Original Article

Antifungal and Plant Growth Regulating Activities of Enantiomers of (*E*)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308L)

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Antifungal and plant growth regulating activities of enantiomers of (*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308L) were examined. In a nutrient agar medium, the *R*(−) isomer was more fungitoxic than the *S*(+) isomer against fungal species belonging to the Ascomycotina, Basidiomycotina and Deuteromycotina. However, against the Oomycetous fungi, the *R*(−) isomer was not so fungitoxic as against the other fungal species. There was not a clear difference in fungitoxicity between the *R*(−) and *S*(+) isomers. In pot-trials, the *R*(−) isomer controlled economically important plant diseases when sprayed preventively or curatively, whereas the *S*(+) isomer was less effective. The *R*(−) isomer also showed protective activity in vapor phase and systemic, whereas the *S*(+) isomer exhibited stronger plant growth regulating activity than the *R*(−) isomer on cucumber seedlings.

INTRODUCTION

In this decade, a remarkable improvement has been achieved on crop protection. The invention of azole fungicides marked one of the noteworthy progresses, contributing to the protection of agricultural crops. This family of fungicides, known to be sterol biosynthetic inhibitors (SI), is fungitoxic to many fungal species. Furthermore, it is generally excellent in both curative and preventive activities.

A new triazole fungicide, (*E*)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308L) (Fig. 1) was found by screening of azole derivatives. It was identified as one of the SIs, which inhibits 14 α -demethylation through the ergosterol biosynthetic pathway in fungi.¹⁾ The compound possesses two enantiomers due to one asymmetric carbon atom. This paper describes the antifungal activity *in vitro* and *in vivo*, and

plant growth regulating activity of enantiomers of S-3308L.

MATERIALS AND METHODS

1. Measurement of Fungitoxicity

Antifungal activity of each enantiomer was evaluated by employing the agar dilution method as previously reported.²⁾

2. Preventive and Curative Activities

The seeds of wheat (cv., Norin No. 73), barley (cv., Akashinriki), cucumber (cv., Sagamihanjiro), peanut (cv., Chibahanritsu) and apple (cv., Fuji) were grown in plastic pots (90 ml in volume) filled with a standardized soil (clay : vermiculite : humus = 2 : 1 : 1, v/v) containing some fertilizer. The pots were kept in a greenhouse and the seedlings were employed as test plants at the cotyledonous (cucumber), second-leaf (wheat and barley) or fifth-leaf (apple) stage. The water suspensions of each S-3308L enantiomer were

Fig. 1 Chemical structures of enantiomers of S-3308L.

prepared as reported by Funaki *et al.*³⁾ and immediately sprayed to the seedlings to run-off before or after inoculation for evaluation of preventive or curative activity. *Erysiphe graminis* f. sp. *tritici*, *Erysiphe graminis* f. sp. *hordei*, and *Sphaerotheca fuliginea* were inoculated to the leaves of wheat, barley and cucumber seedlings, respectively. The inoculated seedlings of wheat and barley were grown for 7 days and cucumber seedlings for 13 days in a greenhouse at 20°C. *Puccinia recondita* was inoculated by dusting uredospores to the leaves of wheat seedlings. The plants were grown in a humid chamber at 23°C in darkness for 20 hr to establish infection and then under fluorescent lamps at 23°C for 7 days. *Cercospora arachidicola* and *Venturia inaequalis* were inoculated by spraying with spore suspension to the leaves of peanut and apple seedlings. Both inoculated plants were covered with a polyethylene bag and grown for 19 days in a greenhouse at 23°C and for 13 days in the air-controlled chamber at 15°C, respectively.

Disease assessment was estimated by the previous method.⁴⁾ Disease severity of leaves was classified according to the following indices; 0 (no symptom), 1 (infection of less than 5% of leaf area), 2 (infection of less than 20%), 4 (infection of less than 50%), 8 (infection of more than 50%). Percentages of control (*A*) were estimated by the following equation;

$$A = 100 - \left(\frac{\sum \text{indices in the treated plot}}{\sum \text{indices in the control plot}} \right) \times 100$$

3. Protective Activity in Vapor Phase

Barley seedlings of the third-leaf stage inoculated with *E. graminis* f. sp. *hordei* were covered with a glass beaker (5 l in volume) with a filter paper hanging inside, which had

been immersed with fungicide solution and dried, and kept in a greenhouse at 20°C. Disease severity was estimated by the previous method after 10 days.

4. Systemic Activity

A pot (90 ml in volume) planted with wheat seedlings of the second-leaf stage was placed in a 280 ml plastic cup containing 50 ml of fungicide solution and kept for 3 days in a greenhouse at 20°C to absorb the solution. Then *E. graminis* f. sp. *tritici* was inoculated and the disease severity was estimated by the aforementioned method 7 days after inoculation.

5. Plant Growth Regulating (P.G.R.) Activity

Cucumber seedlings (cv., Sagamihanjiro) of the second-leaf stage grown in 280 ml plastic pots were sprayed with fungicide solution and maintained in a greenhouse at 23°C. Their stem growth and leaf development were investigated 14 days after treatment.

6. Purity of the Fungicides

Purity of the *R*(-) isomer and the *S*(+) isomer used in the experiments were 99.0% (*R*(-): *S*(+)=99.1:0.9) and 98.6% (*R*(-): *S*(+)=0.6:99.4), respectively.

RESULTS

1. Antifungal Activity

The *R*(-) isomer showed a broad antifungal spectrum and was particularly toxic against species in Ascomycotina, Basidiomycotina, and Deuteromycotina (Table 1). ED_{50} values of the *R*(-) isomer in them were lower than 1 ppm and much lower than those of the *S*(+) isomer. The *R*(-) isomer was extremely toxic to *Venturia inaequalis* and *Sclerotinia sclerotiorum* and its ED_{50} values against both fungi were as low as 0.001 ppm. Difference in antifungal activity between enantiomers varied among fungal species. The ED_{50} value of the *R*(-) isomer against *Ustilago maydis* was more than 300 times higher than that of the *S*(+) isomer, although the difference in their ED_{50} values against *Botrytis cinerea* was small.

Antifungal activity of the *R*(-) isomer against the species in Mastigomycotina and Zygomycotina was generally weak, and it is

Table 1 Antifungal activity of enantiomers of S-3308L.

Fungi	ED ₅₀ (ppm)	
	R(−)	S(+)
Mastigomycotina		
<i>Phytophthora infestans</i>	3.8	5.2
<i>Pythium aphanidermatum</i>	2.2	3.0
Zygomycotina		
<i>Rhizopus nigricans</i>	1.6	33
Ascomycotina		
<i>Diaporthe citri</i>	0.029	1.4
<i>Gibberella fujikuroi</i>	0.46	21
<i>Gibberella zeae</i>	0.42	42
<i>Glomerella cingulata</i>	0.019	5.0
<i>Monilinia fructicola</i>	0.008	0.87
<i>Monilinia mali</i>	0.005	0.6
<i>Mycosphaerella melonis</i>	0.054	6.2
<i>Ophiobolus graminis</i>	0.11	3.2
<i>Sclerotinia cinerea</i>	0.003	0.75
<i>Sclerotinia sclerotiorum</i>	0.001	0.066
<i>Valsa mali</i>	0.17	4.4
<i>Venturia inaequalis</i>	0.001	0.16
Basidiomycotina		
<i>Corticium rolfsii</i>	0.01	5.3
<i>Pellicularia sasakii</i>	0.082	27
<i>Rhizoctonia solani</i>	0.17	19
<i>Ustilago maydis</i>	0.003	1.0
<i>Ustilago nuda</i>	1.0	60
Deuteromycotina		
<i>Alternaria kikuchiana</i>	0.52	30
<i>Alternaria mali</i>	0.6	22
<i>Botrytis cinerea</i>	1.0	3.2
<i>Cercospora beticola</i>	0.17	12
<i>Cladosporium cucumerinum</i>	0.02	2.3
<i>Colletotrichum lagenarium</i>	1.6	12
<i>Fusarium nivale</i>	0.17	4.0
<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	0.22	12
<i>Helminthosporium gramineum</i>	0.35	12
<i>Penicillium expansum</i>	0.31	34
<i>Penicillium digitatum</i>	0.086	18
<i>Penicillium italicum</i>	0.1	9.5
<i>Phoma betae</i>	0.09	15
<i>Phoma destructiva</i>	0.2	30
<i>Pyricularia oryzae</i>	0.078	2.8
<i>Pseudocercospora herpotrichoides</i>	0.1	10
<i>Septoria tritici</i>	0.013	0.2

interesting to note that the activity of two enantiomers against *Phytophthora infestans* and *Pythium aphanidermatum* was not so different.

2. Preventive and Curative Effects

In pot tests, powdery mildew of wheat, barley and cucumber, and apple scab was completely controlled by the preventive application of the R(−) isomer at a concentration as low as 12.5 ppm (Table 2). The R(−) isomer was also effective against leaf spot of peanut with 100% control at 50 ppm. However, the activity of the S(+) isomer was much lower, although it showed 100% control against powdery mildew on wheat and barley at a concentration of 50 ppm.

The R(−) isomer was also effective in curative treatment (Table 3). No disease symptom was observed when the R(−) isomer was treated wheat brown rust at a concentration of 3.1 ppm. On the other hand, the S(+) isomer showed less curative activity as found in the experiment of preventive treatment. These results suggested that the R(−) isomer was about 50 to over 200 times more efficacious than the S(+) isomer in both preventive and curative treatments, as shown in *in vitro* evaluation.

3. Protective Activity in a Vapor Phase

Protective activity of the R(−) isomer in a vapor phase on barley powdery mildew was examined (Fig. 2). When a filter paper containing more than 1 mg of the R(−) isomer was hung in a 5 l glass beaker where test plants were placed, development of powdery mildew was prevented. The result indicated that the R(−) isomer has a strong protective activity in a vapor phase.

4. Systemic Activity

When the R(−) or S(+) isomer of S-3308L was applied to the roots of wheat seedlings, both enantiomers controlled development of wheat powdery mildew, showing a high systemic activity (Fig. 3). The R(−) isomer completely controlled the powdery mildew development at dosages of more than 0.1 mg/pot, whereas the S(+) isomer required higher dosages to obtain the same degree of control. It is supposed that this difference in the activity is attributed to the difference of fungicidal action against *E. graminis*, because such a difference was also recognized in the preventive spraying to leaves.

Table 2 Preventive effect of enantiomers of S-3308L.

Disease (host)	Enantiomer	Concentration of the fungicide (ppm)					
		200	50	12.5	3.1	0.8	0.2
Powdery mildew (wheat)	<i>R</i> (-)	100 ^{a)}	100	100	100	100	80
	<i>S</i> (+)	100	100	96	66	0	0
Powdery mildew (barley)	<i>R</i> (-)	100	100	100	100	100	93
	<i>S</i> (+)	100	100	99	17	0	0
Powdery mildew (cucumber)	<i>R</i> (-)	100	100	100	92	48	0
	<i>S</i> (+)	98	58	3	0	0	0
Scab (apple)	<i>R</i> (-)	— ^{b)}	100	100	100	75	—
	<i>S</i> (+)	87	58	0	0	—	—
Leaf spot (peanut)	<i>R</i> (-)	—	100	89	33	11	—
	<i>S</i> (+)	19	6	12	6	—	—

a) % of disease control.

b) Not determined.

Table 3 Curative effect of enantiomers of S-3308L.

Disease (host)	Enantiomer	Concentration of the fungicide (ppm)					
		200	50	12.5	3.1	0.8	0.2
Brown rust (wheat)	<i>R</i> (-)	100 ^{a)}	100	100	100	99	35
	<i>S</i> (+)	97	66	42	0	— ^{b)}	—
Scab (apple)	<i>R</i> (-)	100	100	97	97	—	—
	<i>S</i> (+)	40	8	0	0	—	—
Leaf spot (peanut)	<i>R</i> (-)	84	80	63	46	—	—
	<i>S</i> (+)	0	0	0	0	—	—

a) % of disease control.

b) Not determined.

Fig. 2 Protective activity of the *R*(-) isomer in a vapor phase to barley powdery mildew.

Fig. 3 Systemic activity of enantiomers of S-3308L in controlling wheat powdery mildew.

●: *R*(-) isomer, ○: *S*(+) isomer.

5. Plant Growth Regulating (P.G.R.) Activity on Cucumber Seedlings

Although the *R*(-) isomer was more active to fungi than the *S*(+) isomer, their activity

to plants were converse (Table 4). The growth of cucumber stems when treated by the *R*(-) isomer at 200 ppm was moderately suppressed, but there was no significant effect at

Table 4 Effect of S-3308L on stem growth and leaf development of cucumber seedlings.

Enantiomer	Treated concentration (ppm)	Stem growth (mm)	Number of leaves
	None	332 ^{a)}	4 ^{b)}
R(—)	3.1	346	4
R(—)	12.5	338	4
R(—)	50	338	4
R(—)	200	222	4
S(+)	3.1	88	4
S(+)	12.5	62	3
S(+)	50	58	3.3
S(+)	200	43	3

^{a)} Mean of the stem growth after treatment in three replications.

^{b)} Mean of the leaf number developed after treatment in three replications.

less than 50 ppm. The S(+) isomer, on the other hand, retarded the stem growth strongly at more than 3.1 ppm. But both isomers showed no particular effect on leaf development, although the S(+) isomer slightly suppressed at more than 12.5 ppm.

DISCUSSION

The R(—) isomer of S-3308L showed a broad antifungal activity against the Ascomycotina, Basidiomycotina and Deuteromycotina *in vitro*, and its activity was stronger than the S(+) isomer's against above fungi. Such a stereochemical specificity has been already reported on the other ergosterol biosynthetic inhibitors, such as dichlobutrazol⁵⁾ and triadimenol.⁶⁾ Both enantiomers also showed moderate antifungal activity against Pythiaceae fungi which are generally considered to be incapable of biosynthesizing sterols,^{7,8)} but in *P. infestans* and *P. aphanidermatum* they were not so different in antifungal activity. These findings suggest that in these fungi, where S-3308L has an inhibiting effect is different from where ergosterol biosynthesis occurs.

The R(—) isomer also showed higher toxicity to fungi in *in vivo* tests. Moreover, it was systemic in plants and showed fungitoxicity in a vapor phase. Both properties may contribute to the disease control in fields.

S-3308L is known as a strong inhibitor of

ergosterol biosynthesis in fungi at a stage of 14 α -demethylation.¹⁾ It is also recognized that the R(—) isomer is more potent in inhibition of 14 α -demethylation than the S(+) isomer.⁹⁾ Therefore the antifungal activity is apparently coincided with the inhibitory potency of 14 α -demethylation in ergosterol biosynthesis.

On the other hand, the S(+) isomer showed higher P.G.R. activity on cucumber seedlings than the R(—) isomer. Izumi *et al.* reported that S-3307, an analog of S-3308L, showed strong P.G.R. activity on rice plants¹⁰⁾ and inhibited the oxidation of kaurene, kaurenol and kaurenal, which are precursors of gibberellin, in the cell-free system of *Cucurbita maxima*.¹¹⁾ Coolbaugh *et al.* also described that fenarimol, nuarimol and triarimol, which are potent inhibitors of 14 α -demethylation in ergosterol biosynthesis in fungi, inhibited the oxidation of kaurene in the cell-free system of *Marah macrocarpus*.¹²⁾ Thus, it is supposed that the S(+) isomer of S-3308L may inhibit the oxidation of kaurene during gibberellin biosynthesis in higher plants. This is a very noteworthy hypothesis, because it has been known that both oxidation systems, 14 α -demethylation of lanosterol in fungi and oxidation of kaurene in higher plants, are catalyzed by microsomal monooxygenase system containing cytochrome P-450.¹³⁻¹⁵⁾ It is speculated that the R(—) isomer has a higher affinity to the enzyme in fungi and reversely the S(+) isomer has a stronger affinity to the enzyme in higher plants. More detailed studies are required to clarify the interaction between the inhibitor and both enzymes.

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要 約

(E)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308L) の光学異性体の抗菌性および植物成長調節活性

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S-3308L の光学異性体 (*R*(-) 異性体および *S*(+) 異性体) について, 抗菌活性および植物に対する作用を比較した. *in vitro* における抗菌活性を調べたところ, 接合菌類, 子囊菌類, 担子菌類, 不完全菌類に対して, *R*(-) 異性体のほうが *S*(+) 異性体に比べて数倍から数百倍以上の高活性を示した. しかし, *R*(-) 異性体の卵菌類に対する抗菌力は弱く, 異性体間の活性差も認められなかった. ポット試験においても, 同様の活性差が認められ, *R*(-) 異性体は, *S*(+) 異性体よりも高い病害防除効果を示した. さらに, *R*(-) 異性体は, 気化作用による防除効果をも有していた. また, 両異性体とも, 植物体への浸透移行性を示し, とくに *R*(-) 異性体では, 鉢当たり 0.1 mg の根部処理で, コムギうどんこ病が完全に防除された. 一方, キュウリに対する作用では, 両異性体とも, 葉の展開に対しては, ほとんど影響を与えなかったが, 茎の伸長に対しては, *S*(+) 異性体が *R*(-) 異性体に比べて, はるかに強い抑制作用を示した.