Original Article

Synthesis of Stereoisomers of Ipconazole and Their Fungicidal and Plant Growth Inhibitory Activities

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Two racemic diastereomers **5a** and **5b**, both of which are active ingredient of a seed treatment fungicide on rice, ipconazole, (1*RS*, 2*SR*, 5*RS* ; 1*RS*, 2*SR*, 5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl)cyclopentanol, and two other racemic diastereomers (1*RS*, 2*RS*, 5*RS*)-**5c** and (1*RS*, 2*RS*, 5*SR*)-**5d** were synthesized, and fungicidal activity and plant growth inhibitory activity were examined. Enantiomers of **5a** and **5b** were prepared, and their fungicidal activities were also examined. Racemic diastereomers **5a**, **5b** and **5d** were more active than **5c** in fungicidal activity (EC_{50} value) *in vitro* on *Gibberella fujikuroi*, *Cochliobolus miyabeanus* and *Pyricularia oryzae*. In plant growth inhibitory activity test for seed treatment on rice, only **5d** was slightly active in the first sheath growth inhibition. The comparative test of enantiomers *in vitro* revealed that the fungicidal activities of (–)-**5a** and (–)-**5b** were higher than those of corresponding (+)-**5a** and (+)-**5b**, respectively.

INTRODUCTION

Ipconazole, (1*RS*, 2*SR*, 5*RS* ; 1*RS*, 2*SR*, 5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl)cyclopentanol is a new seed treatment fungicide which is effective for “Bakanae” disease, brown spot and blast on rice. The fungicidal mechanism is assumed to be the inhibition of cytochrome P450-dependent C-14 demethylation (S. Kumazawa *et al.*: unpubl.) of the intermediate lanosterol in the biosynthesis of sterol in fungi. Ipconazole consists of a 9 : 1 mixture of racemic diastereomers **5a** and **5b**. As for racemic diastereomers of ipconazole, there exist two other isomers, **5c** and **5d** (Fig. 1). Many papers have reported on relationship between stereochemical structure of sterol biosynthesis inhibitor and biological activity.¹⁾ For example, uniconazole, (*E*)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1*H*-1, 2, 4-triazol-1-yl)pent-1-en-3-ol is more active than *Z*-isomer in fungicidal and plant growth inhibitory activities, and *R*-(–) enantiomer of uniconazole is highly active in fungicidal activity, on the other hand, *S*-(+) enantiomer is highly active in plant growth inhibitory activity.²⁾ The *cis*-isomer (SSF-109) of 1-(4-chlorophenyl)-2-(1*H*-1, 2, 4-triazol-1-yl)cycloheptanol is more active than the *trans*-isomer in fungicidal activity, on the other hand, the *trans*-isomer is more active than the *cis*-isomer in plant growth inhibitory activity. Enantiomer (–)-SSF-109 is more active than (+)-SSF-109 in fungicidal and plant growth inhibitory activities.³⁾

It is particularly interesting to investigate the relationship between stereochemical structure and biological activity in the viewpoint of the elucidation of the optimum three-dimensional structure for the target enzyme.⁴⁾ In this paper the synthesis of stereoisomers of ipconazole and fungicidal and plant growth inhibitory activities of each isomer are described.

MATERIALS AND METHODS

1. Synthesis of Compounds

Synthetic scheme of four azole racemic diastereomers (**5**) was shown in Fig. 2 along with methods A and B. Infrared (IR) spectra were recorded on a Hitachi 270-30 spectrophotometer and ¹H NMR were obtained with a JEOL JNM-PMX60SI (60 MHz) or a JEOL GX500 (500 MHz) using tetramethylsilane as an internal standard. Optical rotations were measured with Union Giken PM-201 polarimeter.

1.1 Methyl 3-isopropyl-2-oxocyclopentanecarboxylate (**2**)

Under nitrogen atmosphere, 60% oily sodium hydride (9.2 g, 230 mmol) was washed with hexane, and *N,N*-dimethylformamide (DMF) (160 ml) was added. To the mixture methyl 2-oxocyclopentanecarboxylate (**1**) (30 g, 210 mmol) was slowly added. After stirring for 0.5 hr, isopropyl iodide (42 g, 247 mmol) was added in one portion, and the mixture was stirred for 3 hr at 70–80°C.

After cooling to room temperature, the reaction mixture was poured into water and extracted with benzene.

Fig. 1 Structures of ipiconazole and its racemic diastereomers.

Fig. 2 Synthetic scheme of azole racemic diastereomers (5).

The benzene layer was washed with water and brine, then dried over anhydrous sodium sulfate, and evaporated under reduced pressure. Absolute methanol (100 ml) and 28% sodium methylate in methanol (45 g, 233 mmol) were added to the residue (38 g). The mixture was refluxed for 3 hr, and methanol was distilled off. Toluene (100 ml) was added, and the mixture solvent of toluene-methanol was removed by distilling. This procedure was repeated twice to remove methanol completely.

The residue was poured into 10% acetic acid solution, and extracted with benzene. The benzene layer was washed with water and brine, then dried over anhydrous sodium sulfate. Removal of the solvent followed by distillation afforded ester (2) (26.2 g, 67.8%), bp 84–85°C (5 mmHg). IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} : 2950, 1745, 1720. ^1H NMR (CDCl_3) δ ppm: 0.83 (d, 3H, $J=6$ Hz), 0.87 (d, 3H, $J=6$ Hz), 1.60–2.77 (m, 7H), 3.60 (s, 3H).

1.2 2-(4-Chlorobenzyl)-5-isopropylcyclopentanone (3)

Under nitrogen atmosphere, 60% oily sodium hydride (6.2 g, 155 mmol) was washed with hexane, and DMF (150 ml) was added. To the mixture ester (2) (26 g, 141 mmol) was slowly added. After stirring for 0.5 hr, 4-chlorobenzyl chloride (25 g, 141 mmol) was added in one portion, and the mixture was stirred for 3 hr at room temperature.

The mixture was poured into water and extracted with benzene. The benzene layer was washed with water and brine, then dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue (48 g) was refluxed with 47% hydrobromic acid (160 ml) for 18 hr. After cooling to room temperature, the mixture was poured into water and extracted with benzene. The benzene layer was washed with water and brine, and dried over anhydrous sodium sulfate. Removal of the solvent followed by distillation afforded ketone (3) (19.6 g, 55.5%), bp 143–147°C (1.2 mmHg). IR $\nu_{\text{max}}^{\text{neat}}$ cm^{-1} : 2950, 1720. ^1H NMR (CDCl_3) δ ppm: 0.77, 0.80 (d $\times$ 2, 3H, $J=6$ Hz), 0.93, 0.97 (d $\times$ 2, 3H, $J=6$ Hz), 1.07–3.27 (m, 9H), 6.98 (d, 2H, $J=8$ Hz), 7.18 (d, 2H, $J=8$ Hz).

1.3 4-(4-Chlorobenzyl)-7-isopropyl-1-oxaspiro[2.4]heptane (4) (method A)

Under nitrogen atmosphere, 60% oily sodium hydride (3.2 g, 80.0 mmol) was washed with hexane, and dimethylsulfoxide (DMSO) (30 ml) was added. To the mixture trimethylsulfoxonium iodide (17.4 g, 79.1 mmol) was added over about 0.5 hr. After stirring for 0.5 hr, ketone (3) (10 g, 39.9 mmol) was added and the mixture was stirred for 42 hr at room temperature.

The mixture was poured into water, and extracted with hexane. The hexane layer was washed with water and brine, and dried over anhydrous sodium sulfate.

After removal of solvent, crude oxirane (4) (8.3 g) was obtained as an oil. Purification of the adduct by silicagel column chromatography (hexane:ethyl acetate=100:1) gave 4a (3.2 g) and 4c (1.2 g). It was unable to separate oxirane isomer 4b and 4d from unreacted ketone (3), so the ratio of oxirane isomer (4) was determined by high performance liquid chromatography (HPLC) analysis as follows: 4a : 4b : 4c : 4d = 61 : 13 : 22 : 4.

1.4 4-(4-Chlorobenzyl)-7-isopropyl-1-oxaspiro[2.4]heptane (4) (method B)

Under nitrogen atmosphere, 60% oily sodium hydride (4.8 g, 120 mmol) was washed with hexane, and DMSO (100 ml) was added. The mixture was heated to 70°C until the evolution of hydrogen ceased, and then cooled to room temperature. Methyltriphenylphosphonium bromide (43.0 g, 120 mmol) was added. After stirring for 0.5 hr, ketone (3) (20 g, 79.8 mmol) was added, and the mixture was stirred for 2 hr at room temperature.

The mixture was poured into water, and extracted with hexane. The hexane layer was washed with water and brine, and dried over anhydrous sodium sulfate. After removal of solvent, crude olefin (3') (26.2 g) was obtained as an oil. Purification of the adduct by silicagel column chromatography (hexane) gave 3' (18.7 g).

To the solution of olefin (3') (18.7 g, 75.3 mmol) in

Table 1 Ratios of oxirane isomer (4) by methods A or B.

Method	Ratio ^{a)} of oxirane isomer (4)			
	4a	4b	4c	4d
A	61	13	22	4
B	0	81	6	13

^{a)} Measured by HPLC analysis.

chloroform (100 ml) was added *m*-chloroperbenzoic acid (25.0 g, 145 mmol), and the mixture was stirred for 2 hr at room temperature. Calcium hydroxide (21 g, 284 mmol) was added to the reaction mixture and the mixture was stirred for 10 min. The resulting precipitate was filtered off and the filtrate was dried up. Purification of the adduct by silicagel column chromatography (hexane : ethyl acetate = 100 : 1) gave **4b** (14.6 g), **4c** (1.1 g) and **4d** (2.3 g) in total 85.3% yield. The ratio of oxirane isomer (4) by method B was also measured by HPLC to compare with that of method A (Table 1).

¹H NMR (CDCl₃) δ ppm :

4a : 0.89 (d, 3H, J = 6.64 Hz), 0.90 (d, 3H, J = 6.64 Hz), 1.40–1.45 (m, 1H), 1.54–1.71 (m, 4H), 2.06–2.10 (m, 1H), 2.24–2.28 (m, 1H), 2.34 (dd, 1H, J = 10.08, 13.75 Hz), 2.57 (dd, 1H, J = 4.59, 13.75 Hz), 2.67 (d, 1H, J = 5.04 Hz), 2.69 (d, 1H, J = 5.04 Hz), 7.08 (d, 2H, J = 8.25 Hz), 7.22 (d, 2H, J = 8.25 Hz).

4b : 0.82 (d, 3H, J = 6.65 Hz), 0.88 (d, 3H, J = 6.65 Hz), 1.26–1.42 (m, 2H), 1.63–1.71 (m, 1H), 1.73–1.79 (m, 2H), 1.96–2.03 (m, 1H), 2.11–2.18 (m, 1H), 2.36 (dd, 1H, J = 9.85, 14.10 Hz), 2.50 (dd, 1H, J = 5.04, 14.10 Hz), 2.64 (d, 1H, J = 4.13 Hz), 2.90 (d, 1H, J = 4.13 Hz), 7.08 (d, 2H, J = 8.48 Hz), 7.22 (d, 2H, J = 8.48 Hz).

4c : 0.88 (d, 3H, J = 6.88 Hz), 0.89 (d, 3H, J = 6.88 Hz), 1.15–1.26 (m, 1H), 1.42–1.50 (m, 1H), 1.57–1.62 (m, 1H), 1.70–1.75 (m, 2H), 1.99–2.04 (m, 1H), 2.10–2.17 (m, 1H), 2.23 (dd, 1H, J = 11.22, 12.95 Hz), 2.55 (d, 1H, J = 4.81 Hz), 2.63 (dd, 1H, J = 4.12, 12.95 Hz), 2.87 (d, 1H, J = 4.81 Hz), 7.07 (d, 2H, J = 8.25 Hz), 7.23 (d, 2H, J = 8.25 Hz).

4d : 0.87 (d, 3H, J = 6.87 Hz), 0.91 (d, 3H, J = 6.87 Hz), 1.35–1.43 (m, 1H), 1.55–1.65 (m, 2H), 1.73–1.81 (m, 2H), 1.97–2.02 (m, 1H), 2.15–2.21 (m, 1H), 2.31 (dd, 1H, J = 11.23, 13.52 Hz), 2.65 (dd, 1H, J = 4.36, 13.52 Hz), 2.86 (d, 1H, J = 4.13 Hz), 2.88 (d, 1H, J = 4.13 Hz), 7.06 (d, 2H, J = 8.48 Hz), 7.23 (d, 2H, J = 8.48 Hz).

1.5 *cis*-2-(4-Chlorobenzyl)-*cis*-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl)cyclopentanol (**5a**)

Under nitrogen atmosphere, 60% oily sodium hydride (1.2 g, 30.0 mmol) was washed with hexane, and DMF (20 ml) was added. 1, 2, 4-Triazole (2.1 g, 30.4 mmol) was slowly added and the mixture was stirred for 1 hr. Oxirane (**4a**) (5.0 g, 18.9 mmol) was added, and the mixture was stirred for 2 hr at 80°C.

After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl

acetate. The ethyl acetate layer was washed with water and brine, and dried over anhydrous sodium sulfate. Purification of the adduct by silicagel column chromatography (hexane : ethyl acetate = 1 : 1) gave **5a** (5.0 g, 79.3%) as a solid. Recrystallization from hexane–ethyl acetate gave colorless crystals. mp 91–92°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ : 3250, 2950, 1490. ¹H NMR (CDCl₃) δ ppm : 0.95 (d, 3H, J = 6.88 Hz), 0.98 (d, 3H, J = 6.88 Hz), 1.40–1.44 (m, 1H), 1.49–1.53 (m, 1H), 1.61–1.69 (m, 4H), 1.98–2.05 (m, 1H), 2.24 (dd, 1H, J = 4.58, 13.75 Hz), 2.29 (dd, 1H, J = 10.54, 13.75 Hz), 4.26 (d, 1H, J = 14.21 Hz), 4.41 (d, 1H, J = 14.21 Hz), 7.00 (d, 2H, J = 8.25 Hz), 7.20 (d, 2H, J = 8.25 Hz), 8.03 (s, 1H), 8.47 (s, 1H).

Azole **5b**, **5c** and **5d** were prepared in the same method as described above. Melting point, IR and NMR data of them were shown below.

cis-2-(4-Chlorobenzyl)-*trans*-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl)cyclopentanol (**5b**) : mp 102–103°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ : 3250, 2940, 1480. ¹H NMR (CDCl₃) δ ppm : 0.96 (d, 3H, J = 6.42 Hz), 1.15 (d, 3H, J = 6.42 Hz), 1.13–1.21 (m, 1H), 1.26–1.36 (m, 1H), 1.56–1.62 (m, 1H), 1.69–1.83 (m, 3H), 1.89–1.95 (m, 1H), 2.21 (dd, 1H, J = 10.07, 13.29 Hz), 2.26 (dd, 1H, J = 5.04, 13.29 Hz), 4.15 (d, 1H, J = 13.75 Hz), 4.46 (d, 1H, J = 13.75 Hz), 6.82 (d, 2H, J = 8.25 Hz), 7.16 (d, 2H, J = 8.25 Hz), 8.09 (s, 1H), 8.64 (s, 1H).

trans-2-(4-Chlorobenzyl)-*cis*-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl)cyclopentanol (**5c**) : mp 120–121°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ : 3300, 2950, 1480. ¹H NMR (CDCl₃) δ ppm : 0.75 (d, 3H, J = 6.88 Hz), 0.87 (d, 3H, J = 6.88 Hz), 1.14–1.21 (m, 1H), 1.47–1.54 (m, 2H), 1.57–1.62 (m, 1H), 1.65–1.72 (m, 1H), 1.74–1.81 (m, 1H), 1.99–2.05 (m, 1H), 2.26 (dd, 1H, J = 11.66, 13.08 Hz), 3.04 (dd, 1H, J = 3.43, 13.08 Hz), 4.24 (d, 1H, J = 13.75 Hz), 4.35 (d, 1H, J = 13.75 Hz), 7.05 (d, 2H, J = 8.25 Hz), 7.24 (d, 2H, J = 8.25 Hz), 8.03 (s, 1H), 8.34 (s, 1H).

trans-2-(4-Chlorobenzyl)-*trans*-5-isopropyl-1-(1*H*-1, 2, 4-triazol-1-ylmethyl)cyclopentanol (**5d**) : mp 91–92°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹ : 3200, 2950, 1480. ¹H NMR (CDCl₃) δ ppm : 0.87 (d, 3H, J = 6.19 Hz), 1.00 (d, 3H, J = 6.19 Hz), 1.24–1.36 (m, 2H), 1.49–1.56 (m, 1H), 1.60–1.78 (m, 2H), 1.75 (dd, 1H, J = 11.91, 12.83 Hz), 1.89–1.99 (m, 1H), 2.03–2.09 (m, 1H), 2.47 (dd, 1H, J = 2.75, 12.83 Hz), 4.36 (s, 2H), 6.94 (d, 2H, J = 8.24 Hz), 7.18 (d, 2H, J = 8.24 Hz), 8.01 (s, 1H), 8.43 (s, 1H).

1.6 Optical resolution of azoles **5a** and **5b**

Azole isomer **5a** or **5b** was dissolved in methanol, and the objective compounds were collected fractionally by HPLC with chiral column according to following conditions. After removal of solvent, the residue was recrystallized from hexane–ethyl acetate to give optical pure crystal. Total four enantiomers were obtained. HPLC conditions : column, CHIRALCELL OD (500 × 10 mm i.d., DAICEL Chemical Industries, Ltd.) ; pump, HITACHI 638-30 ; eluent, hexane : isopropanol = 15 : 1 ;

Table 2 Melting points and specific rotations of enantiomers of azoles (**5a**) and (**5b**).

Compound	mp (°C)	$[\alpha]_D^{20}$
(+)- 5a	97–98	+4.17° ($c=1.44$, EtOH)
(-)- 5a	97–98	-3.27° ($c=1.23$, EtOH)
(+)- 5b	129–130	+49.2° ($c=1.59$, EtOH)
(-)- 5b	129–130	-46.8° ($c=1.82$, EtOH)

Table 3 Antifungal activities of azole racemic diastereomers (**5**) *in vitro*.

Compound	EC ₅₀ (μg/ml)		
	<i>Gibberella fujikuroi</i>	<i>Cochliobolus miyabeanus</i>	<i>Pyricularia oryzae</i>
5a	0.05	0.4	0.08
5b	0.06	0.4	0.03
5c	0.9	1	0.5
5d	0.09	0.5	0.04

flow rate, 10 ml/min; detector, UV 268 nm; injection volume, 15 mg/150 μl (methanol).

The melting points and specific rotations of the enantiomers were shown in Table 2. X-ray crystal structure analysis was carried out to assign the absolute configuration.

2. Fungicidal Test

2.1 Antifungal activity assay *in vitro*

The each test compound was dissolved and diluted with DMSO, and then suspended in sterilized potato sucrose agar (PSA) medium to give prescribed concentrations (the final DMSO concentration was 1%). The medium was poured in Petri dishes and was hardened. Mycelial discs (4 mm in diameter) of *Gibberella fujikuroi* (*G. fujikuroi*), *Cochliobolus miyabeanus* (*C. miyabeanus*) and *Pyricularia oryzae* (*P. oryzae*) grown on the PSA alone in advance were transferred to the PSA plates containing the various concentrations of each compound. After incubation for 3 days at 28°C, the diameter of each colony was measured to determine the EC₅₀ (the effective concentration of 50% inhibition of mycelial growth) value.

The EC₅₀ values of azole racemic diastereomers (**5**) were shown in Table 3 and those of azole enantiomers of **5a** and **5b** were in Table 6.

2.2 Antifungal activity assay *in vivo*

2.2.1 "Bakanae" disease of rice

The each test compound was dissolved in acetone, mixed with a detergent, Gramin S, and suspended in water (the final concentrations of acetone and Gramin S were 1 and 0.03%, respectively). The suspension was diluted with water containing 1% of acetone and 0.03% of Gramin S to give concentrations of 300, 75 and 18 μg/ml. Rice seeds (9 g) (cultivar: "Tanginbose" infected

with *G. fujikuroi*) were soaked in 10 ml of the suspension at 20°C for 24 hr. After being immersed in the water at 30°C for 3 days in order to hasten germination, the seeds were sowed in nursery soil and cultivated in a greenhouse for 2 weeks. Disease symptom of the seedlings was investigated and protective value was calculated as follows:

$$\text{Protective value (\%)} = (1 - T/C) \times 100$$

where *T* represents percentage of diseased seedlings on treated plants and *C* represents that on untreated plants. Protective values were shown in Table 4.

2.2.2 Brown spot of rice

Chemical suspensions were prepared as describe above (2.2.1) except concentrations of acetone and Gramin S were 5 and 0.018%, respectively. The suspension was sprayed on rice (cultivar: "Koshihikari") at the fifth leaf stage. After the plants were dried, aqueous spore suspensions of *C. miyabeanus* was inoculated by spraying. The seedlings were maintained in a greenhouse for 6 days, and then the disease spots on the leaf were counted to determine protective value, which was calculated as follows:

$$\text{Protective value (\%)} = (1 - T/C) \times 100$$

where *T* represents number of spots on treated plants and *C* represents that on untreated plants. Protective values were shown in Table 5.

3. Plant Growth Inhibitory Activity Assay

Chemical suspensions prepared as describe above (2.2.2) except for chemical concentration was 250 μg/ml. Rice seeds (5 g) (cultivar: "Koshihikari") were soaked at 20°C for 24 hr in 5 ml of the chemical suspension, then immersed in water at 30°C for 3 days. The seeds were sowed in nursery soil and cultivated in a greenhouse for 10 days. The plant growth inhibitory rate was determined by measuring the first sheath length and shown in Table 7.

RESULTS AND DISCUSSION

1. Chemical Study

The four azole racemic diastereomers (**5**) were synthesized by the method shown in Fig. 2. Isopropyl group was introduced into methyl 2-oxocyclopentanecarboxylate (**1**), the resulting product was subjected to Dieckmann recyclization reaction⁵⁾ to form methyl 3-isopropyl-2-oxocyclopentanecarboxylate (**2**), and subsequently, 4-chlorobenzyl group was introduced,⁶⁾ followed by hydrolysis and decarboxylation⁷⁾ to obtain 2-(4-chlorobenzyl)-5-isopropylcyclopentanone (**3**). To obtain four racemic diastereomers of 4-(4-chlorobenzyl)-7-isopropyl-1-oxaspiro[2.4]heptane (**4**), two methods (A and B) were examined. Method A is a reaction which uses dimethylsulfoxonium methylide,⁸⁾ and method B is a

method in which ketone (3) is converted to 2-(4-chlorobenzyl)-5-isopropyl-1-methylenecyclopentane (3') through Wittig reaction⁹ followed by oxidation.¹⁰ The ratios of resulting diastereomers by these two methods were listed in Table 1. Method A gave oxirane isomer (4a) as a major product and method B gave oxirane isomer (4b) as a major product. The four oxirane isomers (4) were isolated individually and the relative configuration was estimated from NOE (nuclear overhauser effect) difference spectrum.

NOE was examined by irradiating methine proton of the isopropyl group, methylene proton of the oxirane ring, and methylene proton of the benzyl group of the oxirane isomers (4) individually. As shown in Fig. 3, NOE was not found for 4a, NOE was found between Hb-1 of the isopropyl group and Hb-2 of the oxirane ring for 4b, and also found between Hc-3 of the oxirane ring and Hc-4 of the benzyl group for 4c. NOE was found between Hd-1 of the isopropyl group and Hd-2 of the oxirane ring, and between Hd-3 of the oxirane ring and Hd-4 of the benzyl group for 4d.

Based on these results, the relative configuration of oxirane isomers (4) were estimated as shown in Fig. 3. The four oxirane isomers (4) were introduced to azoles (5). The relative configuration ofazole isomers (5) was also estimated by NOE difference spectrum. The result of NOE by irradiation of the methine proton of the isopropyl group, the methylene proton of the triazolyl-methyl group, and the methylene proton of the benzyl group ofazole (5) was the same as that of oxirane (4). This result supports the estimation of relative configuration of four oxirane isomers.

X-ray crystal structure analysis of (–)-5a was carried

out and its crystal structure was determined as follows: monoclinic, $P2_1$, $a=23.592$, $b=8.448$, $c=9.973\text{\AA}$, $\beta=113.54^\circ$, $Z=4$, $R=0.0450$. It assigned the absolute configuration of the (–)-5a as (1*S*, 2*R*, 5*S*). From analyses result of (–)-5a, the absolute configuration of (–)-5b was estimated as (1*S*, 2*R*, 5*R*) (Fig. 4). The relative configuration ofazole isomer (5a) estimated by NOE difference spectrum was confirmed from this result.

2. Antifungal and Plant Growth Inhibitory Activities

In comparison of antifungal activity on *G. fujikuroi* in the *in vitro* test of the fourazole racemic diastereomers, activities of 5a, 5b and 5d were high but that of 5c was rather low. The same results were obtained in the tests on *C. miyabeanus* and *P. oryzae*. The activity of 5a on *G. fujikuroi* was higher about 20 times than that of 5c (Table 3). In the *in vivo* test on "Bakanae" disease at a concentration of 75 $\mu\text{g/ml}$ of seed treatment, the activities of 5a and 5b were high, that of 5d was slightly lower than the formers, and that of 5c was the lowest in the four isomers. In the test on brown spot the same result was obtained (Tables 4 and 5). The result of the *in vivo* test correlated with that of the *in vitro* test. As an overall evaluation of the fourazole racemic diastereomers with the three fungi (*G. fujikuroi*, *C. miyabeanus* and *P. oryzae*), it is found that the activities of 5a and 5b are the highest, 5d is slightly lower, and 5c is the lowest.

Antifungal activity of enantiomers of 5a or 5b was clearly different between (–)- and (+)-isomers. For both 5a and 5b, (–)-isomers were more active than (+)-isomers on *G. fujikuroi*, *C. miyabeanus* and *P. oryzae*. The activity of (–)-isomer of 5a was 330 times

Fig. 3 ^1H NMR chemical shifts (δ ppm) and NOEs in oxirane (4).

Fig. 4 Absolute configurations of (–)-5a and (–)-5b.

Table 4 Control activities ofazole racemic diastereomers (5) on "Bakanae" disease of rice by seed treatment.

Compound	Control (%)		
	Concentration ($\mu\text{g/ml}$)		
	300	75	18
5a	98.7	96.5	61.2
5b	99.6	97.5	52.1
5c	95.2	59.1	0.0
5d	97.5	76.8	9.7

Table 5 Control activities ofazole racemic diastereomers (5) on brown spot of rice by foliar application.

Compound	Control (%)		
	Concentration ($\mu\text{g/ml}$)		
	150	38	9
5a	100	84	86
5b	100	98	71
5c	92	49	25
5d	100	74	53

Table 6 Antifungal activities of enantiomers of azoles (**5a**) and (**5b**) *in vitro*.

Compound	EC ₅₀ (μg/ml)		
	<i>Gibberella fujikuroi</i>	<i>Cochliobolus miyabeanus</i>	<i>Pyricularia oryzae</i>
(+)- 5a	10	6	1
(-)- 5a	0.03	0.2	0.04
(+)- 5b	6	3	0.7
(-)- 5b	0.03	0.2	0.01

higher than that of (+)-isomer on *G. fujikuroi*, 30 times on *C. miyabeanus*, and 25 times on *P. oryzae* (Table 6).

Based on these results, the correlation between the stereochemical structure of azole racemic diastereomers (**5**) and fungicidal activity was examined. Firstly, the relative configuration between hydroxy group and benzyl group of the four racemic diastereomers is correlated with activity as follows. **5a** and **5b**, higher activity isomers, have *cis*-relative configuration, while **5c** and **5d**, lower activity isomers, have *trans*-relative configuration. In other words, *cis*-relative configuration between hydroxy group and benzyl group is favorable for the high activity. Secondly, the relative configuration between hydroxy group and isopropyl group is correlated with activity. The activity of **5a** is not different from that of **5b**, therefore the steric effect of isopropyl group is not clear with those two isomers. However, the fact that the activity of **5d** is higher than that of **5c**, suggests that *trans*-isopropyl group is more favorable than *cis*-isopropyl group for the high activity. Azole isomer (**5d**) shows nearly the same activity as **5a** and **5b** in spite of *trans*-relative configuration between hydroxy group and benzyl group, which is unfavorable configuration for the high activity. We had prepared a series of derivatives which have other alkyl group (e.g., Me, Et, Pr, Bu and Pen) instead of isopropyl group of **5a** and **5b**. In comparison with fungicidal activity among the derivatives, *trans*-alkyl group showed higher activity than *cis*-alkyl group.¹¹⁾ Although the reason for the high activity of **5a**, *cis*-isopropyl group, as well as **5b**, *trans*-isopropyl group, is not clear, the branched-chain of isopropyl group of both **5a** and **5b** likely contributes to the interaction with the binding site of target enzyme, cytochrome P450_{14DM}. Enantiomers (-)-**5a** and (-)-**5b** were more active than (+)-isomers in fungicidal activity. This clear stereochemical discrimination will become important when receptor model is considered.

Among the azole racemic diastereomers, **5a**, **5b** and **5c** are little active on plant growth regulatory effect on rice, but **5d** is slightly active in the first sheath growth inhibition (Table 7). It is known that azole fungicide causes biosynthesis inhibition of a plant hormone, gibberellin, and suppresses elongation of plant cells. Uniconazole¹²⁾ and paclobutorazole¹³⁾ are used as plant growth regula-

Table 7 Plant growth inhibitory activities of azole racemic diastereomers (**5**) on rice by seed treatment.

Compound ^{b)}	Growth rate ^{a)} to control (%)
	Concentration 250 μg/ml
5a	98.3
5b	90.7
5c	91.7
5d	82.5

^{a)} Measured the first sheath length in 30 replications.

^{b)} Treated with 250 μg/ml solution for 24 hr before emergence.

tor. The slight sheath growth inhibition found on **5d** is assumed as a result of gibberellin biosynthesis inhibition based on morphological observation. Further studies will be needed to clarify the correlation between the structure of a series of azolylmethylcyclopentanol derivatives and plant growth inhibitory activity.

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要 約

イプコナゾールの立体異性体の合成とそれらの殺菌および植物生長抑制活性

伊藤篤史, 最勝寺俊英, 熊沢 智
水稻種子消毒剤であるイプコナゾール, (1*RS*, 2*SR*, 5*RS*; 1*RS*, 2*SR*, 5*SR*)-2-(4-クロロベンジル)-5-イソプロピル-1-(1*H*-1, 2, 4-トリアゾール-1-イルメチル)シクロペンタノールの有効成分である2種類のジアステレオ異性体 **5a**, **5b** と他の2種類のジアステレオ異性体 (1*RS*, 2*RS*,

5*RS*)-**5c**, (1*RS*, 2*RS*, 5*SR*)-**5d** を合成し, それらの殺菌および植物生長抑制活性を調べた. *In vitro* 試験でのイネばか苗病菌, イネごま葉枯病菌, イネいもち病菌に対する各ジアステレオ異性体の殺菌活性の比較では, **5a**, **5b** および **5d** が高い活性を示した. *In vivo* 試験では, **5d** の活性が, **5a** と **5b** の活性より少し低下し, 活性の差がみられた. イネ種子処理による植物生長抑制活性試験では, **5d** に弱いながら節間伸長抑制活性がみられた. **5a** と **5b** の光学異性体間の *in vitro* 試験での殺菌活性比較においては, 各(−)体はそれぞれの各(+)体に比べ活性が高かった.