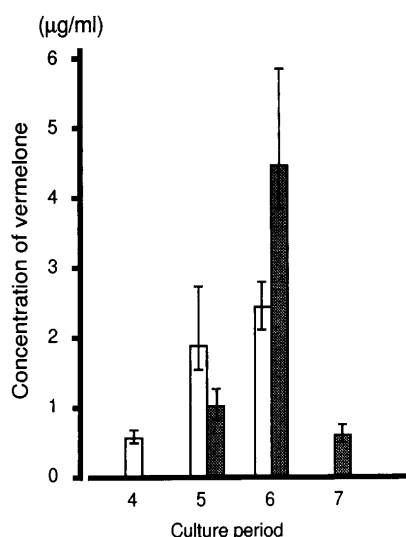


Fig. 2 Accumulation of vermelone in the blast culture treated with carpropamid at 10 µg/ml.

□: 4 to 6-day culture treated at 3-day preculture, ■: 5 to 7-day culture treated at 4-day preculture.

Scytalone extracted from the culture showed a large peak at R_t 8.0 min on HPLC and the characteristic UV spectrum with $\lambda_{\text{max}}^{\text{EtOH}}$ 280 nm was observed. In 5-day culture, amounts of scytalone in the broth increased as the treated concentrations (from 2.5 to 10 µg/ml), and reached a maximum around 60–80 µg/ml. Further accumulation was not observed by the treatment at more than 10 µg/ml of carpropamid after 3-day preculture. The accumulation in the 5-day culture was relatively low when blast inoculation and the chemical treatment were made simultaneously. In the 6-day culture the accumulation of scytalone exceeded 200 µg/ml and reached its maximum when carpropamid was added to the 4-day preculture (Fig. 1). In the 7-day culture, however, the accumulation decreased when the carpropamid was added to the 4-day preculture.

Vermelone was also detected in the culture broth by HPLC analysis. The peak at R_t 14.0 min and its characteristic UV spectrum with $\lambda_{\text{max}}^{\text{EtOH}}$ 259 and 333.5 nm were identical with those of the authentic sample. The accumulation in the blast culture changed according to the application doses and administration timing of carpropamid to the culture; in 6-day culture 1.7 µg/ml of vermelone was detected when 5 µg/ml of carpropamid was added and 3 to 4 µg/ml accumulated when 10 to 50 µg/ml was added to 4-day preculture. As shown in Fig. 2, in 4 to 6 day-culture about 0.6–2.5 µg/ml of vermelone accumulated in the broth when the chemical at 10 µg/ml was added to the culture after 3-day preculture, but vermelone accumulation reached 4.5 µg/ml when added after 4-day preculture.

Thus the application rate and timing of carpropamid to the liquid culture of *P. oryzae* proved to markedly influence scytalone and vermelone accumulation.

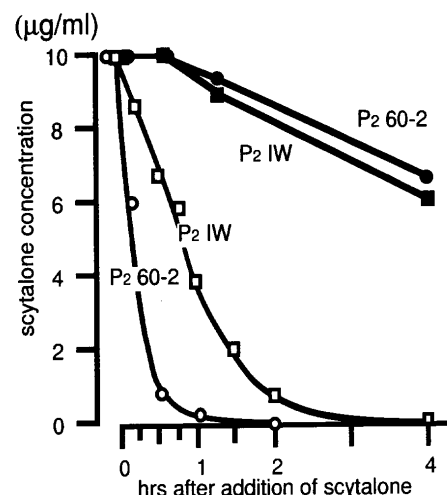


Fig. 3 Effect of carpropamid on scytalone metabolism in the culture of P₂-alb 60-2 and IW.

○, □: without carpropamid; ●, ■: with carpropamid at 10 µg/ml.

Table 2 Pigmentation from scytalone and vermelone on plate culture of P₂ alb 60-2 and IW.

Compound ^{a)}	Color of pigmentation		
	Scytalone ^{b)}	Vermelone ^{b)}	1.8-DHN ^{b)}
Carpropamid	None	None	Black
Tricyclazole	Brown	Black	Black
Untreated	Black	Black	Black

^{a)} PDA was amended with 10 µg/ml of carpropamid or tricyclazole.

^{b)} Melanin intermediates were topically administered at the edge of the colony.

4. Metabolism of Scytalone in the Culture of the Albino Strain

Scytalone externally added to the blast culture was easily metabolized by albino blast strains P₂-alb (60-2 & IW). Scytalone added to the 3-day culture was metabolized to give 50% residue within 20 to 50 min and almost completely disappeared in 2 to 4 hr and the culture broth slightly turned to light brown. But most scytalone was re-isolated from the broth without being metabolized in the culture when carpropamid was applied in advance or simultaneously (Fig. 3).

5. Recovery of Melanization by Scytalone and Vermelone in the Albino Blast Culture

Scytalone and vermelone administered beside the albino blast colony were readily converted to black pigment (Table 2). This suggests that melanin biosynthesis enzyme worked in the culture and melanin was synthesized from scytalone and vermelone. However, the pigmentation was strongly inhibited in the culture medium amended with carpropamid. The pigmentation was also observed in the culture of the wild type of blast

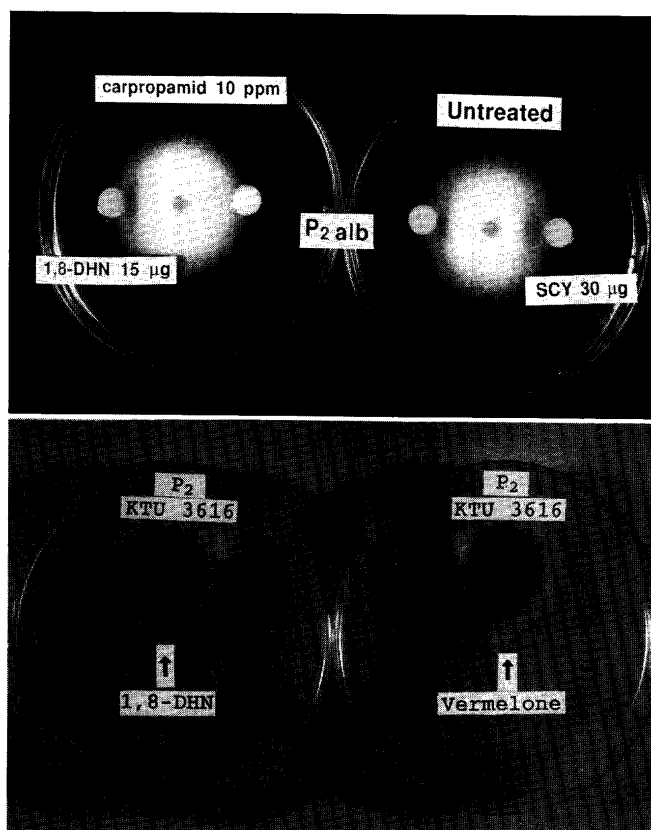


Fig. 4 Melanization from scytalone and vermelone in blast culture plate.

PDA plate was amended with or without carpropamid at 10 $\mu\text{g}/\text{ml}$. Upper picture: the left and right paper disks in the plate contain 1,8-DHN and scytalone, respectively. Lower picture: 1,8-DHN (left) and vermelone (right) contained in paper disks were administered beside blast colony in the plates amended with carpropamid.

fungus P_2 but changing of the color was not clear due to the presence of its own pigment in the culture. Carpropamid and tricyclazole did not inhibit the pigmentation from 1,8-DHN.

DISCUSSION

The present study proved that carpropamid interfered with the biosynthesis of DHN melanin in *P. oryzae* and *V. dahliae*. Scytalone was predominantly isolated from the culture of *P. oryzae* and *V. dahliae* treated with carpropamid. Tricyclazole and pyroquilon were reported to inhibit the melanization and to induce accumulation of scytalone and 2-HJ as the result of the action.^{13,18-20} Yamaguchi *et al.* showed this accumulation of scytalone and 2-HJ in the culture with four MBI fungicides which primarily block the reaction from 1,3,8-trihydroxynaphthalene (1,3,8-THN) to vermelone.¹³ The precursor, 1,3,8-THN, once accumulated in the culture, appears to be easily converted to 2-HJ in the branched pathway. In the culture with carpropamid, a relatively high amount of scytalone accumulates in the culture but 2-HJ and 1,3,8-THN were not

detected by TLC and HPLC analyses. From these data carpropamid is supposed not to block 1,3,8-THN reductase but specifically inhibit scytalone dehydratase. In addition, a small amount of vermelone was detected in the liquid blast culture treated with the carpropamid.

Carpropamid did not show strong influence on blast mycelial growth and there was little difference in the fresh weights of the mycelia treated or not. However, the amounts of scytalone and vermelone in the culture broth increased in parallel by the application of carpropamid and reached a maximum by the treatment at more than 10 $\mu\text{g}/\text{ml}$ in 5-day culture broth. This means that inhibition activity of carpropamid against the two dehydration steps may be in the same level. Culture periods and application timing influenced accumulation of scytalone and vermelone. In 5 to 6-day culture the amount gradually increased from 0 day (inoculated and treated simultaneously) to 4-day preculture and maximum accumulation of scytalone was found at 4-day preculture in 6-day culture. As accumulation of the scytalone and vermelone was not larger in amount by earlier application of the chemical than 3 to 4-day preculture, it is assumed that carpropamid may interfere with the earlier steps than scytalone dehydration in melanin biosynthesis or to synthesis of the dehydratase. In our experiments, however, flaviolin, which accumulates in the culture treated with tricyclazole or pyroquilon,^{13,22,23} has never been detected in the culture with carpropamid.

Vermelone proved to accumulate in the blast culture by the treatment of carpropamid (10 $\mu\text{g}/\text{ml}$), as shown in Fig. 2. This might have occurred due to the imperfect inhibition of scytalone dehydration by carpropamid to allow transformation from leaking 1,3,8-THN to vermelone. However, the results that scytalone accumulation reaches the maximum by the treatment of 10 $\mu\text{g}/\text{ml}$ carpropamid and that vermelone accumulates more significantly when the chemical is treated at the later stage of the blast culture suggest that existing 1,3,8-THN already formed in the culture is reduced to vermelone but dehydration of vermelone was inhibited by carpropamid. This is supported by the fact that vermelone is detected even in the untreated blast culture.¹³

In the shaking culture of the albino mutants (P_2 -alb IW and P_2 -alb 60-2), scytalone added to the culture completely disappeared in a short time and the medium was slightly pigmented to light brown. This means that melanin synthesis ability from scytalone fulfills the function in the albino strains. The metabolism was significantly inhibited by carpropamid and scytalone added to the culture remained in the medium. This suggests that carpropamid specifically inhibits scytalone dehydration process in the melanin biosynthesis.

In addition, scytalone, vermelone and 1,8-DHN were easily converted to black pigment when they were fed at the edge of the colony of the albino mutants. Car-

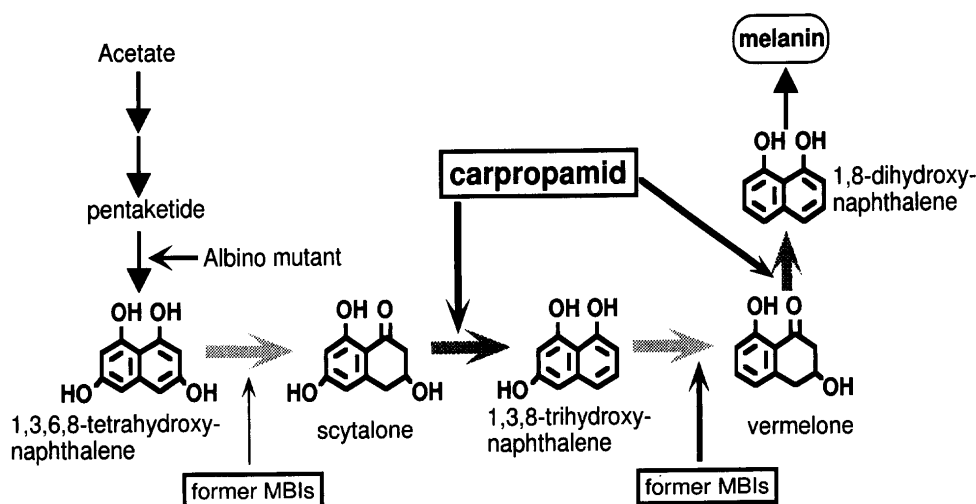


Fig. 5 Inhibition sites of carpropamid in fungal melanin biosynthesis pathway.

→, dehydration; ⇨, reduction.

propamid effectively inhibited the metabolism from scytalone and vermeline, but the conversion of 1,8-DHN to black pigment was not inhibited by the chemical. In other words, dehydration of scytalone and vermeline is blocked by carpropamid but polymerization of 1,8-DHN is not affected by the chemical. These results indicate that the action site of carpropamid is not the reduction process from 1,3,8-THN to vermeline but dehydration from scytalone to 1,3,8-THN and from vermeline to 1,8-DHN in the melanin biosynthesis pathway (Fig. 5).

As shown in Fig. 5 there are two reduction and two dehydration steps in the DHN melanin biosynthesis pathway.^{19,21)} Phthalazine derivatives, which were designed on the basis of the stereochemistry of 1,3,8-THN and the known MBIs, were shown to act as a reductase inhibitor and to afford a good effect against rice blast.²⁴⁾ The substrates and reductase inhibitors have some similarity in their chemical structures. But a resemblance between carpropamid and scytalone can hardly be seen in their chemical structures. Carpropamid may be inferred, therefore, to have a different inhibition mechanism from conventional MBIs.

As to blast appressorial penetration, two mechanisms are suggested, *i.e.* enzymatic and physical penetration into rice epidermis. In fact, activity of such enzymes as cutinase and cellulase concerning metabolism of plant epidermal constituents were found in the culture of various fungi.^{22,26)} The role of melanin in blast appressoria is suggested to induce the rigidity of cell wall to resist an increase of the inner osmotic pressure of the organ generated for penetration into host plant epidermis and to determine the direction of development of penetration peg.^{12,14,25)} Kubo *et al.* reported a lateral germination from unmelanized appressoria of *Colletotrichum lagenarium* treated with tricyclazole and then suggested that infection hyphae to be formed vertically in the

melanized appressoria has lost the direction due to the insufficient rigidity.²⁷⁻²⁹⁾ The rise of the pressure in blast appressoria have been measured by Howard *et al.* and estimated to be as high as 8.0 MPa.^{30,31)} But it is thought that the melanin layer in appressorial cells not only works for producing rigidity of the wall but also blocks the efflux of any larger molecule by reducing porosity of the membrane and generates the internal pressure by influx of water caused by osmotic gradient.³⁰⁻³²⁾

Melanin biosynthesis inhibition by carpropamid in the appressoria is supposed primarily to cause the penetration failure because of insufficient rigidity of the cell wall or an unsatisfactory rise of turgor pressure in the cells. On the other hand, the melanization of appressoria of albino mutants and unmelanized appressoria of wild type strains treated with MBIs is relatively easily restored by administration of vermeline and 1,8-DHN but the penetration into epidermal cells is less recovered. Chida and Sisler showed that restoration of penetration into onion epidermis from the unmelanized appressorium in which toxic intermediates such as scytalone and 2-HJ are not accumulated by the action of cerulenin, increased at relatively high rates.^{15,33)} Accumulation of scytalone and vermeline induced by carpropamid may hinder secondarily the appressorium function for their infection since these intermediates accumulated in the blast cell reportedly exert some cell toxicity.^{13,22)}

Reductase inhibitors of melanin biosynthesis such as tricyclazole, pyroquilon and fthalide have been developed and practically used for rice blast control. Although the chemicals with dehydratase inhibitory activity on melanin biosynthesis have been predicted, carpropamid is the first one to have been found out as a blast controller with inhibitory activity on the dehydration step in the fungal melanin biosynthesis. In fact, carpropamid can provide an excellent effect against rice

blast disease by one shot application as nursery box treatment.

Recently a few patents by Sumitomo Chem. Ind. Co. Ltd.,³⁴⁾ Kumiai Chem. Ind. Co. Ltd.³⁵⁾ and Rhone Poulenc Co. Ltd.³⁶⁾ have been applied for, and similar biological properties were reported on the compounds. They may have the same mode of action as carpropamid because these have a common moiety in their chemical structure with carpropamid.

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要 約

カルプロパミドによるもち病菌のメラニン生合成中間体の蓄積とその作用点

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カルプロパミド (ウィン®) は *P. oryzae* や *V. dahliae* などの平面培養やもち病菌の振とう培養での菌の色素沈着を強く抑制した。そしてそれらの野生株の培養物の抽出によって白色の結晶が単離された。薄層クロマト *R_f* 0.36 付近のスポットから得られた白色結晶の化学分析値は既に報告されているシタロンのデータとよく一致した。カルプロパミド 10 µg/ml を処理したそれらの培養物中には多量のシタロンと少量のバーメロンが蓄積したが、2-ハイドロキシジグロンは検出されなかった。*P. oryzae* のアルビノ変異株 (*P₂-alb*) の振とう培養にシタロンを加えると速やかに代謝され、その培養は薄い褐色となった。しかしその培養にあらかじめカルプロパミドを加えるとシタロンはあまり代謝されないで培地中に残った。*P. oryzae* のアルビノ変異株 (*P₂-alb*) の平面培養の菌叢先端に局所処理したシタロンとバーメロンは黒色色素に変換されたがその着色はカルプロパミドによって阻害された。これらの試験結果はカルプロパミドが菌類メラニン生合成においてシタロン及びバーメロンの脱水反応を阻害することを示唆している。