Original Article

Synthesis and Fungicidal Activity of α -Cyanoacetamide Derivatives: Discovery of a New Rice Blast Fungicide, Diclocymet (S-2900)

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In order to search for a new systemic rice blast fungicide, a number of *N*-benzyl- α -cyanoacetamide derivatives and related compounds were prepared and their preventive activity against rice blast, *Magnaporthe grisea* was examined both in a foliar spray test and in a soil drench test. Several *N*-[1-(substituted-phenyl)ethyl]-2-cyano-3,3-dimethylbutanamides and -2-pentenamides showed high activity in both tests. Among them, (*RS*)-2-cyano-*N*-[(*R*)-1-(2,4-dichlorophenyl)ethyl]-3,3-dimethylbutyramide (diclocymet, S-2900) was selected as a practical new systemic rice blast fungicide, which shows potent fungicidal activity with little crop injury.

Key words: rice blast fungicide, systemic activity, α -cyanoacetamide derivatives, stereoisomer, diclocymet, S-2900.

INTRODUCTION

It has been reported that certain kinds of sterically hindered *N*-benzylbutanamides such as 2-bromo-*N*-[1-(4-chlorophenyl)ethyl]-3,3-dimethylbutanamide **1** were effective in controlling rice blast.¹⁾ As part of a continuing screening program to search for a new systemic rice blast fungicide, we reinvestigated the efficacy of **1** against rice blast and found that its activity in a soil drench test was moderate, although it showed good preventive activity in a foliar spray test. This result might be attributed to its low systemic action due to a high degree of hydrophobicity. On the basis of this working hypothesis, we reasoned that one could enhance its systemic action by introducing a hydrophilic group into an appropriate site on the molecule. In this context, we initiated structural modifications of **1** and found that replacement of the bromo group with the more hydrophilic cyano group (**5**) greatly increased the systemic action of **1** without decreasing its preventive activity. Further structural optimization from the view points of fungicidal potency and degree of phytotoxicity, including a steric structure-activity correlation study, led to the discovery of a new systemic blast fungicide, diclocymet (S-2900, (*RS*)-2-

cyano-*N*-[(*R*)-1-(2,4-dichlorophenyl)ethyl]-3,3-dimethylbutyramide, **56e**). We report here the synthesis and fungicidal activity of the α -cyanoacetamide derivatives. The mode of action of **56e** will be briefly referred to.

MATERIALS AND METHODS

1. Synthesis of Compounds

The structures of the synthesized compounds were confirmed using ¹H NMR spectra, and in some cases, mass spectra. Melting points and boiling points are uncorrected.

1.1 Synthesis of amides other than α -cyanoacetamide compounds

The routes to synthesize amides other than α -cyanoacetamide compounds are shown in Fig. 1. The sulfoxide **8** and the sulfone **9** were obtained by oxidation of the sulfide **2** which was prepared by the reaction of **1**¹⁾ with NaSMe. Compound **6** having a methoxy group was prepared by the reaction of the corresponding amine and the acid chloride derived from the acid, which was prepared by the reaction of the α -bromo acid with NaOMe. The fluorine compound **3** was obtained by the reaction of the alcohol **7** with diethylaminosulfur trifluoride,²⁾ which was prepared³⁾ by the reaction of pivalaldehyde and an isonitrile⁴⁾ in the presence of an

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To an ice-cooled solution of 2-bromo-3,3-dimethylbutanoic acid (9.5 g) in DMF (50 ml) was added dropwise NaOMe (28% solution in methanol, 27.4 g). Methanol was removed under reduced pressure and the residue was stirred at reflux under N₂ for 6 hr. The mixture was treated with water, acidified with conc. HCl with ice-cooling and extracted with ethyl acetate. The organic layer was washed three times with brine, dried and distilled (bp 76–92°C at 0.2 mmHg) to give crude 2-methoxy-3,3-dimethylbutanoic acid (2.57 g). The mixture of the acid (1.46 g) and thionyl chloride (1.43 g) was stirred at room temperature in the presence of a drop of DMF and stirred at 60–70°C for 1 hr to give crude 2-methoxy-3,3-dimethylbutanoyl chloride. To an ice-cooled solution of 1-(4-chlorophenyl)ethylamine (1.56 g) and triethylamine (1.6 g) was added dropwise the crude acid chloride. After stirring at room temperature for 2 hr, the mixture was washed with water, dried and concentrated. The residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (5:1, v/v)) to give **6 l** (the less polar diastereomer, 0.30 g), followed by **6 m** (the more polar diastereomer, 0.35 g).

1.1.5 *N*-[1-(4-Chlorophenyl)ethyl]-2-hydroxy-3,3-dimethylbutanamide 7

A mixture of 4'-chloroacetophenone (46.4 g), formamide (55.7 g) and 90% aq. formic acid (6 ml) was heated at 160–170°C for 6 hr. The mixture was treated with water and extracted with CHCl_3 . The organic layer was washed twice with brine, dried and concentrated. The solid residue was recrystallized from diisopropyl ether to give *N*-[1-(4-chlorophenyl)ethyl]formamide (39.6 g, 72%).

A mixture of the amide (9.18 g), CCl_4 (8.01 g), triethylamine (5.11 g) and triphenylphosphine (16.3 g) in dry CHCl_3 was stirred at 60°C for 2.5 hr and concentrated. The residue was triturated with hexane and filtered. The filtrate was distilled (bp 79–81°C at 0.55 mmHg) to give 1-(4-chlorophenyl)ethyl isocyanide (6.0 g, 72%).

To an ice-cooled solution of the isocyanide (0.83 g) in pivalaldehyde (5 ml) was added dropwise conc. H_2SO_4 (0.52 g) in water (5 ml). After stirring at room temperature for 1 hr, the mixture was diluted with ethyl acetate, washed successively with K_2CO_3 aq. and brine, dried and concentrated. The residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (3:1 then 1:1, v/v)) to give **7l** (the less polar diastereomer, 0.47 g, 35%), followed by **7m** (the more polar diastereomer, 0.45 g, 33%).

1.1.6 *N*-[1-(4-Chlorophenyl)ethyl]-2-fluoro-3,3-dimethylbutanamide 3

To a solution of diethylaminosulfur trifluoride (0.64 g) in dry CH_2Cl_2 (13 ml) was added dropwise **7** (1.08 g) in dry CH_2Cl_2 (5 ml) with stirring at -78°C , and the reaction mixture was then allowed to rise to room temperature. The stirring was continued overnight and the resultant mixture was washed twice with water, dried and concentrated. The residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (10:1, v/v)) to give crude **3** (0.06 g), which was subjected to a preparative TLC on silica gel (developer, benzene) to give **3** (0.03 g, 3%).

1.1.7 2-*tert*-Butyl-*N*-[1-(4-chlorophenyl)ethyl]-3-butanamide 4

To a solution of diisopropylamine (10.12 g) in dry THF (50 ml) was added dropwise butyllithium (1.6 M in hexane, 62.5 ml) below 0°C . The mixture was stirred at 0°C for 10 min. To the mixture was added *tert*-butyl 3,3-dimethylbutanoate (15.48 g) in dry THF (20 ml) below 0°C and the stirring was continued for 30 min. To the mixture was added trichloroacetaldehyde (13.54 g) in dry THF (20 ml) at -60°C . The mixture was stirred at -60°C for 1 hr and allowed to rise to room temperature with stirring overnight. The mixture was poured into ice-water, neutralized with conc. HCl and extracted with ether. The organic layer was washed with water, dried and distilled (bp 105–110°C at 0.4 mmHg) to give *tert*-butyl 2-*tert*-butyl-4,4,4-trichloro-3-

hydroxybutanoate (15.6 g, 49%).

To an ice-cooled solution of the hydroxy ester (9.58 g), triethylamine (4.64 g) and 4-dimethylaminopyridine (0.77 g) in dry THF (100 ml) was added acetic anhydride (3.99 g). After stirring at room temperature for 2 hr, the mixture was diluted with ether, washed successively with HCl aq., NaHCO_3 aq. and water, dried and concentrated to give *tert*-butyl 3-acetyloxy-2-*tert*-butyl-4,4,4-trichlorobutanoate (9.73 g, 90%).

To a solution of the *tert*-butyl ester (8.65 g) in acetic acid (100 ml) was added zinc powder (18.38 g) and the mixture was stirred at 60°C for 7 hr. The mixture was filtered and washed with ether. The filtrate was washed successively with 5% NaOH aq., NaHCO_3 aq. and water, dried and concentrated to give *tert*-butyl 2-*tert*-butyl-4,4-dichloro-3-butenate (5.68 g, 89%).

To a solution of the *tert*-butyl ester (3.93 g) in CH_2Cl_2 (10 ml) was added trifluoroacetic acid (5 ml) and the solution was allowed to stand at room temperature overnight. The mixture was washed twice with water, dried and concentrated to give 2-*tert*-butyl-4,4-dichloro-3-butenic acid (3.12 g). To a solution of the acid (2.7 g) in dry benzene was added dropwise thionyl chloride (2.28 g) and a drop of DMF. The mixture was heated under reflux for 3 hr and filtered. The filtrate was concentrated to give 2-*tert*-butyl-4,4-dichloro-3-butenoyl chloride (2.78 g). To an ice-cooled solution of 1-(4-chlorophenyl)ethylamine (1.55 g) and triethylamine (1.21 g) in dry THF was added the acid chloride (2.30 g). After stirring at room temperature for 2 hr, the mixture was treated with water and extracted with ethyl acetate. The organic layer was washed with water, dried and concentrated. The residue was chromatographed on silica gel (eluent, CHCl_3) to give 2-*tert*-butyl-4,4-dichloro-*N*-[1-(4-chlorophenyl)ethyl]-3-butanamide.

To a solution of diisopropylamine (0.81 g) in dry THF (10 ml) and ether (10 ml) was added dropwise butyllithium (1.6 M in hexane, 5 ml) below 0°C . The mixture was stirred at 0°C for 10 min. To the mixture was added the dichlorovinyl amide (0.70 g) in dry THF (5 ml) and ether (5 ml) at -70°C . The mixture was stirred at -70°C for 3 hr and then allowed to rise to room temperature. After stirring overnight, the mixture was quenched with 2*N* H_2SO_4 and extracted with ether. The organic layer was washed with water, dried and concentrated. The residue was subjected to a preparative TLC on silica gel (developer, hexane-ethyl acetate (5:1, v/v)), followed by trituration with hexane-ether and filtration to give **4** (0.1 g, 18%).

1.2 Synthesis of α -cyanoacetamide compounds

α -Cyanoacetamide compounds were prepared by one of the following six methods:

- the reaction of the corresponding acid chlorides with the corresponding amines in the presence of a base.
- the reaction of the corresponding amines with the

Fig. 2 Examples of α -cyanoacetamide derivatives prepared by synthetic methods a to f.

corresponding acyl imidazoles which were prepared *in situ* by the reaction of the acid with 1,1'-carbonyldiimidazole.

- c) the reaction of the corresponding acid ethyl esters with the corresponding amines at elevated temperatures.
- d) α -alkylation or α -bromination of the corresponding α -cyanoacetamide compounds in the presence of a base.
- e) the Knoevenagel-type condensation of *N*-[1-(4-chlorophenyl)ethyl]-2-cyanoethanamide with the corresponding ketones.
- f) the reduction of an acyl imidate with NaBH_4 .⁵⁾

Examples of the preparations obtained by these methods are shown in Fig. 2 and experimental details are as follows:

1.2.1 a) *N*-[1-(4-Bromophenyl)ethyl]-2-cyano-3,3-dimethylbutanamide **47**

To an ice-cooled solution of 1-(4-bromophenyl)ethan-1-amine (0.80 g) and triethylamine (0.48 g) in dry ether (10 ml) was added dropwise 2-cyano-3,3-dimethylbutanoyl chloride (0.64 g). After stirring at room temperature for 2 hr, the mixture was diluted with ether, washed with water, dried and concentrated. The residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (5:1, v/v)) to give **47** (0.94 g, 73%).

1.2.2 b) *N*-[1-(4-Chlorophenyl)ethyl]-2-cyano-3,3-dimethyl-4-pentanamide **17**

To a solution of 2-cyano-3,3-dimethyl-4-pentenoic acid

(0.76 g) in dry THF (5 ml), was added 1,1'-carbonyldiimidazole (0.97 g) in small portions. The mixture was stirred at room temperature for 1 hr. To this solution was added dropwise 1-(4-chlorophenyl)ethan-1-amine (0.78 g) and the stirring was continued at room temperature for 2 hr. The mixture was diluted with ether and washed successively with HCl aq., sat. NaHCO_3 and sat. brine, dried and concentrated. The residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (5:1, v/v)) to give **17** (0.72 g, 50%).

1.2.3 c) *N*-[1-(4-Chlorophenyl)ethyl]-2-cyano-2-phenylethanamide **21**

A mixture of ethyl 2-cyano-2-phenylethanoate (0.98 g) and 1-(4-chlorophenyl)ethan-1-amine (0.78 g) was stirred at 150–160°C for 1 hr, while removing the ethanol produced. The mixture was chromatographed on silica gel (eluent, hexane-ethyl acetate (5:1, v/v)) to give **21** (1.0 g, 67%).

1.2.4 d) *N*-[1-(4-Chlorophenyl)ethyl]-2-cyano-2-cyclopentylethanamide **19**

To an ice-cooled solution of *N*-[1-(4-chlorophenyl)ethyl]-2-cyanoethanamide (0.89 g) in dry DMF (5 ml) was added sodium hydride (60% in oil, 0.17 g) under N_2 . The mixture was stirred with ice-cooling for 0.5 hr. To this solution was added dropwise cyclopentyl bromide (0.73 g) and the stirring was continued at room temperature for 4 hr. The mixture was treated with water and extracted with ethyl acetate. The organic layer was washed twice with brine, dried and concentrated. The

residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (5:1, v/v)) to give **19** (0.58 g, 50%).

1.2.5 e) *N*-[1-(4-Chlorophenyl)ethyl]-2-cyano-3-ethyl-2-pentenamide **31**

A mixture of *N*-[1-(4-chlorophenyl)ethyl]-2-cyanoethanamide (2.22 g), 3-pentanone (5 ml), glacial acetic acid (0.2 g), benzene (10 ml) and 4-aminophenol (28 mg) was stirred at reflux for 3 hr, while removing the water produced. The mixture was filtered through Celite, washed with CHCl_3 and concentrated. The residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (5:1, v/v)) to give **31** (1.87 g, 64%).

1.2.6 f) *N*-[1-(4-Chlorophenyl)-1-methoxymethyl]-2-cyano-3,3-dimethylbutanamide **39**

To an ice-cooled solution of methyl 4-chlorobenzene-carboximate⁶⁾ (1.70 g) and triethylamine (1.24 g) in dry toluene (15 ml) was added dropwise a solution of 2-cyano-3,3-dimethylbutanoyl chloride in dry toluene (5 ml). After stirring at room temperature for 12 hr, the mixture was cooled in an ice bath, filtered and washed with toluene. The filtrate was concentrated to give methyl 4-chloro-*N*-(2-cyano-3,3-dimethylbutanoyl)-benzenecarboximate (3.09 g).

To an ice-cooled mixture of the acylimide (1.17 g) in methanol (7 ml) was added NaBH_4 (0.17 g). The mixture was stirred with ice cooling for 10 min, poured into an ice-cooled solution of 1% aq. acetic acid (50 ml) and extracted with ether. The organic layer was washed successively with NaHCO_3 aq. and brine, dried and concentrated. The residue was chromatographed on silica gel (eluent, hexane-ethyl acetate (2:1, v/v)) to give **39** (0.53 g, 45%).

1.3 Synthesis of acids and esters

The α -substituted cyanoacetic acids and their esters were prepared as described in the literature,⁷⁻¹⁰⁾ except the ester shown below.

1.3.1 Ethyl 1-cyano-2,2-dimethylcyclopropanecarboxylate

To a mixture of sodium hydride (60% in oil, 0.42 g) in dry DMF (20 ml) was added trimethylsulfoxonium iodide (2.25 g) under N_2 . The mixture was stirred at room temperature for 15 min. To the mixture was added ethyl 2-cyano-3-methylbutenoate (1.53 g) in dry DMF (5 ml). After stirring at room temperature for 1 hr, the mixture was poured into ice-water, extracted with ether, washed twice with brine, dried and concentrated. The residue was chromatographed on silica gel (eluent: hexane-ethyl acetate (5:1, v/v)) to give the desired ester (0.88 g, 53%).

1.4 Synthesis of amines

Both 1-(2-chloro-5-pyridyl)ethylamine and 1-(3-chloro-6-pyridyl)ethylamine were prepared from the corresponding *O*-methyl oxime ethers by reduction with $\text{BH}_3 \cdot \text{S}(\text{Me})_2$. All 1-(substituted-phenyl or -thienyl)ethylamines were prepared from the corresponding ketones

by the Leukart reaction¹¹⁾ as exemplified below:

1.4.1 1-(4-Chloro-2-methoxyphenyl)ethylamine

A mixture of 4-chloro-2-methoxyacetophenone (7.43 g), formamide (7.48 g) and 90% aq. formic acid (1 ml) was heated at 180–190°C. The mixture was treated with water and extracted with CHCl_3 . The organic layer was concentrated. To the residue was added conc. HCl and the mixture was stirred at 100°C (bath temperature) for 1 hr. The mixture was treated with water with ice-cooling, and extracted twice with CHCl_3 . The aqueous layer was made alkaline by adding NaOH aq. with ice-cooling, extracted twice with ether, dried and concentrated to give the desired amine (5.24 g, 70%).

1.5 Preparation of stereoisomers

Each of the four stereoisomers constituting the compound **56** was directly isolated from **56** by preparative HPLC. The conditions for the isolation were as follows: Apparatus: HITACHI L-6200; Column: SUMIPAX YMC-GEL SIL 120A (5 μm , 4 mm diameter $\times$ 25 cm length) + SUMICHIRAL OA-4700 (5 μm , 4.6 mm diameter $\times$ 25 cm length); Mobile phase: hexane/ethanol/trifluoroacetic acid = 240:10:1; Detection: UV 254 nm.

Each of the four stereoisomers was named after the order eluted in the HPLC as **56a**, **56b**, **56c** and **56d**, respectively. The absolute configuration of each of the stereoisomers was determined as follows: the first eluent **56a** was determined to be a (*R,R*)-configuration by X-ray structural analysis. Furthermore, a mixture of the third and the fourth eluent **56c** + **56d** was determined to be the (*R,S*) + (*S,R*)-configuration by X-ray analysis. From these results, the remaining second eluent **56b** was determined to be the (*S,S*)-configuration. On the other hand, the reaction of (*R*)-1-(2,4-dichlorophenyl)ethylamine (obtained below) with ethyl (*RS*)-2-cyano-3,3-dimethylbutanoate at an elevated temperature gave a product **56e** which was found to be identical with **56a** + **56d** by analytical HPLC. Therefore, **56c** and **56d** were determined to be the [(*R*)acid, (*S*)amine] and [(*S*)acid, (*R*)amine] configuration, respectively.

1.5.1 (*R*)-(+) -1-(2,4-Dichlorophenyl)ethylamine

To a mixture of water (1.2 l) and 95% ethanol (2.8 l) were added L-aspartic acid (Special Grade Reagent available from Wako Pure Chemical Industries, Ltd.; chemical purity > 99.0%; $[\alpha]_D^{25} = +24.9$ – 25.8°) and (*RS*)-1-(2,4-dichlorophenyl)ethylamine (76.5 g), and the mixture was heated at reflux with stirring for 1 hr. While stirring was continued, the solution was allowed to cool to 40°C and then cooled to 20°C in a water bath. The solution was stirred at 23°C for 30 min, and the resulting crystals were collected by filtration, washed with chilled ethanol (100 ml) and dried to give white crystals (25.6 g), which were recrystallized from 50% aq. ethanol (200 ml) to give (+)-1-(2,4-dichlorophenyl)ethylamine L-aspartate (20.4 g). These were treated with 20% aq.

NaOH (100 ml), and the liberated amine was extracted with toluene. The organic layer was dried and concentrated to give (R)-(+)-1-(2,4-dichlorophenyl)ethylamine (11.9 g); $[\alpha]_D^{25} = +44.86^\circ$ ($c = 1.114$). The optical purity was 99.9% ee by HPLC analysis under the following conditions: Apparatus: HITACHI L-6200; Column: SUMICHIRAL OA-4100 (6 mm diameter $\times$ 25 cm length); Mobile phase: hexane/ethanol/trifluoroacetic acid = 240:10:1; Detection: UV 254 nm. The absolute configuration of the amine was determined to be (R) as follows: The resolved amine was reacted with (RS)-2-cyano-3,3-dimethylbutanoyl imidazole to give a diastereomeric mixture, part of which was determined to be (R)-2-cyano-N-[(R)-1-(2,4-dichlorophenyl)ethyl]-3,3-dimethylbutanamide by X-ray analysis. Therefore, the absolute configuration of the resolved amine was determined to be (R).

2. Biological Tests

2.1 Preventive activity against rice blast

2.1.1 Method A: Preventive activity in the foliar spray test

Sandy loam soil was placed in plastic pots (5 cm in diameter, 90 ml in volume), and rice (var., Nihonbare) was sowed and cultivated into seedlings for 20 days in a greenhouse. The emulsifiable concentrate of each test compound was diluted with water to a prescribed concentration (400, 200, 50, 12.5 or 3.1 ppm; 25, 6.3, 1.6 or 0.4 ppm) and applied to the seedlings. The seedlings were air-dried and inoculated by spraying a conidial suspension of *Magnaporthe grisea*. The seedlings were cultivated in a greenhouse for 8 days under dark and highly humid conditions at 20°C in the nighttime and under light and slightly humid conditions at 24°C in the daytime to examine the control. The activity to control the disease was assessed by visual observation of the disease severity in test plants, i.e., the extent of damage to the leaves and stems, and represented in most cases by the 5 ranks of S, A, B, C and D based on the concentration giving 10% or less lesions, i.e., "S" for 3.1 ppm; "A" for 12.5 ppm; "B" for 50 ppm; "C" for 200 ppm; "D" for less active than C. In some cases the activity was represented by the following 6 ranks, i.e., "5" for 0% damage; "4" for 10%; "3" for 30%; "2" for 50%; "1" for 70%; "0" for more than 70% with no difference from the disease severity of the untreated plants.

2.1.2 Method B: Preventive activity in the soil drench test

Sandy loam soil was placed in plastic pots (5 cm in diameter, 90 ml in volume), and rice (var., Nihonbare) was sowed and cultivated into seedlings for 14 days in a greenhouse. The emulsifiable concentrate of each test compound was diluted with water and the soil was drenched with a prescribed amount (2, 1, 0.25, 0.063 mg/pot) of this aqueous solution. The seedlings were cultivated

for 7 days in a greenhouse, and then inoculated and cultivated as described for method A. The activity to control the disease was determined as described for method A, and ranked as A, B, C and D based on the dosage giving 10% or less lesions, i.e., "A" for 0.063 mg/pot; "B" for 0.25 mg/pot; "C" for 1 mg/pot; "D" for less active than C.

2.1.3 Method C: Preventive effect of the seedling box treatment

Seedling boxes for rice (30 cm $\times$ 60 cm $\times$ 3 cm) were filled with soil (Bonsol No. 2, available from Koura Sangyo Co., Ltd.) and sowed with germinated rice seeds (var. Nihonbare) at 200 g per box. Twenty days after the seeding, a granule formulation of each test compound was uniformly distributed over the surface of the seedbed in the seedling boxes. Five rice seedlings were then transplanted in a Wagner pot (1/5000 are), which had been filled with sandy loam soil and flooded with water. The rice seedlings were cultivated in a greenhouse with the water leaking from the bottom of the Wagner pot at a rate of 3 cm per day. Four or six weeks after the transplanting, these rice seedlings, together with other rice seedlings which were previously inoculated with *Magnaporthe grisea*, were placed under a plastic cover and maintained in a moistened state to infect them with the fungus. After eleven days, the rice seedlings were examined and an index of disease attack by leaf rice blast was established based on the following criteria to determine the control value using the Equations 1 and 2. The disease attack ratio in the non-treated plants was 91% and 95%, respectively, when the period from transplant to infection was four and six weeks. The criteria of disease attack were as follows: Disease attack index "0": lesion area 0%; Disease attack index 1: 1% to 5%; Disease attack index "2": 6% to 25%; Disease attack index "4": 26% to 50%; Disease attack index "8": 51% or higher. (Equation 1)

$$\begin{aligned} \text{Disease attack ratio (\%)} \\ = \{[(1 \times n_1) + (2 \times n_2) + (4 \times n_3) \\ + (8 \times n_4)] / 8 \times N\} \times 100 \end{aligned}$$

where N is the total number of leaves examined and n_1 to n_4 are the numbers of leaves having a disease attack index of 1, 2, 4 and 8, respectively.

(Equation 2)

$$\begin{aligned} \text{Control value (\%)} \\ = \frac{((\text{Dar in non-treated field}) \\ - (\text{Dar in treated field}))}{\text{Dar in non-treated field}} \times 100 \end{aligned}$$

where Dar stands for Disease attack ratio (%).

2.2 Inhibitory potency of melanization

Method D

Each of the test compounds dissolved in DMSO was added to an agar medium in a petri dish to prepare an

agar plate. On the agar plate was placed a piece of cellophane paper, which was then inoculated with the conidial suspension of *Magnaporthe grisea*. After incubation at 27°C for two days, the inhibitory potency of the fungal appressorial melanization was evaluated by observing color changes microscopically.

RESULTS AND DISCUSSION

1. Effect of the α -Cyano Group

Table 1 shows the fungicidal activity against rice blast of a variety of 2-substituted-*N*-[1-(4-chlorophenyl)ethyl]-3,3-dimethylbutanamides. These 2-substituents were selected in order to improve the hydrophilicity of the molecule. As reported earlier,¹⁾ the bromine derivative **1** showed good activity in a foliar spray test, but its activity in a soil drench test was found to be moderate. In marked contrast to **1**, the nitrile derivative **5** was found to be highly effective both in the foliar spray test and in the soil drench test, demonstrating that **5** has excellent systemic activity as well as excellent fundamental fungicidal activity. No remarkable activity was observed in other derivatives having a methylsulphenyl (**2**), a fluoro (**3**), an ethynyl (**4**), a methoxy (**6**), a hydroxy (**7**), a methylsulfinyl (**8**) and a methylsulfonyl group (**9**) as an α -substituent. It is noteworthy that the ethynyl derivative **4** shows only weak activity even in the foliar spray test, in view of the fact that the ethynyl group is well known as a structural analogue of the cyano group and that the pyrethroid insecticide empenethrin with an ethynyl group was far more active than its cyano analogue in a fundamental insecticidal test.¹²⁾ Moreover, the compact and electron-withdrawing fluorine (**3**) is far less suited to the α -substituent even in comparison with

bromine (**1**). These facts suggest that the hydrogen-bonding ability of the nitrogen atom in the cyano group together with its ability to impart hydrophilicity contributes to the remarkably high activity.

2. Effect of the α -Alkyl Group

Table 2 shows the fungicidal activity of a wide range of 2-substituted-*N*-[1-(4-chlorophenyl)ethyl]-2-cyanoacetamides. The activity was found to be greatly influenced by the α -alkyl group. The 2-ethylpropyl (**15**), 1,1-dimethyl-2-propenyl (**17**) and 1,1-dimethyl-2-propynyl (**18**) derivatives showed activity comparable to the *tert*-butyl derivative **5**. By contrast, the isopropyl (**11**), *n*-butyl (**12**), isobutyl (**13**), *sec*-butyl (**14**), neopentyl (**16**), cyclopentyl (**19**) and cyclohexyl (**20**) derivatives were less active. The methyl (**10**) and phenyl (**21**) derivatives were inactive even at 400 ppm and 1 mg/pot.

No highly active compound was found in 2,2-disubstituted-*N*-[1-(4-chlorophenyl)ethyl]-2-cyanoacetamides such as α,α -dialkyl derivatives (**22** to **24**, **26**), the α -alkyl- α -bromo derivative (**25**), cyclic derivatives (**27**–**29**) and α -alkylidene derivatives (**30**–**33**), as shown in Table 3. These results suggest the importance of the presence of α -hydrogen for activity.

3. Effect of the Amine Moiety

Table 4 shows the fungicidal activity of *N*-substituted-2-cyano-3,3-dimethylbutanamides. The high level of activity shown by the α -methyl-4-chlorobenzyl derivative **5** was almost lost on transformation of the α -methyl-4-chlorobenzyl group to alkyl (**34**, **35**), aryl (**36**) and

Table 1 2-Substituted-*N*-[1-(4-chlorophenyl)ethyl]-3,3-dimethylbutanamides and their preventive activity against rice blast.

Compound			Preventive Activity	
No.	X	mp(°C)	Foliar Spray ^{c)}	Soil Drench ^{d)}
1	Br	106-108	A	C
2	SMe	117-119	C	D
3	F	63-67	D	D
4	C≡CH	100-105	C	D
5	CN	99-103	S	A
6 ^{a)}	OMe	70-72.5	D	D
6 ^{m)}	OMe	115-118	C	D
7 ^{b)}	OH	102	C	D
7 ^{m)}	OH	159	D	D
8	S(O)Me	119-121	D	D
9	S(O) ₂ Me	140-145	C	D

^{a)} The less polar diastereomer on silica gel TLC developed by a hexane-ethyl acetate system. ^{b)} The more polar diastereomer on silica gel TLC developed by a hexane-ethyl acetate system. ^{c)} For the indexes of the activity and the experimental details, see the text (method A). ^{d)} For the indexes of the activity and the experimental details, see the text (method B).

Table 2 2-Substituted-*N*-[1-(4-chlorophenyl)ethyl]-2-cyanoacetamides and their preventive activity against rice blast.

Compound				Preventive Activity	
No.	R¹	Synthetic Method ^{a)}	mp(°C)	Foliar Spray ^{b)}	Soil Drench ^{c)}
10	Me	c	114-116	D	D
11	<i>i</i> Pr	d	89-92	C	B
12	<i>n</i> Bu	d	77-79	B	C
13	<i>i</i> Bu	d	122-125	C	C
14	<i>s</i> Bu	a	78-82	B	B
5	<i>t</i> Bu	a	99-103	S	A
15	(Et) ₂ CH	d	73-76	S	B
16	<i>t</i> BuCH ₂	b	81-83	C	B
17	(H ₂ C=CH)C(Me) ₂	b	102-105	S	A
18	(HC≡C)C(Me) ₂	b	147-149	S	A
19	<i>c</i> Pent	d	106-108	B	C
20	<i>c</i> Hex	d	149-152	C	D
21	Ph	c	134-136	D	D

^{a)} For the indexes of the synthetic method and the experimental details, see the text. ^{b)} For the indexes of the activity and the experimental details, see the text (method A). ^{c)} For the indexes of the activity and the experimental details, see the text (method B).

Table 3 2,2-Disubstituted-*N*-[1-(4-chlorophenyl)ethyl]-2-cyanoacetamides and their preventive activity against rice blast.

Compound					Preventive Activity	
No.	R ¹	R ²	Synthetic Method ^{a)}	mp(°C)	Foliar Spray ^{b)}	Soil Drench ^{c)}
5	<i>i</i> Bu	H	a	99-103	S	A
22	Me	Me	d	85-87	D	D
23	<i>i</i> Pr	Me	d	96-98	D	D
24	<i>i</i> Bu	Me	d	43-46	C	D
25	<i>i</i> Bu	Br	d	70-72	C	B
26	Et	Et	d	141-143	D	D
27	-CH ₂ CH ₂ CH ₂ CH ₂ -		d	90-92	D	D
28	-CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ -		d	114-116	D	D
29	-CH ₂ -C(Me) ₂ -		b	133-136	D	D
30	Me(Me)C=		e	74-76	D	D
31	Et(Et)C=		e	67-69	B	C
32	(CH ₂ CH ₂ CH ₂ CH ₂)C=		e	79-82	C	C
33	(CH ₂ CH ₂ CH ₂ CH ₂ CH ₂)C=		e	78-80	D	D

^{a)} For the indexes of the synthetic method and the experimental details, see the text. ^{b)} For the indexes of the activity and the experimental details, see the text (method A). ^{c)} For the indexes of the activity and the experimental details, see the text (method B).

Table 4 *N*-Substituted-2-cyano-3,3-dimethylbutanamides and their preventive activity against rice blast.

Compound				Preventive Activity	
No.	R ³	Synthetic Method ^{a)}	mp(°C)	Foliar Spray ^{b)}	Soil Drench ^{c)}
34	<i>i</i> Pr	b	112-114	D	D
35	CH(Me)- <i>i</i> Pr	b	124-127	D	D
36	C ₆ H ₅ -4-Cl	b	136-137	D	D
37	CH ₂ -C ₆ H ₄ -4-Cl	c	134-137	C	C
5	CH(Me)-C ₆ H ₄ -4-Cl	a	99-103	S	A
38	CH(Et)-C ₆ H ₄ -4-Cl	b	163-166	D	D
39	CH(OMe)-C ₆ H ₄ -4-Cl	f	91-93	D	D
40	CH ₂ CH ₂ -C ₆ H ₄ -4-Cl	b	95-97	C	D
41	CH(Me)-CH ₂ -C ₆ H ₄ -4-Cl	c	95-98	C	D
42	CH(Me)-	b	141-143	D	D
43	CH(Me)-	b	50-55	C	D
44	CH(Me)-	a	126-128	C	D

^{a)} For the indexes of the synthetic method and the experimental details, see the text. ^{b)} For the indexes of the activity and the experimental details, see the text (method A). ^{c)} For the indexes of the activity and the experimental details, see the text (method B).

aralkyl (37-44) derivatives. Weak activity was observed for 4-chlorobenzyl (37), 2-(4-chlorophenyl)ethyl (40), 2-(4-chlorophenyl)-1-methylethyl (41), 1-(2-chloro-5-pyridyl)ethyl (43) and 1-(2-chloro-5-thienyl)ethyl (44)

Table 5 *N*-[1-(Substituted phenyl)ethyl]-2-cyano-3,3-dimethylbutanamides and -2-pentanamides and their preventive activity against rice blast.

Compound				Preventive Activity	
No.	(Y) ⁿ	R ⁴	mp(°C)	Foliar Spray ^{a)}	Soil Drench ^{b)}
45	H	Me	137-139	C	D
46	4-F	Me	121-125	C	D
5	4-Cl	Me	99-103	S	A
47	4-Br	Me	102-104	S	A
48	4-CF ₃	Me	92-94	S	B
49	4-Me	Me	80-83	S	B
50	4-OMe	Me	142-145	C	C
51	4-OCHF ₂	Me	110-115	B	B
52	4-OCF ₃	Me	91-94	B	B
53	4-OCF ₂ CF ₂ H	Me	141-144	C	C
54	2-Cl	Me	161-162	D	C
55	3-Cl	Me	141-143	C	C
56	2,4-Cl ₂	Me	186-188	S	B
57	3,4-Cl ₂	Me	146-148	B	B
17	4-Cl	CH ₂ =CH	102-105	S	A
58	2-F,4-Cl	CH ₂ =CH	115-118	S	A
59	2,4-Cl ₂	CH ₂ =CH	153-155	A	B
60	2-Me,4-Cl	CH ₂ =CH	283	D	D
61	2-OMe,4-Cl	CH ₂ =CH	142-147	S	B

^{a)} For the indexes of the activity and the experimental details, see the text (method A). ^{b)} For the indexes of the activity and the experimental details, see the text (method B).

derivatives even in the foliar spray test. It is noted that even isosteric transformations as from methyl to ethyl (38), and from phenyl to pyridyl (42, 43) or thienyl (44), are detrimental to the activity.

4. Effect of Phenyl Ring Substituents

Table 5 shows the fungicidal activity of *N*-[1-(substituted-phenyl)ethyl]-2-cyano-3,3-dimethylbutanamides and *N*-[1-(substituted-phenyl)ethyl]-2-cyano-3,3-dimethyl-3-pentanamides. It seems clear from the table that the 4-substituent in the phenyl ring has an important role in the activity. Although the 4-chloro (5, 17), 4-bromo (47) and 2-fluoro-4-chloro (58) derivatives showed strong activity in both tests, they were also phytotoxic to rice plants at high concentrations. Thus, an extensive evaluation of highly active compounds such as 4-trifluoromethyl (48), 4-methyl (49), 2,4-dichloro (56), 4-chloro-2-methoxy (61) derivatives was conducted, and the 2,4-dichloro derivative 56 was selected for development based on fungicidal potency, lack of phytotoxicity and manufacturing cost.

5. Steric Structure-Activity Correlation of 56¹³⁾

Compound 56 has two stereogenic centers, one in the acid moiety and another in the amine part. To examine the steric structure/biological activity relationships of the molecule, the four possible stereoisomers were isolated using preparative HPLC with optically active columns

and the absolute configuration of each of the four isomers was determined as described in Materials and Methods (1.5 Preparation of stereoisomers).

As shown in Table 6, the stereoisomers with an (*R*)-configuration at the amine moiety (**56a**, **56d**) exhibited potent preventive activity, while those with an (*S*)-configuration (**56b**, **56c**) were inactive. The [(*S*)-acid, (*R*)-amine]-isomer (**56d**) was found to be most active, both in inhibiting fungal melanization (see the following section) and in pot tests on rice blast disease. On the other hand, it was found that racemization at the C-2 position of the acid moiety bearing the cyano group proceeds easily under basic conditions. Thus, the compound **56e** (diclocymet, S-2900) having the (*RS*)-configuration at the acid moiety and the (*R*)-configuration at the amine moiety was selected and has been developed as a new systemic blast fungicide.

6. Fungicidal Activity of Diclocymet **56e**

56e has superior preventive activity compared to the blast fungicide, fthalide (4,5,6,7-tetrachlorophthalide), as shown in Table 6. Furthermore, in Table 7 its remarkable residual preventive activity is noted in the seedling box treatment test, where **56e** shows superior preventive activity compared to the systemic blast fungicide, tricyclazole (5-methyl-1,2,4-triazolo[3,4-*b*]benzothiazole), demonstrating that both the excellent systemic activity and the residual activity of **56e** are highlighted in the seedling box treatment for rice blast control. Judging from the fact that **56e** scarcely affects fungal mycelial growth *in vitro* and has no curative activity in pot tests in spite of its excellent preventive and systemic action, its mode of action is presumed to be the inhibition of the fungal infection of rice plants.¹⁴⁾ Moreover, as seen in Table 6, it inhibits fungal melanization strongly *in vitro* without

Table 6 Stereoisomers of 2-cyano-*N*-[2,4-dichlorophenyl]-ethyl]-3,3-dimethylbutanamides and their preventive activity against rice blast and inhibitory potency for appressorial melanization of the fungus.

Compound				Preventive Activity ^{d)}				Minimum inhibitory concentration for melanization ^{e)} (ppm)
				Foliar Spray				
No.	Isomer (*,**)	mp(°C)	[α] _D ²⁵ in CH ₂ Cl ₂	25	6.3	1.6	0.4 ppm	
56a	(<i>R,R</i>)	180-186	+45.9° (c=1.17)	4	3	2	0	0.16-0.31
56b	(<i>S,S</i>)	180-182	-46.0° (c=0.98)	0	0	0	0	>20
56c	(<i>R,S</i>)	173-174	-1.4° (c=0.96)	0	0	0	0	>20
56d	(<i>S,R</i>)	168-170	+2.7° (c=0.80)	5	4	4	3	0.04-0.08
56e^{a)}	(<i>RS,R</i>)	160-161	+18.0° (c=1.01) ^{d)}	4	4	3	2	0.08-0.16
	fthalide ^{b)}	-	-	4	1	0	0	-

a) Diclocymet (S-2900). b) A commercial rice blast fungicide. c) Measured at 20°C. d) For the indexes of the activity and the experimental details, see the text (method A). e) For the experimental details, see the text (method D).

Table 7 Preventive activity of diclocymet (S-2900) against rice blast by seedling box treatment.

Compound		Preventive Activity ^{b)}					
		4 weeks ^{c)}		6 weeks ^{c)}			
No.	Common name	1.5	0.75	3	2	1.5	0.75g/box ^{d)}
56e	diclocymet	98	88	98	-	92	80
-	tricyclazole ^{a)}	-	-	-	39	-	-

a) A commercial systemic rice blast fungicide. b) For the indexes of the activity and the experimental details, see the text (method C). c) Period of time from transplant to infection with a rice blast pathogen. d) Applied amount of active ingredient.

affecting the formation of the fungal appressoria. Therefore, its main mechanism of action was suggested to be the inhibition of melanin biosynthesis in the fungal appressoria.¹⁴⁾ In fact, scytalone, an intermediate of melanin biosynthesis, was found to be accumulated in the fungal fermentation medium that had been treated with **56e**, suggesting that its target enzyme is scytalone dehydratase catalyzing the conversion of scytalone to 1,3,8-trihydroxynaphthalene.¹⁴⁾ Recently, it has been reported that a structurally related amide compound, carpropamid((1*R*,3*S*/1*S*,3*R*)-2,2-dichloro-*N*-(*R*)-1-(4-chlorophenyl)ethyl-1-ethyl-3-methylcyclopropanecarboxamide), is also effective for rice blast control with a similar mode of action.^{15,16)} In the meanwhile, **56e** has been registered in Japan and marketed as the rice blast fungicide, Delaus® by Sumitomo Chemical Co., Ltd., since April, 2000.

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要 約

α -Cyanoacetamide 誘導体の合成と殺菌活性：新規 イネいもち病防除剤ジクロシメット(S-2900)の創製

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新しいイネいもち病防除剤を見つけるため, 多くの α -シアノアセトアミド誘導体および関連化合物を合成し, イネいもち病に対する予防効果を茎葉散布試験および灌漑試験で調べた。いくつかの *N*-[1-(置換フェニル)エチル]-2-シアノ-3,3-ジメチル-ブタンアミドおよび-2-ペンテンアミドが両試験において高活性を示した。それらの中から, (*RS*)-2-シアノ-*N*-[(*R*)-1-(2,4-ジクロロフェニル)エチル]-3,3-ジメチルブチルアミド(ジクロシメット, S-2900)を浸透移行性を有する実用的なイネいもち病防除剤として選抜した。