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Original Article

Stereochemistry of Fungicidal *N*-Phenylformamidoximes*

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It is possible to draw four isomeric structures, *i.e.* *E*/*Z* *N*-phenylimine forms and *E*/*Z* oxime forms, of the formamidine group of fungicidal *N*-phenylformamidoximes. In this study the structure of *N*-(3,5-dichloro-4-propinyloxyphenyl)-*N'*-methoxyformamidine (DCPF), a representative compound of *N*-phenylformamidoximes, was investigated by means of NMR techniques. DCPF was stabilized in the *Z* oxime form by intramolecular hydrogen bonding in $CDCl_3$, while an isomerization to the *E* oxime form was observed in DMSO-d_6. Furthermore, HPLC analysis gave two separate peaks suggesting *E* and *Z* isomers. The active structure of *N*-phenylformamidoximes was suggested to be one of the *E* oxime forms shown in our previous paper. This active *E*-configuration seemed to emerge through *E*/*Z* isomerization involving the N-H dissociation caused by interaction with polar components in the biological system.

INTRODUCTION

N-Phenylformamidoximes have fungicidal properties similar to *N*-phenylcarbamates. In our preceding papers,[1,2] an active conformation of *N*-phenylformamidoximes was proposed by studying their electrostatic and steric similarity to *N*-phenylcarbamates, and the structural features of *N*-phenylformamidoximes to govern the antifungal activity were discussed by QSAR analysis. In the structure-activity studies, the structural configuration of the formamidoxime moiety was considered to be important to understand the bioisosterism between *N*-phenylformamidoximes and *N*-phenylcarbamates. In order to clarify the stereochemical behavior of fungicidal *N*-phenylformamidoximes, we have studied the configuration of the amidoxime structure by means of NMR techniques. Configuration analyses of amidoxime structures have been reported on alkylcarboxamidoximes, benzamidoximes and heterocyclic carboxamidoximes by means of NMR techniques or X-ray analysis,[3-8] but stereochemistry of *N*-phenylformamidoximes has not. In this report, configuration of a formamidoxime part of *N*-(3,5-dichloro-4-propinyloxyphenyl)-*N'*-methoxyformamidine (DCPF), a representative compound of *N*-phenylformamidoximes, is discussed from the aspects of NMR spectra.

MATERIALS AND METHODS

1. Chemicals

*1.1 Synthesis of N-(3,5-dichloro-4-propinyloxyphenyl)-N'-methoxyformamidine (DCPF; **1**)*

DCPF was prepared by reacting methoxyamine with ethyl *N*-(3,5-dichloro-4-propinyloxyphenyl)formimidate, which had been synthesized by reacting 3,5-dichloro-4-propinyloxyaniline with triethyl orthoformate. The synthetic method has been described in detail in the patent of *N*-phenylformamidoximes.[9]

*1.2 Synthesis of N-(3,5-dichloro-4-propinyloxyphenyl)-N'-methoxy-N-methylformamidine (**2**)*

DCPF (8.0 g, 29 mmol) was added in small portions to 60% oil-dispersed sodium hydride (1.2 g, 30 mmol) in DMF (30 ml) at 0–5°C. After stirring at 5–10°C for 30 min, methyl-

* Studies on Fungicidal *N*-Phenylformamidoximes (Part 3). For Part 1 and 2, see Refs. 1) and 2).

iodide (5 g, 35 mmol) in DMF (10 ml) was dropped to the solution at 5–10°C. After stirring at room temperature for 1 hr, the solution was poured into ice water (150 ml), extracted with ethyl acetate and dried over anhyd. magnesium sulfate. Evaporation of the organic solvent gave a crude oil, which was purified by column chromatography to yield a described product (1.2 g, Y 13.9%; mp 82–85°C). ^{1}H NMR δ $CDCl_3$ ppm: 2.54 (1H, t, J=2.4 Hz, HC≡C), 3.36 (3H, s, NCH_3), 3.82 (3H, s, OCH_3), 4.73 (2H, d, J=2.8 Hz, OCH_2), 6.88 (2H, s, aromatic), 8.20 (1H, s, CH=N).

Along with compound **2**, *N*-(3,5-dichloro-4-propinyloxyphenyl)-*N*-methyl-*N*-cyanoaniline, which may be a degradate of **2**, was isolated by the column chromatography.

1.3 Synthesis of N-(3,5-dichloro-4-propinyloxyphenyl)-N′-methoxy-N′-methylformamidine (***3***)

N-Methyl-*O*-methylhydroxylamine hydrochloride (1.6 g, 16 mmol) and sodium carbonate (1.8 g, 17 mmol) were mixed in methanol (30 ml) at room temperature for 1 hr, and ethyl *N*-(3,5-dichloro-4-propinyloxy)-formimidate (3.8 g, 14 mmol) in methanol (20 ml) was added in one portion at room temperature. After stirring at room temperature for 1 hr, evaporation of the methanol gave a crude oil, which was extracted with ethyl acetate and water, and the organic layer was dried over anhyd. magnesium sulfate. The organic solvent was evaporated and the residual crystal was recrystallized from *n*-hexane to yield a described product (3.6 g, Y 90.2%; mp 63–65°C). ^{1}H NMR δ $CDCl_3$ ppm: 2.53 (1H, t, J=2.4 Hz, HC≡C), 3.21 (3H, s, NCH_3), 3.77 (3H, s, OCH_3), 4.75 (2H, d, OCH_2), 7.00 (2H, s, aromatic), 7.87 (1H, s, N=CH).

2. Nuclear Magnetic Resonance

NMR spectra were measured on a JEOL GSX-400 spectrometer. The referencial standard for measuring resonance shifts was tetramethylsilane as an internal standard in ^{1}H NMR and nitromethane as an external standard in ^{15}N NMR.

RESULTS AND DISCUSSION

The formamidine group of *N*-phenylformamidoximes has four possible forms, *i.e.* *E*/*Z* oxime forms (**A**, **B**) and *E*/*Z* *N*-phenylimine forms (**C**, **D**) (Fig. 1). *N*-(3,5-dichloro-4-propinyloxyphenyl)-*N*′-methoxyformamidine (DCPF, **1**) was chosen as a representative compound of *N*-phenylformamidoximes, and its configuration was analyzed mainly by means of NMR techniques.

Figure 2 shows the ^{1}H NMR of DCPF in $CDCl_3$ at room temperature. Each proton was assigned to a single chemical shift. When the temperature was lowered to −50°C, proton resonances of H_2O, H-2 (–CH=N–) and H-3 (NH) showed downfield shifts, but each proton still resonated in one set of signals, which indicated that the molecule was in one of the isomers or in the mixture of fast exchanging isomers.

Whether it had an oxime form or an *N*-phenylimine form was determined by comparing ^{15}N NMR spectroscopies of DCPF (**1**), *N*-(3,5-dichloro-4-propinyloxyphenyl)-*N*′-methoxy-*N*-methylformamidine (**2**, oxime form) and *N*-(3,5-dichloro-4-propinyloxyphenyl)-*N*′-methoxy-*N*′-methylformamidine (**3**, *N*-phenylimine form) in $CDCl_3$ (Table 1). In each chemical shifts of nitrogens, DCPF was similar to compound **2** and remarkably different from compound **3**. Therefore, the structure of DCPF was determined to be an oxime form, and fast exchange between an oxime form and an *N*-phenylimine form was

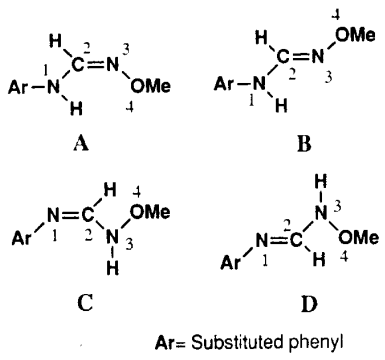

Fig. 1 Configuration of *N*-phenylformamidoximes.

A: *Z* oxime, **B**: *E* oxime, **C**: *Z* *N*-phenylimine, **D**: *E* *N*-phenylimine.

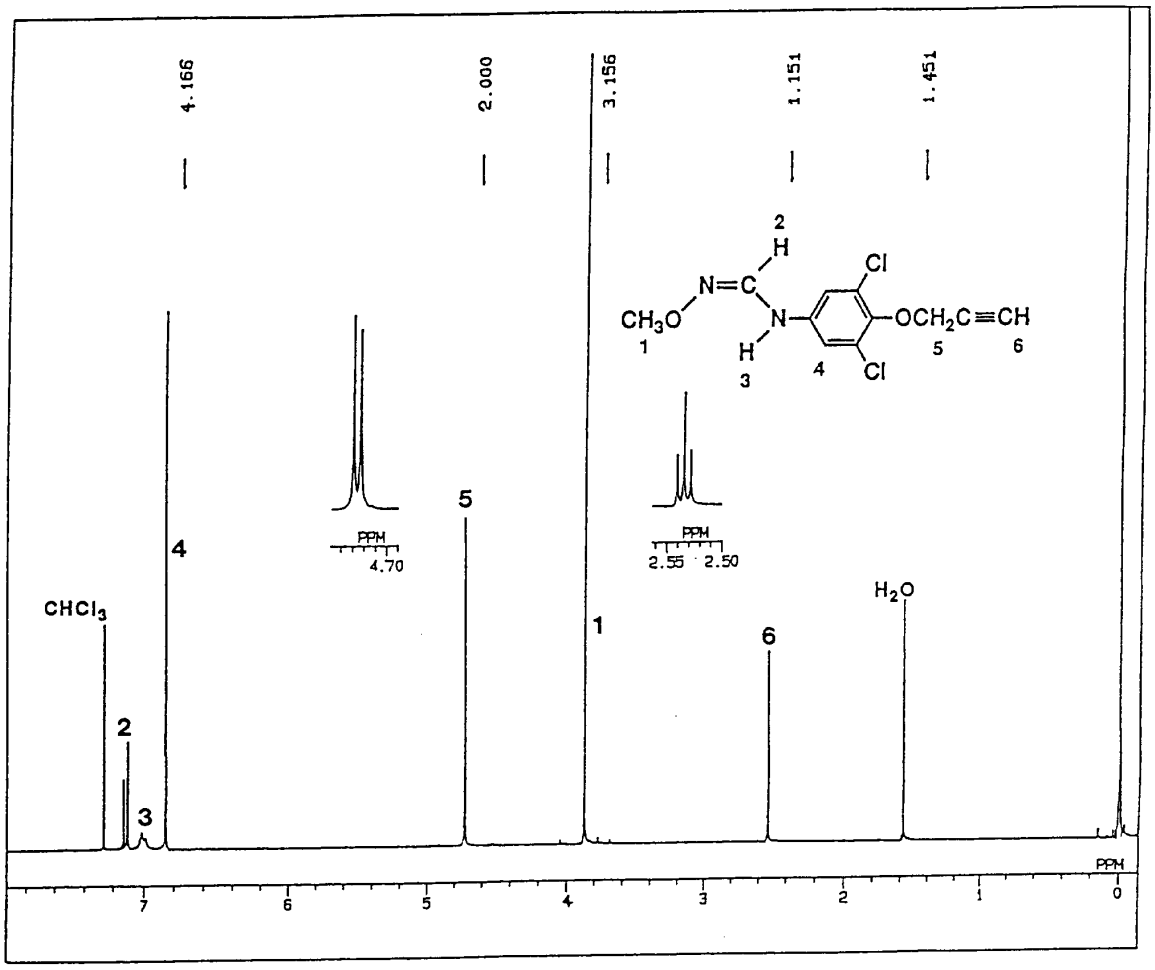

Fig. 2 ^{1}H NMR spectrum of DCPF in $CDCl_3$.

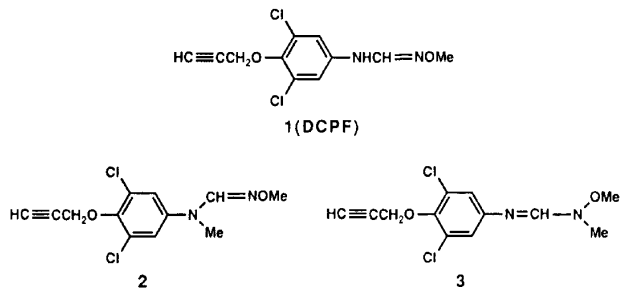

Fig. 3 Structures of compounds **1**, **2** and **3**.

considered to be impossible. If a fast exchange had occurred in $CDCl_3$, the chemical shifts of two nitrogens must have indicated the average value of the two forms.

Figure 4 is the ^{1}H NMR of DCPF in DMSO-d_6 at room temperature. The resonance of each proton was apparently one set, but a minute doublet peak was observed at about 8.2 ppm which was assigned to H-2. When the sensitivity of the NMR was raised, two pairs of minute doublet peaks assigned to H-2 and H-3 became distinct (Fig. 5). The intensity of minute doublet peaks was significantly increased when the temperature was raised to 130°C and returned to the original level when it was lowered to room temperature (Fig. 5). This suggested that *E*/*Z* oxime isomers of DCPF were present in the DMSO-d_6 solution and that the ratio of the isomers had changed by temperature.

E/*Z* configuration was also studied by N~H coupling constant ($^2J_{NH}$) of the oxime nitrogen and the formoxime proton of DCPF in $CDCl_3$ and DMSO-d_6. It is generally known that $^2J_{NH}$ are positive small values (0–3 Hz) in the *E*-isomer and negative moderate values (−10–−20 Hz) in the *Z*-isomer.[10,11] The measured coupling constants shown in Table 1 indicate that the predominant structure of DCPF is in a *Z*-form both in $CDCl_3$ and DMSO-d_6.

The downfield shift of NH proton at lower

Table 1 Chemical shift and coupling constant of compounds **1**, **2** and **3** in ^{15}N NMR.

Compounds	Chemical shift (ppm) and coupling constant [J (Hz)]			
	in $CDCl_3$		in DMSO-d_6	
	1N[a]	3N[a]	1N[a]	3N[a]
1 (DCPF)	−282.4 [−92.8]	−66.8 [−17.6]	−279.5 [−92.6]	−64.7 [−15.6]
2 (Oxime)	−293.1 [0]	−52.6 [0]	—	—
3 (Imine)	−146.1 [0]	−204.5 [0]	—	—

[a] Numbering of nitrogen is indicated in Fig. 1.

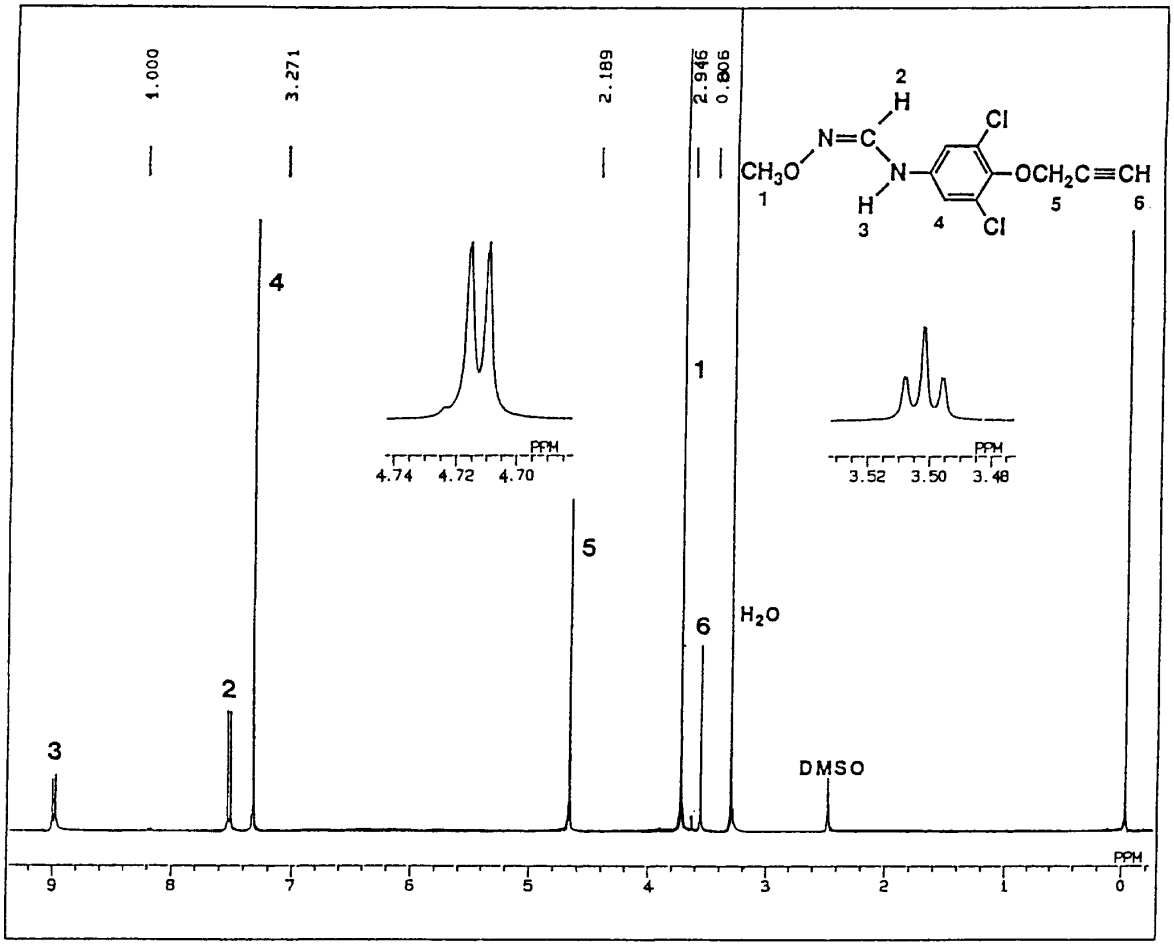

Fig. 4 ^1H NMR spectrum of DCPF in DMSO-d_6.

temperatures in $CDCl_3$, mentioned above, suggests its hydrogen bonding character. This hydrogen bond was ascertained to be an intramolecular one, because the chemical shift did not depend on the concentrations of the compound. The exchange of NH proton by deuterium (D_2O) was examined at room temperature. It took about 24 hr in $CDCl_3$ and less than 1 min in DMSO-d_6. This means that strong intramolecular hydrogen bonding occurs in $CDCl_3$ but not in DMSO-d_6. The NH proton may interact with DMSO oxygen in the DMSO-d_6 solution.

E/*Z* isomers observed on ^1H NMR in DMSO-d_6 solution were also detected in HPLC analysis. Two separate peaks were observed on the Zorbax-Sil column eluted with *n*-hexane–dichloromethane–methanol (300: 100: 1, v/v),

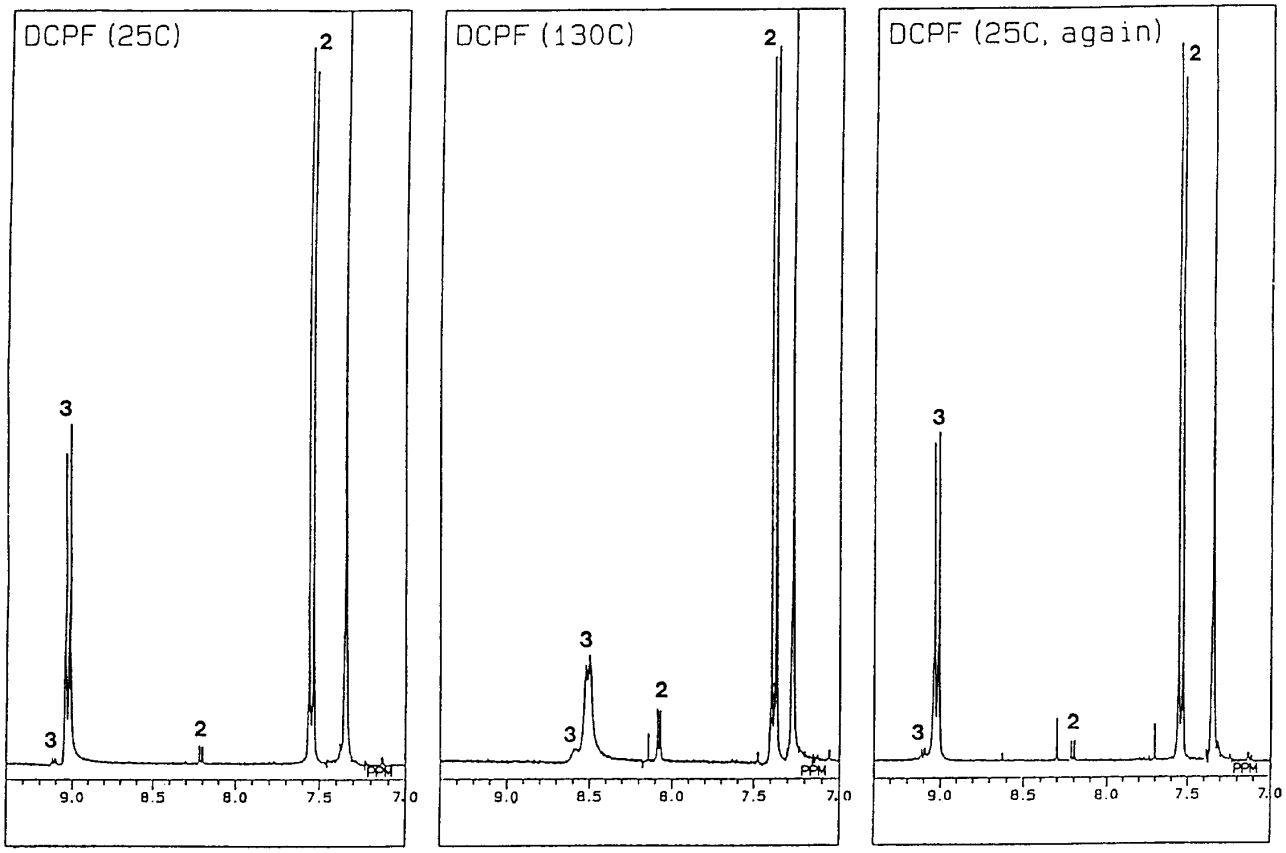

Fig. 5 ^{1}H NMR spectrum of DCPF in DMSO-d_6 at 25°C and 130°C (high sensitivity).

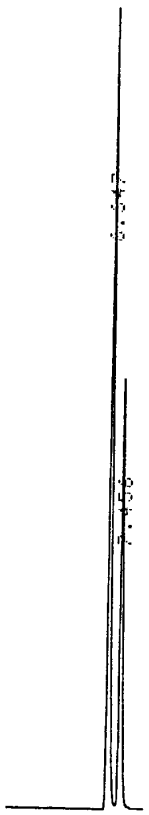

Fig. 6 HPLC chromatogram of DCPF.

and each isolated fraction also gave two peaks at the same retention time as in the initial analysis (Fig. 6). Furthermore, each fraction gave the same mass spectra.

Considering the experimental aspects shown above, the structural features of DCPF are summarized as follows:

(1) DCPF is in an oxime form. The NMR spectroscopies showed no tautomeric *N*-phenylimine form.

(2) The oxime is mainly in the *Z*-isomer, which is stabilized by intramolecular hydrogen bonding in $CDCl_3$.

(3) DCPF is in the mixture of *E* and *Z* isomers ($E<Z$) in a polar solvent such as DMSO-d_6.

(4) HPLC analysis gives two separate peaks of *E* and *Z* isomers, but they rapidly change to the other in the eluted solution.

These results demonstrate the existence of *E* and *Z* oxime isomers of DCPF, and the *Z*-configuration seems to be more stable in $CDCl_3$ because intramolecular hydrogen bonding significantly contributes to the stabiliza-

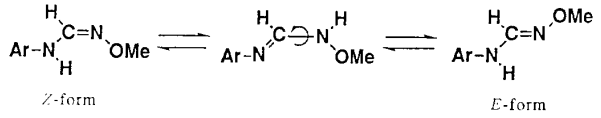

Fig. 7 *E*/*Z* isomerization of *N*-phenylformamidoximes.

tion of the *Z*-isomer. In DMSO-d_6, however, the *E*-configuration was detected as a minor component, suggesting the possibility of *E*/*Z* isomerization, which is also supported by HPLC analysis. The process of the isomerization is considered to involve the dissociation of NH proton (Fig. 7). DMSO plays a role of hydrogen-bonding acceptor to cleave the intramolecular hydrogen bond. Johnson *et al.* have reported that benzamidoximes are also stabilized in the *Z*-configuration form by intramolecular hydrogen bonding, but that the content of the *E*-configuration increases when the amide nitrogen is substituted by a bulky group or two substituents because of the cleavage of the intramolecular hydrogen bonding.[3] This suggests that effects to cleave the intramolecular hydrogen bonding in the *Z*-isomer, *e.g.* steric hindrance in benzamidoximes or intermolecular hydrogen bonding with a polar solvent, are important for isomerization to *E*-isomer. Although the configuration of *N*-phenylformamidoximes in the biological system is not clear, both *E* and *Z* oximes may exist because N-H dissociation may be promoted by intermolecular hydrogen bonding with polar components in the biological system. In our previous paper,[1] we proposed that the active structure of *N*-phenylformamidoximes is an *E* oxime structure based on the structural similarity to *N*-phenylcarbamates. We speculate that the active *E*-configuration emerges through *E*/*Z* isomerization involving the N-H dissociation caused by interaction with polar groups at the target site.

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要　　約

殺菌活性を有する *N*-フェニルホルムアミドキシム類の立体構造*

早川公一，江口禎之，中山　章

ベンズイミダゾール系殺菌剤に対して負相関交差耐性を示す *N*-フェニルホルムアミドキシム系殺菌剤のホルムアミジン部分の構造を ^{1}H NMR および ^{15}N NMR を用いて解析した．その結果非極性溶媒である $CDCl_3$ 中では強い分子内水素結合により *Z*-オキシム型が安定に存在することがわかった．一方，極性溶媒である DMSO-d_6 中では一部 *E*-オキシム型も存在し，温度を上げるとこの *E* 体の増加がみられた．さらにシリカ系カラムを用いて *n*Hx-CH_2Cl_2-MeOH 系溶媒で溶出した HPLC では約 6:4 に分離するピークを与え，これらは *Z*-オキシムと *E*-オキシムのピークであると考えられた．われわれは先に *N*-フェニルカーバメート系殺菌剤との構造比較からホルムアミジン部分の活性構造は *E*-オキシム型であると推定したが，極性の高い生体系においては，安定構造である *Z* 体から *E* 体への異性化が容易となり，活性化するものと考えられた．

* 殺菌活性を有する *N*-Phenylformamidoximes の研究 (第3報)