新規イミダゾール系殺菌剤OK-8705,OK-8801光学異性体の合成と灰色かび病菌,ばか苗病菌に対する抗菌活性

誌名	日本農薬学会誌
ISSN	03851559
著者名	今井,哲弥 内田,俊郎 山口,国夫
発行元	日本農薬学会
巻/号	18巻4号
掲載ページ	p. 375-380
発行年月	1993年11月

J. Pesticide Sci. **18**, 375–380 (1993)

Original Article

Preparation of Enantiomers of the New Imidazole Fungicides OK-8705 and OK-8801 and Their Antifungal Activity against *Botrytis cinerea* and *Gibberella fujikuroi*

Tetsuya IMAI, Toshiro UCHIDA, Kunio YAMAGUCHI, Hisashi TAKAO and Takeshi GOTO

Naruto Research Center, Otsuka Chemical Co., Ltd., Satoura, Naruto 772, Japan

(Received June 2, 1993; Accepted August 18, 1993)

New Imidazole fungicides, OK-8705 (1-(4-methoxyphenoxymethyl)-2,2-dimethylpropyl imidazole-1-carboxylate) and OK-8801 (the thiocarboxylate derivative of OK-8705), have one chiral center in the molecule. We determined the absolute configurations of the common starting material for synthesis of the fungicides, (−)- and (+)-1-(4-methoxyphenoxy)-3,3-dimethyl-2-butanol, as (*R*)- and (*S*)-enantiomers, respectively, by Mosher's method. (*R*)- and (*S*)-OK-8705 and OK-8801 prepared from the (*R*)- and (*S*)-alcohols, respectively, were evaluated for their antifungal activity against *Botrytis cinerea* and *Gibberella fujikuroi* using the agar dilution method. The antifungal activities of the (*R*)-isomers of OK-8705 and OK-8801 against *B. cinerea* were 513 and 265 times higher than those of the (*S*)-isomers, respectively; against *G. fujikuroi* 38 and 143 times higher, respectively. We concluded that the (*R*)-isomers were mainly responsible for the antifungal activity.

INTRODUCTION

OK-8705 and OK-8801 are new imidazole fungicides for foliar application and seed-treatment, respectively, under development by Otsuka Chemical Co., Ltd.[1-3] The former is effective against grey mould caused by *Botrytis cinerea* on eggplant, and the latter is effective against bakanae disease caused by *Gibberella fujikuroi* on rice. A mode of action study indicated that the fungicides act as inhibitors of 14α-demethylase in the ergosterol biosynthesis pathway.[4]

Both fungicides possess one chiral center, and consequently a pair of enantiomers exists for each fungicide. Some current azole fungicides similarly possess one or two chiral centers in the molecule, and the relationship between the stereochemistry of the fungicides and the fungicidal activity has been studied thoroughly.[5-12] Studies indicated that there was a distinct difference in activity between the isomers, and this suggested that the inhibition of 14α-demethylase in fungi by azole fungicides was very stereospecific.

To determine the absolute configuration of an optically active carbinol, Mosher's method has been developed.[13-18] The method is based on chemical shift differences of nuclear magnetic resonance (NMR) between diastereomeric α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA) esters obtained by the reactions of the carbinol with (+)- and (−)-MTPACl. Using the method, many absolute configurational assignments of secondary carbinols have been conducted.[19,20] The common starting alcohol, 1-(4-methoxyphenoxy)-3,3-dimethyl-2-butanol, for the synthesis of OK-8705 and OK-8801 has a chiral center at the carbinol carbon, and we determined the absolute configuration of each isomer of the alcohol by the above method.

In this paper, we describe the absolute configurational assignment of each enantiomer of the alcohols, preparation of OK-8705 and OK-8801 isomers and their antifungal activities against *B. cinerea* and *G. fujikuroi*.

MATERIALS AND METHODS

1. *Preparation of Optically Active Compounds*

The synthetic scheme of the compounds is shown in Fig. 1. Two enantiomers of 1-(4-methoxyphenoxy)-3,3-dimethyl-2-butanol (**Ia** and **Ib**) were purchased from Daicel Chemical Industries, Ltd. The optical rotations were $[\alpha]_D^{25}$ −42.2° (c=2.03, CCl_4) and $[\alpha]_D^{26}$ +42.0° (c=2.17, CCl_4) for **Ia** and **Ib**, respectively. The reaction of **Ia** with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*S*)-MTPACl), which was synthesized by the method of Dale *et al.*,[18] afforded (−)-1-(4-methoxyphenoxymethyl)-2,2-dimethylpropyl α-methoxy-α-(trifluoromethyl) phenylacetate (**IIa**). The reaction of **Ia** with 1,1′-carbonyldiimidazole (CDI) and with 1,1′-thiocarbonyldiimidazole (TCDI) gave (−)-1-(4-methoxyphenoxymethyl)-2,2-dimethylpropyl imidazole-1-carboxylate (**IIIa**) and (+)-1-(4-methoxyphenoxymethyl)-2,2-dimethylpropyl imidazole-1-thiocarboxylate (**IVa**), respectively. Similarly, **IIb**, **IIIb** and **IVb** were prepared from **Ib**.

Melting points were uncorrected. Refractive indices were measured with a Bausch & Lomb Abbe-3L refractometer. Optical rotations were measured with a Jasco DIP-370 digital polarimeter. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian VXR-300S NMR spectrometer with deuterochloroform as solvent. Mass spectra (MS) were recorded on a Shimadzu GCMS-QP 1000EX. Satisfactory ^{1}H NMR and MS were obtained for all the compounds.

1.1 (−)-*1-(4-Methoxyphenoxymethyl)-2,2-dimethylpropyl α-methoxy-α-(trifluoromethyl)-phenylacetate* (***IIa***) *and its diastereomeric isomer* (***IIb***)

(*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (5 g, 21 mmol; 99 ee%, Aldrich), thionyl chloride (9.1 ml, 126 mmol) and sodium chloride (0.06 g) were heated together under reflux for 47 hr. The excess thionyl chloride was removed by vacuum evaporation (18 mmHg at room temperature), and then the residue was distilled to give 4.8 g of (*S*)-(+)-MTPACl (yield=89%) as a colourless oil; bp 58.5°C (3 mmHg), $[\alpha]_D^{26}$ +133.2° (c

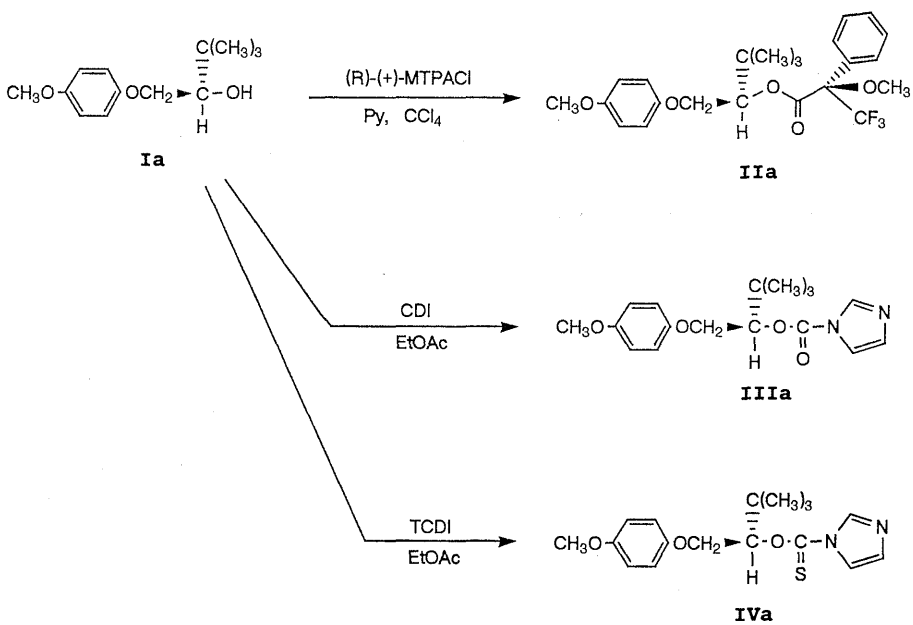


Fig. 1 Synthetic scheme of (−)-MTPA ester (**IIa**), (*R*)-(−)-OK-8705 (**IIIa**) and (*R*)-(+)-OK-8801 (**IVa**).

$=10.1$, CCl_4).

To a solution of **Ia** (224 mg, 1 mmol) and (*S*)-(+)-MTPACl (227 mg, 1.1 mmol) in carbon tetrachloride (0.7 ml), pyridine (0.44 ml, 5.5 mmol) was added with stirring in an ice bath. The mixture was stirred at room temperature for 15 hr and diluted with ether (70 ml). The ethereal mixture was washed successively with dil. hydrochloric acid, saturated sodium carbonate solution and water, dried over anhyd. magnesium sulphate, and evaporated to dryness. The residue was also subjected to silica gel column chromatography [Merck silica gel 60 (70–230 mesh)] using an eluent of 12.5% ethyl acetate in hexane (v/v) to give 333 mg of **IIa** (yield=75.7%) as a colourless oil; n_D^{20} 1.5068, $[\alpha]_D^{24}$ −14.0° ($c=1.89$, CCl_4), EI-MS *m/z*: 317 ((M-methoxyphenoxy)$^+$), 189, 124, 83 (base peak).

Similarly, the reaction of **Ib** (224 mg, 1 mmol) with (*S*)-(+)-MTPACl (227 mg, 1.1 mmol) afforded 326 mg of **IIb** (yield=74.1%) as a colourless oil; n_D^{20} 1.5069, $[\alpha]_D^{25}$ +46.5° ($c=2.01$, CCl_4), EI-MS *m/z*: 317 ((M-methoxyphenoxy)$^+$), 189, 124, 83 (base peak).

*1.2 (−)-1-(4-Methoxyphenoxymethyl)-2, 2-dimethylpropyl imidazole-1-carboxylate (**IIIa**) and its (+)-isomer (**IIIb**)*

A mixture of **Ia** (336 mg, 1.5 mmol) and CDI (365 mg, 2.25 mmol) in ethyl acetate (30 ml) was refluxed with stirring for 3 hr. The reaction mixture was concentrated under reduced pressure, and the resulting residue was subjected to silica gel column chromatography (Merck silica gel 60) using an eluent of 20% ethyl acetate in benzene (v/v) to give 458 mg of **IIIa** (yield=96%) as a colourless prism; mp 102.4°C, $[\alpha]_D^{26}$ −11.0° ($c=1.86$, CCl_4), EI-MS *m/z*: 318 (M^+), 195, 124, 83 (base peak), ^{1}H NMR (in $CDCl_3$) δ ppm: 1.08 (9H, s, $-C(CH_3)_3$), 3.74 (3H, s, $O-CH_3$), 4.10 (1H, dd, $J=8.4$, 10.8 Hz, $O-CH_2-CH-$), 4.25 (1H, dd, $J=2.7$, 10.8 Hz, $O-CH_2-CH-$), 5.25 (1H, dd, $J=2.7$, 8.4 Hz, $O-CH-CH_2-$), 6.79 (4H, s, arom. H), 7.08 (1H, bs, imidazole), 7.44 (1H, bs, imidazole), 8.17 (1H, bs, imidazole).

Similarly, the reaction of **Ib** with CDI gave **IIIb** (yield=97%) as a colourless prism; mp 102.6°C, $[\alpha]_D^{26}$ +10.9° ($c=1.81$, CCl_4)

*1.3 (+)-1-(4-Methoxyphenoxymethyl)-2, 2-dimethylpropyl imidazole-1-thiocarboxylate (**IVa**) and its (−)-isomer (**IVb**)*

A mixture of **Ia** (336 mg, 1.5 mmol) and TCDI (341 mg, 1.72 mmol; 90%, Aldrich) in ethyl acetate (30 ml) was heated under reflux for 18 hr. The reaction mixture was worked up and purified in the same manner as described in *1.2* to give 422 mg of **IVa** (yield=84%) as a colourless prism; mp 65.0°C, $[\alpha]_D^{26}$ +19.2° ($c=2.02$, CCl_4), EI-MS *m/z*: 334 (M^+), 211, 191, 124, 83 (base peak), ^{1}H NMR (in $CDCl_3$) δ ppm: 1.12 (9H, s, $-C(CH_3)_3$), 3.74 (3H, s, $O-CH_3$), 4.19 (1H, dd, $J=6.9$, 10.8 Hz, $O-CH_2-CH-$), 4.33 (1H, dd, $J=3.3$, 10.8 Hz, $O-CH_2-CH-$), 5.86 (1H, dd, $J=3.3$, 6.9 Hz, $O-CH-CH_2-$), 6.79 (4H, s, arom. H), 7.05 (1H, bs, imidazole), 7.64 (1H, bs, imidazole), 8.36 (1H, bs, imidazole).

Similarly, the reaction of **Ib** with TCDI afforded **IVb** (yield=70%) as a colourless prism; mp 62.6°C, $[\alpha]_D^{27}$ −19.4° ($c=0.732$, CCl_4).

2. Determination of Chemical and Optical Purities

2.1 Chemical purity

Chemical purity of **Ia** and **Ib** was determined by gas-liquid chromatography (GC) using a glass column (3.0 mm i.d.×1.1 m) packed with 2% Silicone OV-101 on Chromosorb W (100–120 mesh). The GC instrument used was a Shimadzu GC-7AG with a flame ionization detector and connected with a Shimadzu C-R1B data processor. The GC conditions were as follows: injection temperature, 200°C; column temperature, 160°C; detection temperature, 200°C; and nitrogen flow rate, 50 ml/min.

Chemical purity of **IIIa**, **IIIb**, **IVa** and **IVb** was determined by HPLC with Zorbax BP-ODS (4.6 mm i.d.×25 cm). The HPLC instrument used was a Shimadzu LC-6A equipped with an SPD-6A UV spectrophotometric detector and connected with a Shimadzu C-R3A data processor. The HPLC conditions were as follows: column temperature, 55°C; AUFS, 0.64; detection wavelength, 254 nm; mobile phase, 20% water in acetonitrile (v/v); and flow rate, 1.0 ml/min.

2.2 Optical purity

Optical purity of all isomers of **I**, **III** and **IV**

was determined by HPLC with Daicel CHIRALPAK AD (4.6 mm i.d.×25 cm). The HPLC instrument used was a Hitachi L-6000 liquid chromatograph equipped with an L-4000 UV detector. The HPLC conditions were as follows: column temperature, 25°C; AUFS, 2.5; detection wavelength, 254 nm; mobile phase, 10% ethyl alcohol in hexane (v/v); and flow rate, 0.7–1.0 ml/min.

3. Biological Tests

The fungicidal activity was determined by the agar dilution method. A solution of test compound, dissolved in ethyl alcohol to give final concentrations of 0.004–100 mg/*l*, was added to potato dextrose agar medium. The resulting solution was thoroughly mixed and approximately 20 ml was poured into 9-cm diameter sterile plastic Petri dishes. The resulting agar plates were inoculated with 5-mm diameter mycelial discs of *B. cinerea* or *G. fujikuroi* from freshly prepared, actively growing cultures and incubated at 25°C in the dark. The diameter of each colony was measured after 3 days. Three replicates were used for each concentration of a test compound and for each test fungus. Control cultures, also comprising three replicates, received an equivalent amount of the solvent used to dissolve the test compound. The fungitoxic activity was expressed as ED_{50}, the concentration required for a 50% inhibition of mycelial growth.

RESULTS AND DISCUSSION

1. Preparation and Purity of Optically Active Compounds

Chemical purities of **Ia** and **Ib** were measured to be 96.6 and 98.2%, respectively, by GC based on the detection linearity using the racemic compound as a standard. The (−)-isomer (**Ia**) and (+)-isomer (**Ib**) were detected at 7.7 and 13.9 min, respectively, on HPLC chromatograms, and each optical purity was almost 100 ee%.

The reaction of **Ia** and **Ib** with excess CDI (1.5 equivalents) afforded **IIIa** and **IIIb**, respectively, with a high yield. Chemical purities for **IIIa** and **IIIb** were 96.7 and 98.4%, respectively, in terms of HPLC analysis. The (−)-isomer (**IIIa**) and (+)-isomer (**IIIb**) were eluted at 9.9 and 17.2 min, respectively. The optical purity of both isomers was almost 100 ee%.

To obtain a high yield based on the optically active alcohol, we applied an excess of TCDI for the synthesis of thiocarboxylates. The reactions, however, of the racemic alcohol with 2.0 and 1.5 equivalents of TCDI resulted in very low yields of 24.0 and 60.4%, respectively. Finally we obtained the (+)-isomer (**IVa**) and (−)-isomer (**IVb**) with the purified yields of 84 and 70%, respectively, by reacting each optically active alcohol with 1.15 equivalents of TCDI. We have not yet elucidated this incompatibility, but some impurity of the purchased TCDI seems to have affected the yield. The HPLC analysis indicated that chemical purities of **IVa** and **IVb** were 96.6 and 97.8%, respectively, and the optical purity for both isomers was almost 100 ee%. The retention times of **IVa** and **IVb** were 10.1 and 12.3 min, respectively.

In these reactions, racemization or inversion at the chiral center does not seem to have occurred since all four isomers showed a very high optical purity as mentioned above. Moreover, the hydrolysis of **IIIa** and **IVa** with dil. hydrochloric acid or dil. sodium hydroxide solution equally afforded the starting alcohol (**Ia**) with 100 ee% (data not shown).

*2. Determination of Absolute Configurations of (−)-Alcohol (**Ia**) and (+)-Alcohol (**Ib**)*

Mosher's method for the determination of the absolute configuration of an optically active alcohol is fundamentally based on chemical shift differences of NMR between (*R*)- and (*S*)-MTPA esters of the alcohol, but is applicable to (*R*)-MTPA esters of each isomeric alcohol as well. As shown in Fig. 2, the chemical shifts of protons (**Ha**), which are located in the same direction as the phenyl group of (*R*)-MTPA moiety, of diastereomer **1** (**D-1**) are upper than those of protons (**Ha**) of diastereomer **2** (**D-2**) due to an anisotropic effect exerted by the phenyl group of (*R*)-MTPA moiety. Similarly, protons (**Hb**) of **D-1** show a lower chemical shift than those of **D-2**. The proton directly attached to the carbinyl carbon is, however, not applicable since the difference of the chemical shifts between the diastereomeric esters is irregularly ranged

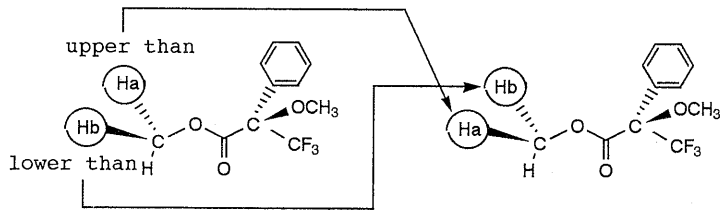
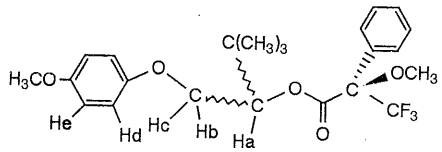


Fig. 2 Relationship between configurations of diastereomeric (*R*)-MTPA esters (**D-1** and **D-2**) and ^{1}H NMR chemical shift of carbinol moiety.

Table 1 ^{1}H NMR chemical shift difference between MTPA esters of (+)- and (−)-alcohols.



Protons[a]	Chemical shift of alcohol moiety		δ (**Ia**−**Ib**)
	(−)-alcohol (**Ia**)	(+)-alcohol (**Ib**)	
$-C(CH_3)_3$	0.986	1.033	−0.047
Ha	5.376	5.352	0.024
Hb	4.199	4.152	0.047
Hc	4.043	3.948	0.095
Hd	6.846	6.807	0.039
He	6.804	6.749	0.055
OCH_3	3.778	3.760	0.018

[a] Protons corresponding to those in the structure described above.

from positive to negative region.[20]

Table 1 shows the chemical shifts of protons of the alcohol moiety of each MTPA ester and chemical shift differences. The difference of *tert*-butyl group was negative, and the others were positive. These results mean that *tert*-butyl group of MTPA ester of (−)-alcohol (**Ia**) is equivalent to the **Ha** of **D-1** in Fig. 2, and the others equivalent to the **Hb**.

From the above results, we concluded that (−)-alcohol (**Ia**) and (+)-alcohol (**Ib**) were (*R*)-isomer and (*S*)-isomer, respectively.

3. *Antifungal Activity*

The ED_{50} values of each compound to both fungi are listed in Table 2.

Table 2 Antifungal activity of OK-8705, 8801 and their isomers to *B. cinerea* and *G. fujikuroi*.

Compound	ED_{50} (ppm)	
	Bc[a]	Gf[b]
(±)-8705	0.170	1.03
(*R*)-(−)-8705 (**IIIa**)	0.069	0.675
(*S*)-(+)-8705 (**IIIb**)	35.4	25.6
(±)-8801	0.077	0.225
(*R*)-(+)-8801 (**IVa**)	0.032	0.127
(*S*)-(−)-8801 (**IVb**)	8.47	18.2

[a] Activity against *B. cinerea* 3 days after treatment.

[b] Activity against *G. fujikuroi* 3 days after treatment.

The antifungal activities of the (*R*)-isomers (**IIIa** and **IVa**) were 513 and 265 times as high as those of the (*S*)-isomers (**IIIb** and **IVb**), respectively, and almost twice as high as those of the racemates. These results mean that the (*R*)-isomers (**IIIa** and **IVa**) of OK-8705 and OK-8801 are responsible for the antifungal activity against *B. cinerea*. Similar results were also obtained with *G. fujikuroi*. We would conclude that the (*R*)-forms of OK-8705 and OK-8801 intrinsically represent fungicidal activity of the racemates.

REFERENCES

1) T. Imai, H. Takao, K. Yamaguchi, T. Uchida, M. Aoki, T. Goto & N. Umetsu: Abstr.; 13th Pestic. Sci. Soc. Jpn. B210, 1988 (in Japanese)
2) T. Imai, H. Takao, K. Yamaguchi, T. Uchida, T. Goto & N. Umetsu: Abstr.; 13th Int. Chem. Congr. Pacific Basin Soc. AGRO 266, 1989
3) T. Imai, H. Takao, K. Yamaguchi, T. Uchida,

T. Goto & N. Umetsu: *Pestic. Sci.* **34**, 119 (1992)
4) K. Yanosaka, T. Imai, K. Yamaguchi, T. Uchida & N. Umetsu: *Pestic. Biochem. Physiol.* **41**, 250 (1991)
5) B. Sugavanam: *Pestic. Sci.* **15**, 296 (1984)
6) Y. Funaki, Y. Ishiguri, T. Kato & S. Tanaka: *J. Pesticide Sci.* **9**, 229 (1984)
7) H. Takano, T. Oguri & T. Kato: *J. Pesticide Sci.* **11**, 373 (1986)
8) W. Köller: *Pestic. Sci.* **18**, 129 (1987)
9) R. S. Burden, G. A. Carter, T. Clark, D. T. Cooke, S. J. Croker, A. H. B. Deas, P. Hedden, C. S. James & J. R. Lenton: *Pestic. Sci.* **21**, 253 (1987)
10) R. S. Burden, T. Clark & P. J. Holloway: *Pestic. Biochem. Physiol.* **27**, 289 (1987)
11) P. A. Haughan, R. S. Burden, J. R. Lenton & J. Goad: *Phytochemistry* **28**, 781 (1989)
12) M. Takenaka, S. Miura, T. Tanaka & T. Wada: *J. Pesticide Sci.* **17**, 205 (1992)
13) J. A. Dale, D. L. Dull & H. S. Mosher: *J. Org. Chem.* **34**, 2543 (1969)
14) G. Barth, W. Voelter, H. S. Mosher, E. Bunnenberg & C. Djerassi: *J. Am. Chem. Soc.* **92**, 875 (1970)
15) J. A. Dale & H. S. Mosher: *J. Am. Chem. Soc.* **95**, 512 (1973)
16) G. R. Sullivan, J. A. Dale & H. S. Mosher: *J. Org. Chem.* **38**, 2143 (1973)
17) S. Yamaguchi, F. Yasuhara & K. Kabuto: *Tetrahedron* **32**, 1363 (1976)
18) S. K. Balani, D. R. Boyd, E. S. Cassidy, G. I. Devine, J. F. Malone, K. M. McCombe, N. D. Sharma & W. B. Jennings: *J. Chem. Soc. Perkin Trans. I.* **1983**, 2751
19) S. Takano, M. Takahashi, M. Yanase, Y. Sekiguchi, Y. Iwabuchi & K. Ogasawara: *Chemistry Lett.* **1988**, 1827
20) I. Ohtani, T. Kusumi, M. O. Ishitsuka & H. Kakisawa: *Tetrahedron Lett.* **30**, 3147 (1989)

要　　約

新規イミダゾール系殺菌剤 OK-8705, OK-8801 光学異性体の合成と灰色かび病菌，ばか苗病菌に対する抗菌活性

今井哲弥，内田俊郎，山口国夫
高尾　久，後藤武司

OK-8705 および OK-8801 は分子内に 1 個の不斉炭素を有する (*RS*)-1-(4-メトキシフェノキシ)-3, 3-ジメチル-2-ブタノールから合成され，ラセミ体から成るイミダゾール系殺菌剤である．本稿ではアルコール異性体の絶体構造を Mosher らの方法により決定し，各異性体から合成した OK-8705 および OK-8801 光学異性体の灰色かび病菌，ばか苗病菌に対する抗菌活性を検討した．(*R*)-OK-8705 および (*R*)-OK-8801 はそれらの (*S*)-異性体に比べ *in vitro* 試験で灰色かび病菌に対し，それぞれ 513, 265 倍活性が強かった．一方，ばか苗病菌に対しても (*R*)-OK-8705 および (*R*)-OK-8801 はそれぞれ 38, 143 倍強い活性を示した．以上の結果より OK-8705 および OK-8801 の活性に主として関与しているのは，(*R*)-異性体であることが明らかとなった．