

Syntheses and Biological Activities of α -Isocyanoacetic Acid Derivatives¹⁾

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Received April 10, 1983

A variety of α -isocyanoacetic acid and α -isocyanoacrylic acid derivatives were synthesized, and inhibitory activities of germination against rice, cucumber, and radish seeds were examined. Of these, a series of α -isocyanoacetic acid anilides showed fairly strong inhibitory activities. The structure-activity relationships are also discussed.

KEY WORDS: α -Isocyanoacetic Acids/ α -Isocyanoacrylic Acids/
Inhibitory Activity/ Germination/ Structure-Activity
Relationship/

An isonitrile is well known as a product of Hofmann carbylamine reaction and as a compound possessing the "horrid odor".²⁾

In recent years, the isonitrile (isocyano) compounds have been noted as a biologically active substance, since they have been frequently isolated from natural products such as culture of microorganisms and marine sponges, and most of them exhibit antimicrobial activities.³⁾

On the other hand, synthetic study of the isonitriles had been lack of enthusiasm over the unpleasant odor. However, since Ugi *et al.*⁴⁾ have reported an improved synthetic method of the isocyano compounds, they have been widely employed for the syntheses of various kinds of amino acids and heterocyclic compounds using the peculiar reactivities of the isocyano group.⁵⁾ In this way, the isocyano compounds have recently become attractive as a class of chemically and biologically useful compounds.

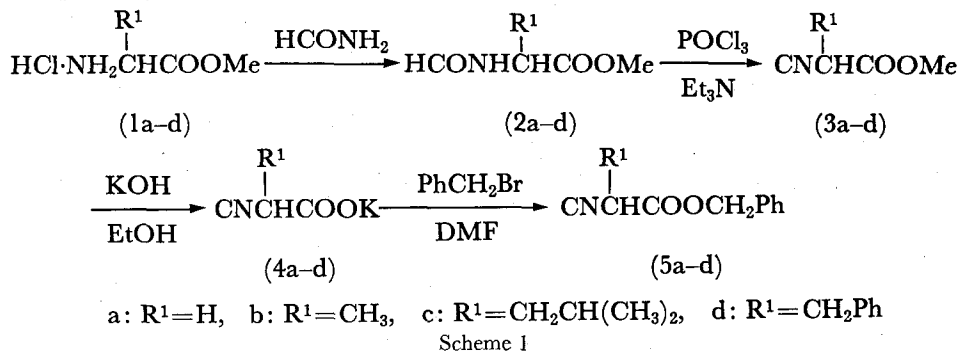
The syntheses of pharmaceuticals and agriculturals from amino acids are of continuing interest and one of the major subject in our laboratories. To this, in the course of our investigation into the pharmaceutical usefulness of the α -isocyanoacetic acid derivatives, which is one of amino acid analogs, we previously reported the synthesis and antimicrobial activities of α -isocyanoacrylic acid derivatives.⁶⁾ In connection with this, to develop an effective and safe agriculturals, systematic preparation and bioassay of the α -isocyanoacetic acid derivatives were carried out in this study. Especially, we wish to report the structure-activity relationship against the inhibitory activities of germination using some seeds.

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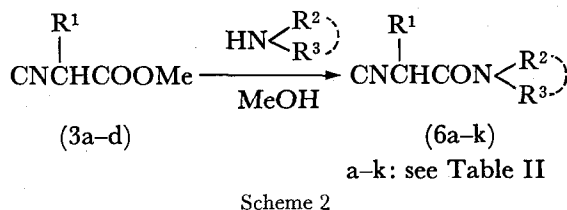
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Synthesis

Generally, lower alkyl α -isocyanoacetates seem not to become suitable agents for agriculturals because of the unpleasant odor and instability. Therefore, from the viewpoint of practical usage, we selected the benzyl ester, which is less volatile and stable compounds. A variety of benzyl isocyanoacetates (5a-e; Table I) were synthesized by the esterification of potassium α -isocyanoacetates (4a-d) derived from the corresponding α -amino acids as shown in Scheme 1.⁶⁾



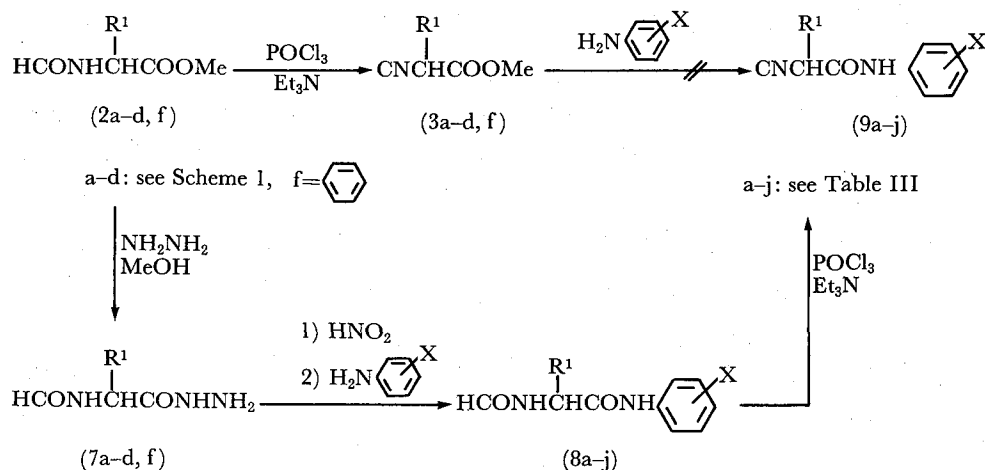
In the synthesis of α -isocyanoacetic acid amides (6a-k) possessing cyclic amines such as piperidine and aliphatic amines such as cyclohexylamine, an amidation reaction of the corresponding methyl α -isocyanoacetates (3a-d) was carried out according to the reported method.⁷⁾ These results were summarized in Table II.



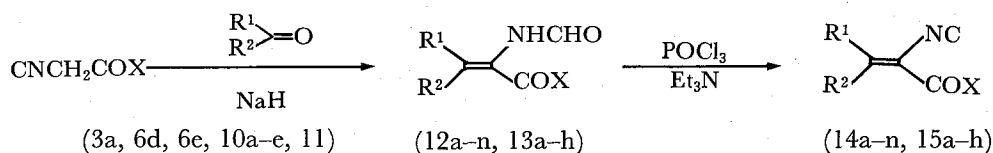
On the other hand, in the case of formation of α -isocyanoacetic acid anilides, the direct amidation using aniline analogs did not proceed under the same conditions as described above, only the starting materials being recovered. Then, we attempted the coupling reaction using an azide method; N-formylamino acid methyl esters were changed to the hydrazides (7a-d, f) and successive azidation followed by coupling with anilines gave the corresponding anilides (8a-j) in good yields. Subsequently, these compounds were converted to the isocyano anilide derivatives (9a-j; Table III) by the usual way as shown in Scheme 3.

α -Isocyanoacrylic acid esters (14a-n) and amides (15a-h) were obtained by dehydration of the corresponding α -formylamino acrylic acid derivatives, which were derived from the reaction of α -isocyanoacetic esters or amides with carbonyl compounds in the presence of sodium hydride according to the methods previously reported^{6,8)} as shown in Scheme 4. These results were summarized in Tables IV and V.

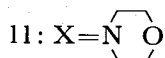
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Scheme 3



- 10a: X=OC₂H₅
 b: X=OCH₂C $\equiv$ CH
 c: X=OCH₂CH=CH₂
 d: X=CH(CH₃)₂
 e: X=C(CH₃)₃



- 12, 14a-n: see Table IV
 13, 15a-h: see Table V

Scheme 4

Biological Activities

All α -isocyanoacetic acid and α -isocyanoacrylic acid derivatives obtained in this study were examined on the inhibitory activity of the germination against rice seeds, radish seeds, and cucumber seeds according to the Koaze's method⁹⁾ as described in the experimental section. The results were indicated as follows; —: no effect for germination; $\pm$: 50–80% inhibition of growth for seedlings; +: 80–100% inhibition of growth for seedlings. The activities of a series of isocyano compounds in the concentration of 100 ppm were summarized in Table I–V.

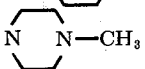
In the benzyl isocyanoacetate (5a–e), the compounds derived from glycine (5a), alanine (5b) and phenylalanine (5d) showed the inhibitory activities of germination against all seeds, whereas the isocyano compound (5e) corresponding to aspartic acid was inactive. Especially, 5b exhibited fairly strong activities against both stem and root.

Table I. Inhibitory Activities of Germination of α -Isocyanoacetic Acid Benzyl Esters (5)

Compd. No.	R	Bp (°C/mmHg)	Inhibitory Activities (100 ppm)					
			rice		cucumber		radish	
			stem	root	stem	root	stem	root
5a	H	130/0.9	+	+	+	+	±	±
5b	CH ₃	123/2	+	+	+	+	+	+
5c	CH ₂ CH(CH ₃) ₂	125/1	±	—	±	±	±	±
5d	CH ₂ Ph	syrup ^{a)}	±	±	±	±	+	+
5e	CH ₂ COOCH ₂ Ph	syrup ^{a)}	—	—	—	—	—	—

a) Purified by column chromatography

Table II. Inhibitory Activities of Germination of α -Isocyanoacetic Acid Amides (6)

Compd. No.	R	X	Mp (°C)	Inhibitory Activities (100 ppm)					
				rice		cucumber		radish	
				stem	root	stem	root	stem	root
6a	H	NHCH ₂ Ph	120–122 ^{a)}	±	±	±	±	±	±
6b	H	NH(CH ₂) ₂ CH ₃	40–41 ^{a)}	—	±	±	±	—	±
6c	H	NH- 	121–122 ^{a)}	—	±	+	±	±	±
6d	H	N- 	75–77 ^{b)}	—	±	—	±	—	—
6e	H	N- 	86–88 ^{b)}	—	—	±	±	—	±
6f	H	N- 	102–105	—	±	—	±	±	+
6g	H	N- 	71–73 ^{b)}	—	—	—	—	—	—
6h	CH ₃	NH- 	90–91	—	±	+	±	±	±
6i	CH ₂ Ph	NH- 	114–115	+	+	+	+	+	+
6j	CH ₂ Ph	NHCH ₂ Ph	124–125	—	±	+	+	±	±
6k	CH ₂ CH(CH ₃) ₂	NHCH ₂ Ph	80–82	±	+	+	+	+	+

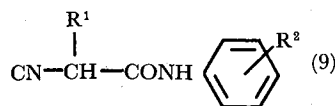
a) See Lit. 7b. b) See Lit. 7a.

Among the α -isocyanoacetic acid amide derivatives (6a–k), the compounds condensed with benzylamine or cyclohexylamine showed generally high inhibitory activities, while the amide compounds containing alkylamines such as dimethyl amine or cyclic amines such as pyrrolidine were weak. Furthermore, the amides cor-

responding to phenylalanine (6i, j) and leucine (6k) exhibited stronger activities than glycine analogs (6a, c). Among them, the compound 6i completely inhibited the germination against both stems and roots of all seeds.

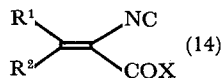
As shown in Table III, most of the isocyanoacetic acid anilides (9a-d) exhibited potent activities and it was obvious that bulkier substituents at α -position led to decrease the activity. Moreover, an introduction of substituents into aromatic ring inclined to cause decreasing the activity. This tendency was also observed notably in the case of anilides (9e, f and 9h, i) derived from alanine and phenylglycine. These results manifested that side chains (R^1) of α -position and substituents (R^2) of aniline moiety seem to have an important effect upon the inhibitory activity. Interestingly, isocyanoacetic acid anilide (9a), which is the simplest structure in this series, showed the strongest activity.

Table III. Inhibitory Activities of Germination of α -Isocyanoacetic Acid Anilides (9)



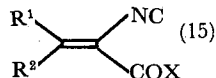
Compd. No	R^1	R^2	Mp ($^{\circ}\text{C}$)	Inhibitory Activities (100 ppm)					
				rice		cucumber		radish	
				stem	root	stem	root	stem	root
9a	H	H	160-163	+	+	+	+	+	+
9b	H	3-Cl	140-142	-	+	+	+	±	+
9c	H	4-CH ₃	163-165	-	+	+	+	-	±
9d	H	2-OCH ₃	99-101	+	+	+	+	+	+
9e	CH ₃	H	86-88	±	±	+	+	+	+
9f	CH ₃	4-Cl	114-116	-	-	-	-	-	-
9g	CH ₂ CH(CH ₃) ₂	H	88-89	-	±	±	±	±	±
9h	Ph	H	110-113	±	±	+	+	-	±
9i	Ph	4-CH ₃	129-131	-	-	±	±	-	±
9j	CH ₂ Ph	H	143-145	-	±	+	±	±	+

Next, a series of α -isocyanoacrylic acid esters (14a-n) and amides (15a-h), which possess potent antifungal activities as reported previously by us,⁶⁾ were also examined on the inhibitory activities of the germination. The results were summarized in Tables IV and V. In the case of ester derivatives, the inhibitory activities against cucumber and radish were generally effective but not against rice. Of these, (Z)-methyl α -isocyanocinnamate (14a) and *p*-mono-halogeno compounds (14c, d, g) exhibited fairly strong activities, whereas dichloro (14e) and *p*-isopropyl compounds (14f) showed no activities. The α -isocyanoacrylate compound (14i) substituted with thiophene moiety exhibited the strongest activities of these derivatives. Then, in order to investigate the effect of ester groups on the activity, the various ester derivatives (14j-n) were synthesized and tested. As a result, the effective activity was observed in the low alkyl esters such as ethyl, propargyl or allyl group, while an increase of the carbon chain length reduced the activity. On the other hand, the results of α -iso-

Table IV. Inhibitory Activities of Germination of α -Isocyanoacrylic Acid Esters (14)^{a)}

Compd. No	R ¹	R ²	X	Inhibitory Activities (100 ppm)					
				rice		cucumber		radish	
				stem	root	stem	root	stem	root
14a	Ph	H	OCH ₃	—	—	+	+	±	±
14b	2-Cl-Ph	H	OCH ₃	±	±	+	±	—	—
14c	4-Cl-Ph	H	OCH ₃	+	+	+	+	±	+
14d	4-F-Ph	H	OCH ₃	—	—	+	±	±	±
14e	2, 4-diCl-Ph	H	OCH ₃	—	—	—	—	—	—
14f	4-iPr-Ph	H	OCH ₃	—	—	—	—	—	—
14g	4-Cl-Ph	CH ₃	OCH ₃	—	—	±	±	±	—
14h	Ph	Ph	OCH ₃	—	—	±	±	+	+
14i	2-Thienyl	CH ₃	OCH ₃	+	+	+	+	+	+
14j	2-Thienyl	CH ₃	OC(CH ₃) ₃	—	—	—	—	—	—
14k	2-Thienyl	H	OC ₂ H ₅	—	—	+	+	+	+
14l	2-Thienyl	H	CH ₂ C≡CH	±	±	±	±	±	±
14m	2-Thienyl	H	CH ₂ CH=CH ₂	+	+	—	+	±	±
14n	2-Thienyl	H	OCH(CH ₃) ₂	—	—	—	—	+	+

a) See Lit. 6.

Table V. Inhibitory Activities of Germination of α -Isocyanoacrylic Acid Amides (15)

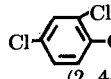
Compd. No	R ¹	R ²	X	Mp (°C)	Inhibitory Activities (100 ppm)					
					rice		cucumber		radish	
					stem	root	stem	root	stem	root
15a	Ph	H		51-52	—	—	±	±	±	±
15b	4-Cl-Ph	H		56-58 ^{a)}	—	—	—	—	—	±
15c	4-Me-Ph	H		syrup ^{b)}	—	—	±	±	±	±
15d	4-MeO-Ph	H		79-80 ^{a)}	—	±	+	+	+	+
15e	2, 4-diCl-Ph	H		syrup ^{b)}	±	±	±	±	±	±
15f	3, 4-diCl-Ph	H		76-78	±	±	±	±	±	±
15g	4-Cl-Ph	CH ₃		syrup ^{b)}	—	—	±	±	+	+
15h	Ph	Ph		131-133	—	±	±	±	—	—

a) See Lit. 11. b) Purified by column chromatography.

cyanoacrylic acid amides (15a-h) as shown in Table V were similar to those of the corresponding ester compounds.

Furthermore, in the several isocyano compounds showing strong activities from these results, we also examined on inhibitory effects of the germination with the concentration of 10 ppm. Consequently, as shown in Table VI, isocyanoacetanilide (9a) exhibited considerably strong inhibitory activities even in low concentration. These activities were almost as equal as 2, 4-dichlorophenoxyacetic acid (2, 4-D) as a standard agent.

Table VI. Inhibitory Activities of Germination at the concentration of 10 ppm

Compd. No	Inhibitory Activities					
	rice		cucumber		radish	
	stem	root	stem	root	stem	root
5b	±	+	+	+	—	—
6i	—	±	+	+	±	±
9a	±	+	+	+	—	±
9d	—	±	±	±	±	±
14i	—	—	±	±	±	±
 (2, 4-D)	—	+	+	+	+	+

EXPERIMENTAL

Melting points (which were measured by the use of a Yamato melting point apparatus) and boiling points are uncorrected. The infrared (IR) spectra were recorded on a Shimadzu IR-27G infrared spectrophotometer. The ^1H -NMR spectra were obtained using a Hitachi Perkin-Elmer R-20A high resolution NMR spectrometer with tetramethylsilane as an internal standard. Column chromatography was carried out on silica gel (Kieselgel 60, 0.063–0.200 mm, E. Merck).

The structures of all compounds were elucidated by IR and NMR spectroscopy in addition to the elementary analyses. Typical procedures were as follows.

Benzyl 2-Isocyanopropionate (5b). Methyl 2-isocyanopropionate (3b) (11.3 g, 0.1 mol), which was prepared from methyl alaninate hydrochloride (1b) *via* N-formylation followed by dehydration by the usual manner,⁸⁾ was added to the solution of KOH (7.8 g, 0.12 mol) in EtOH (120 ml) at 5°C. The reaction mixture was stirred for 1 h at room temperature and the resulting crystals (4b) were isolated by suction and washed with EtOH. A solution of potassium 2-isocyanopropionate (4b) and benzylbromide (17.1 g, 0.1 mol) in DMF (100 ml) was stirred for 3 hr at 50°C. The reaction mixture was concentrated *in vacuo* and the resulting residue was extracted with Et₂O. The extract was washed with H₂O, dried over MgSO₄, and concentrated *in vacuo*. The oily residue was distilled to give the title compound (5b) as a colorless oil (18 g, 90.5%), bp 123°C/2 mmHg. IR $\nu_{\text{max}}^{\text{film}}$ cm⁻¹: 2150 and

1755. NMR (in CDCl_3) δ : 1.57 (d, $J=7\text{Hz}$, 3H, CH_3), 4.31 (q, $J=7\text{Hz}$, 1H, CH), 5.21 (s, 2H, CH_2), and 7.34 (s, 5H, aromatic H).

2-Isocyano-4-methylpentanoic Acid Benzylamide (6k). A solution of methyl 2-isocyano-4-methylpentanoate (3c) (15.5 g, 0.1 mol) and benzylamine (16 g, 0.15 mol) in MeOH (300 ml) was stirred for 1 day at room temperature. The reaction mixture was concentrated to dryness and the resulting crystals (6k) were filtered by suction and washed with $i\text{Pr}_2\text{O}$ (12 g, 52.2%), mp $80-2^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{NaJol}} \text{cm}^{-1}$: 3250, 3100, 2150, and 1660. NMR (in CDCl_3) δ : 0.95, 1.00 (dd, $J=5\text{Hz}$, 6H, 2CH_3), 1.5–2.1 (m, 3H, CH_2+CH), 4.20 (t, $J=7\text{Hz}$, 1H, CH), 4.44 (d, $J=6\text{Hz}$, 2H, NCH_2), 6.90 (broad s, 1H, NH), and 7.30 (s, 5H, aromatic H). Anal. Calcd for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}$: C, 73.01; H, 7.88; N, 12.16%. Found: C, 72.89; H, 7.98; N, 12.11%.

Dibenzyl 2-Isocyanosuccinate (5e). To a suspension of dibenzyl N-formylaspartate (16.4 g, 48 mmol) and triethylamine 19.2 g (0.192 mol) in CH_2Cl_2 (100 ml) was added dropwise phosphoryl chloride (12.5 g, 82 mmol) at $0-5^\circ\text{C}$ over a period of 20 min. After stirring for 1 h under ice cooling, 25% aqueous Na_2CO_3 (80 ml) was added to the reaction mixture and CH_2Cl_2 layer was separated. The organic layer was washed with H_2O , dried over MgSO_4 , and then evaporated. The resulting residue was chromatographed eluting with CHCl_3 to afford 5e as brown syrup (9.8 g, 63%). IR $\nu_{\text{max}}^{\text{film}} \text{cm}^{-1}$: 2150 and 1740. NMR (in CDCl_3) δ : 3.00 (d, $J=6\text{Hz}$, 2H, CH_2), 4.68 (t, $J=6\text{Hz}$, 1H, CH), 5.15 (s, 2H, CH_2), 5.20 (s, 2H, CH_2), and 7.35 (s, 10H, aromatic H).

Isocyanoacetic Acid 2-Methoxyanilide (9d). Hydrazine hydrate (6 g, 0.12 mol) was added to the solution of methyl N-formylglycinate (2a) (11.7 g, 0.1 mol) in MeOH (50 ml) at $30-40^\circ\text{C}$ and the solution was stirred for 3 h at room temperature. The crystals (7a) obtained were isolated by suction and washed with EtOH and Et_2O (15.8 g, 89.8%), mp $129-130^\circ\text{C}$.¹⁰ To a solution of the hydrazide (7a) (4.0 g, 34 mmol) in 1N-HCl (40 ml) was added dropwise NaNO_2 (2.36 g, 34 mmol) in H_2O (20 ml) at 0°C over a period of 15 min. After stirring for 20 min at the same temperature, the mixture was neutralized with sat. aqueous NaHCO_3 and then 2-methoxyaniline (13.2 g, 0.11 mol) in THF (40 ml) was added to the mixture at 0°C over a period of 20 min. After stirring for 3 h at the same temperature, THF was evaporated *in vacuo* and the residue was extracted with AcOEt. The extract was washed with 2% HCl and sat. aqueous NaCl, dried over MgSO_4 , and then evaporated *in vacuo*. The resulting crystals were recrystallized from EtOH- H_2O to give 8d as colorless needles (4.05 g, 57.2%), mp $134-5^\circ\text{C}$. IR $\nu_{\text{max}}^{\text{NaJol}} \text{cm}^{-1}$: 3360, 3250, 1655, and 1600. NMR (in $\text{DMSO}-d_6$) δ : 3.85 (s, 3H, OCH_3), 4.01 (d, $J=7\text{Hz}$, 2H, CH_2), 6.7–7.2 (m, 3H, aromatic H), 7.9–8.1 (m, 1H, aromatic H), 8.16 (s, 1H, HCO), 8.35 (broad d, $J=7\text{Hz}$, 1H, NH), and 9.18 (broad s, 1H, NH). Anal. Calcd for $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_3$: C, 57.68; H, 5.80; N, 13.45%. Found: C, 57.56; H, 5.84; N, 13.47%.

To a suspension of 8d (10 g, 48 mmol) and triethylamine 19.2 g (0.192 mol) in CH_2Cl_2 (100 ml) was added dropwise phosphoryl chloride 12.5 g (82 mmol) at $10-15^\circ\text{C}$ over a period of 20 min. After stirring for 30 min at room temperature, 25% aqueous Na_2CO_3 (80 ml) was added to the reaction mixture under ice cooling and CH_2Cl_2 layer was separated. The aqueous layer was extracted with CH_2Cl_2 . The combined

organic layer was washed with H_2O , dried over MgSO_4 , and then evaporated *in vacuo*. The residual crystals were recrystallized from $\text{EtOH-H}_2\text{O}$ to give the title compound (9d) as brown prisms (8.5 g, 93.2%), mp 99–101°C. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 2150, 1678, 1605, and 1595. NMR (in DMSO-d_6) δ : 3.82 (s, 3H, OCH_3), 4.65 (s, 2H, CH_2), 6.7–7.2 (m, 3H, aromatic H), 7.8–8.1 (m, 1H, aromatic H), and 9.56 (broad, 1H, NH). *Anal.* Calcd for $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$: C, 63.14; H, 5.29; N, 14.72%. Found: C, 63.00; H, 5.30; N, 14.74%.

(Z)- α -Isocyano-3, 4-dichlorocinnamoylpiperidine (15f). This compound was prepared by a method similar to that described by us:⁶⁾ a mixture of isocyanoacetyl-piperidine^{7a)} (6d) (3.0 g, 0.02 mol) and 3, 4-dichlorobenzaldehyde (3.5 g, 0.02 mol) in DMF was added dropwise to a suspension of NaH (65% in oil) (0.89 g, 0.024 mol) in DMF (20 ml) at 30–35°C. After stirring for 2 h at room temperature, 10% AcOH (20 ml) was added to the mixture under cooling and the solvent was removed under reduced pressure. The residue was extracted with CHCl_3 and the extract was washed with H_2O , dried over MgSO_4 , and then concentrated *in vacuo*. Et_2O was added to the resulting residue and the crystals obtained were filtered by suction and recrystallization from EtOH gave (Z)- α -formylamino-3, 4-dichlorocinnamoylpiperidine (13f) (3.49 g, 53.5%), mp 171–172.5°C. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3270, 1675, 1650, and 1603. NMR (in CDCl_3) δ : 1.4–1.8 (m, 6H, 3CH_2), 3.3–3.8 (m, 4H, 2NCH_2), 5.71 (s, 1H, CH), 7.1–7.6 (m, 3H, aromatic H), 8.20 (s, 1H, CHO), and 9.52 (broad, 1H, NH). *Anal.* Calcd for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_2\text{Cl}_2$: C, 55.06; H, 4.93; N, 8.56; Cl, 21.67%. Found: C, 55.15; H, 5.13; N, 8.64; Cl, 21.46%.

A solution of phosphoryl chloride (2.3 g, 15 mmol) in CH_2Cl_2 (2 ml) was added dropwise to a mixture of 13f (3.26 g, 10 mmol) and triethylamine (4.9 ml, 35 mmol) in CH_2Cl_2 (10 ml) at 25–30°C over a period of 10 min with stirring. After stirring for 1 h at room temperature, 15% aqueous K_2CO_3 (15 ml) was added to the mixture under ice cooling. The organic layer was washed with H_2O , dried over MgSO_4 , and concentrated *in vacuo*. The residue was chromatographed on silica gel (50 g), eluting with CHCl_3 , to afford the title compound as a colorless crystals (2.76 g, 89.7%), mp 76–8°C. IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 2100, 1648, and 1610. NMR (in CDCl_3) δ : 1.5–1.95 (m, 6H, 3CH_2), 3.4–3.8 (m, 4H, 2NCH_2), 6.85 (s, 1H, CH), and 7.3–7.9 (m, 3H, aromatic H). *Anal.* Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{OCl}_2$: C, 58.26; H, 4.56; N, 9.06; Cl, 22.93%. Found: C, 58.19; H, 4.66; N, 9.13; Cl, 22.76%.

Germination Test. The following seeds were used in all the tests; rice plant: Nihon-masari harvested in Saitama Pref., cucumber: Shinjibae harvested in the field of Nohara Seed Co., Ltd., radish: Kotomaru Shogoin harvested in the field of Takayama Seed Co., Ltd., Germination test was carried out according to the Method reported by Koaze *et al.*⁹⁾ as follows: rice plant seeds (25 grains), radish seeds (25 grains), and cucumber seeds (7 grains) were put on a piece of filter paper placed in each petri dish (diameter of dish is 9 cm). Then, each test compound (2.0 mg) was dissolved or suspended in acetone (0.3 ml), and distilled water (19.7 ml) was added to the mixture to make up the concentration of 100 ppm. A 10 ppm solution was made by 10 times dilution with distilled water. The resulting solution (18–20 ml) was poured into each dish, which was place in an incubation. After the seeds were grown for

88 h at 27°C, the length of stems and seminal roots were measured. The activity for the germination test was determined by the comparison of length of stems (coleoptiles) and seminal roots between non-treated dish with chemicals and the treated one.

ACKNOWLEDGMENT

We wish to express our thanks to Dr. I. Chibata, Director, of Research and Development Headquarters, Tanabe Seiyaku Co., Ltd., to Dr. M. Miyoshi, former Vice Director, of the Research Laboratory of Applied Biochemistry, and to Dr. M. Kawanishi, Director, and Dr. N. Ito, Manager, of the Microbiological Research Laboratory, for their encouragement during this study.

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