Preventive Activity of *N*-Allylamino Acids against Fusarium Diseases and Their Mode of Action*

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Thirty-two *N*-allylamino acids were prepared, and their preventive activity against Fusarium diseases was determined. Esters of *N*-allyl-glycine and -sarcosine showed a strong effect in preventing yellows of the Japanese radish caused by *Fusarium oxysporum* f. sp. *raphani*. These compounds were also effective against Fusarium wilt of tomato by foliage treatment. The preventive activity of *N*-allylsarcosinates varied insignificantly with the variation of the alcohol moiety of the esters. Although *n*-dodecyl *N*-allylsarcosinate was shown to effectively control Fusarium diseases, it did not affect the growth of *Fusarium* on an agar medium. The preventive effect was dependent on the application time. Enhancement of the peroxidase activity and the accumulation of total phenols were seen in the plants treated with these chemicals.

The strong preventive activity of *N*-allylaminoacetonitrile and *N*-allyl-*N*-methylaminoacetonitrile against Fusarium diseases was reported previously.[1] We also found that *N*-allylglycine is highly active.[2] Speziale and Jaworsky[3] described the systemic protecting activity of esters of *N*-substituted glycine and alanine against Fusarium wilt of tomato. In this paper, we discuss the relationship between chemical structure and preventive activity of *N*-allylamino acid derivatives against Fusarium diseases and their biological effects.

MATERIALS AND METHODS

Synthesis of compounds. The structures of all compounds were confirmed by IR and NMR spectroscopy including the elementary analyses. Typical procedures were as below:

1) *N-Allylglycine Esters.* The compounds shown in Table I were prepared according to the method of Speziale and Jaworsky.[3]

2) *Ethyl N-allylsarcosinate* (12). To a mixture of ethyl sarcosinate (11.7 g), triethylamine (12.1 g) and benzene (100 ml), was added allyl bromide (13.3 g) with stirring. The mixture was then heated under reflux for 4 hr. The reaction mixture was then washed with water. After drying with sodium sulfate, the solvent was removed and the residue was distilled under reduced pressure to give 11.5 g of a colorless oil (bp 116～118°C/110 mmHg, n_D^{27} 1.4308). *Anal.* Found: C, 60.95; H, 9.38; N, 8.76. Calcd. for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91.

By similar methods, the methyl, propyl and butyl derivatives were prepared (see Table II).

3) *n-Dodecyl N-allylsarcosinate* (30). A mixture of ethyl *N*-allylsarcosinate (15.7 g), sodium (0.2 g) and *n*-dodecylalcohol (50 g) was heated and the generated ethanol distilled off (final temperature, 140°C). The reaction mixture was then washed with water, and distilled under reduced pressure to produce 15.7 g of a colorless oil (bp 132～134°C/0.2 mmHg, $n_D^{28.5}$ 1.4492). *Anal.* Found: C, 72.51; H, 12.03; N, 4.75. Calcd. for $C_{18}H_{35}NO_2$: C, 72.68; H, 11.86; N, 4.71.

Similarly, the other alkyl esters were prepared (see Table II).

4) *^{14}C-Labeled n-dodecyl N-allylsarcosinate.* ^{14}C-Labeled *N*-allyl-*N*-methylaminoacetonitrile was prepared from K^{14}CN by the method previously reported.[1] ^{14}C-Labeled ethyl *N*-allylsarcosinate was derived[4] from the aminonitrile by ethanolysis and hydrolysis.[5] ^{14}C-Labeled *n*-dodecyl *N*-allylsarcosinate (specific activity: 2.43 mCi/mmol) was synthesized by a method similar to that described above.

5) *N-Allylsarcosine* (10). *N*-Allylsarcosine, mp 99～101°C (uncorrected), was prepared from ethyl *N*-allylsarcosinate by hydrolysis with a 10% solution of potassium hydroxide. *Anal.* Found: C, 55.65;

*Studies on Anti-Fusarium Disease Activity of Aminonitrile Derivatives. Part III. See ref. 2.

H, 8.81; N, 11.03. Calcd. for $C_6H_{11}NO_2$: C, 55.80; H, 8.58; N, 10.84.

Biological tests.

1) *The pot test for the preventive activity against Fusarium diseases.* Preventive activities against yellows of the Japanese radish (*Fusarium oxysporum* f. sp. *raphani*), Fusarium wilt of cucumber (*F. oxysporum* f. sp. *cucumerinum*) and Fusarium wilt of tomato (*F. oxysporum* f. sp. *lycopersici*) were determined by the methods described prevously.[1)]

2) *The bed test for the preventive activity against Fusarium wilt of tomato.* The preventive activity of *n*-dodecyl *N*-allylsarcosinate, *N*-allylaminoacetonitrile hydrochloride and benomyl was determined. Tomatoes (cv.; Beiju) were planted in plastic pots (6.5 cm in diameter, 150 ml in volume) and grown in a greenhouse. At the fourth true leaf stage, the tomato seedlings were transplanted into soil which had previously been inoculated with *F. oxysporum* f. sp. *lycopersici* J_3 by mixing with infected oat grains. *n*-Dodecyl *N*-allylsarcosinate was made up as a 50% (by weight for this and the following components) emulsifiable concentrate with 10% Sorpol 1200 (emulsifier, Toho Chemical Co.), 20% *N,N*-dimethylformamide and 20% xylene. Benomyl (du Pont) was a 50% wettable powder formulation. The formulated componds were diluted with water to the desired concentrations. A solution was applied to the infected soil 5 times. One week before transplanting, the amount was 50 ml per plant, but at the time of transplanting and 2, 4, and 6 weeks after transplanting, the amount was 300 ml per plant. The plants were grown at 20°C during the day-time and 15°C during the night in a greenhouse. Assessment of disease severity was made 8 weeks after transplanting. The plants were cut off, at the bottom of the stems, and discoloration of xylem tissues was rated. The preventive value was expressed as the inhibition percentage of the vascular discoloration.

The preventive value against *F. oxysporum* f. sp. *lycopersici* J_1 was similarly determined.

3) *Relationship between preventive activity and application time.* Tomato plants (cv.; Beiju, 12 days old) were transplanted into plastic pots (8.0 cm in diameter, 270 ml in volume) and grown in a greenhouse. Twenty milliliters of an emulsion containing 500 ppm of *n*-dodecyl *N*-allylsarcosinate was administered to each pot at an appropriate time before the inoculation. One week after the transplantation, the roots of the plants were washed and the inoculation was made by dipping them in a conidial suspension (1×10^6 cells/ml) of *F. oxysporum* f. sp. *lycopersici*. The infected plants were then transplanted into larger pots (11 cm in diameter, 500 ml in volume). The preventive value was determined 11 days after the inoculation in the same manner as described above.

4) *Antifungal activity.* Ten milliliters of potato-sucrose-agar medium containing *n*-dodecyl *N*-allylsarcosinate was kept at 45~55°C and then poured into a petri dish (90 mm in diameter). After cooling, a 5 mm mycelial disc was placed on the agar plate and cultured at 26°C. After 4 days, the diameter of the mycelial colony was measured and the inhibition percentage of mycelial growth was calculated.

5) *Peroxidase activity assay and total phenol measurement.* The peroxidase activity was determined according to the method of Retig.[6)] The protein content was measured according to the method of Lowry *et al.*[7)] The peroxidase activity relative to the unit protein content was then calculated. Total phenol was determined according to the method of Retig and Chet.[8)]

RESULTS AND DISCUSSION

Preventive activity of N-allylamino acids and esters against Fusarium diseases

As shown in Table I, *N*-allylglycine(1) and its esters (2~4) display potent preventive activity against yellows of the Japanese radish. The methyl esters of *N*-allyl-alanine(5), -valine(6), -leucine(7), -methionine(8) and -phenylalanine(9) show lower activity. *N*-Allylsarcosinates (shown in Table II) possess preventive activity against Fusarium wilts of cucumber and tomato by soil treatment as well as against yellows of the Japanese radish. The preventive activity does not vary significantly with variation of the alcohol moiety of the esters.

Twenty natural amino acids includng glycine, sarcosine, alanine, valine, leucine, methionine and phenylalanine were found to be inactive in controlling yellows of the Japanese radish. Dittmer *et al.* reported[9)] that α-allylglycine (Fig. 1) inhibits the growth of

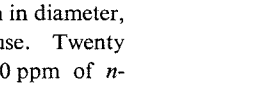


FIG. 1. α-allylglycine

Escherichia coli. However, it does not show inhibition of yellows of the Japanese radish. Only the *N*-allyl derivatives of glycine and sarcosine are active. Therefore, the structural requirements for the preventive activity seem to be highly specific.

We reported previously[1)] that *N*-allylaminoacetonitrile hydrochloride shows systemic movement in plants and possesses preventive

TABLE I. PREVENTIVE ACTIVITY OF *N*-ALLYLAMINO ACID ESTERS AGAINST YELLOWS OF THE JAPANESE RADISH

$$CH_2{=}CH{-}CH_2{-}NH{-}\underset{\displaystyle R_1}{\underset{|}{C}}H{-}COOR_2$$

No	R_1[a]	R_2[a]	mp (°C)[b] or n_D (°C)	Preventive value (%) 500 ppm
1	H	H[c]	161–162.5	100
2	H	Me	1.4408 (19.5)	92
3	H	Et[d]	1.4357 (24)	100
4	H	*n*-Dodec[d]	1.4508 (23)	100
5	Me	Me	1.4410 (19)	3
6	*i*-Pr	Me	1.4340 (22)	23
7	*i*-Bu	Me	1.4362 (20.5)	25
8	$MeSCH_2CH_2$	Me	1.4852 (20.5)	0
9	Benzyl	Me	1.5115 (20.5)	57

[a] *n* and *i* denote normal and iso.
[b] Uncorrected.
[c] Ref. 2.
[d] Ref. 3.

TABLE. II. PREVENTIVE ACTIVITIES OF *N*-ALLYLSARCOSINATES AGAINST YELLOWS OF THE JAPANESE RADISH AND FUSARIUM WILTS OF CUCUMBER AND TOMATO BY SOIL TREATMENT

$$CH_2{=}CH{-}CH_2{-}\underset{\displaystyle CH_3}{\underset{|}{N}}{-}CH_2{-}COOR$$

No	R[a]	mp (°C)[b] or n_D (°C)	Preventive value (%)		
			Japanese radish 500 ppm	Cucumber 500 ppm	Tomato 500 ppm
10	H	99–101	100	67	91
11	Me	1.4340 (26)	86	45	86
12	Et	1.4308 (27)	78	37	—
13	*n*-Pr	1.4348 (24)	81	61	69
14	*i*-Pr	1.4295 (26)	90	45	—
15	*n*-Bu	1.4366 (24.5)	66	24	—
16	*s*-Bu	1.4330 (24)	89	39	94
17	*n*-Pent	1.4382 (24.5)	52	58	—
18	*i*-Pent	1.4371 (24)	96	51	51
19	2-Me-Bu	1.4338 (24)	93	39	60
20	*n*-Hex	1 4402 (24.5)	73	45	—
21	2,3-Me_2-Bu	1.4320 (24)	92	27	89
22	*c*-Hex	1.4631 (24.5)	74	36	—
23	*n*-Hep	1.4405 (24.5)	87	21	—
24	*n*-Oct	1.4432 (24.5)	87	48	—
25	2-Me-Hep	1.4415 (24)	90	15	77
26	1,1,3,3-Me_4-Bu	1.4422 (24)	100	76	100
27	*n*-Non	1.4452 (24)	92	45	—
28	*n*-Dec	1.4486 (28.5)	100	27	—
29	*n*-Undec	1.4488 (25)	92	56	—
30	*n*-Dodec	1.4492 (28.5)	100	51	100
31	*n*-Tetradec	1.4524 (25)	79	45	—
32	*n*-Octadec	1.4542 (25)	55	33	—

[a] *n*, *i*, *s*, and *c* denote normal, iso, secondary and cyclo.
[b] Uncorrected.

TABLE III. PREVENTIVE ACTIVITY OF *N*-ALLYLAMINO ACIDS AGAINST FUSARIUM WILT OF TOMATO BY FOLIAGE TREATMENT

$$CH_2=CH-CH_2-\underset{\displaystyle R_1}{\overset{|}{N}}-CH_2-COOR_2$$

No.	R_1	R_2[a]	Preventive value (%) 2000 ppm
1	H	H	86
3	H	Et	80
4	H	*n*-Dodec	53[b]
10	Me	H	45
12	Me	Et	66
30	Me	*n*-Dodec	35

[a] *n* denotes normal.
[b] Slight phytotoxicity.

activity against Fusarium diseases by foliage treatment. Speziale and Jaworski also reported[3] that *N*-allylglycine esters possess the activity against Fusarium wilt of tomato by foliage treatment. The ^{14}C-labeled *n*-dodecyl *N*-allylsarcosinate was rapidly absorbed by the tomato plants from Hoagland's nutrient solution.[10] The rate of uptake at 1, 2, 4 and 7 days after application was, respectively, 9%, 15%, 36% and 68% of the initially added radioactivity. *N*-Allyl-glycine(1) and -sarcosine(10) and their esters have the preventive activity against Fusarium wilt of tomato by foliage treatment (Table III).

Mode of action of N-allylamino acids

n-Dodecyl *N*-allylsarcosinate(30) exhibits potent preventive activity in spite of the poor curative effect against Fusarium diseases. This compound slightly prevents, but only at high concentrations (500 and 1000 ppm), the mycelial growth of *F. oxysporum* f. sp. *raphani*, *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *lycopersici* on nutrient agar medium (Table IV). *F. oxysporum* f. sp. *lycopersici* was incubated for 6 days in a nutrient solution containing 500 ppm of the compound and the conidial suspension (1×10^6 cells/ml) obtained after the incubation was administered to the roots of tomato plants at the second true leaf stage. After greenhouse cultivation for 2 weeks, the disease symptoms developed normally. Therefore, this compound does not seem to influence either the growth of the pathogen or the pathogenicity itself.

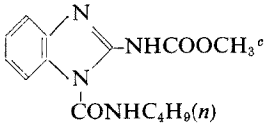
TABLE IV. ANTIFUNGAL ACTIVITIES OF *n*-DODECYL *N*-ALLYLSARCOSINATE AGAINST *Fusarium* spp.

Concentration (ppm)	Inhibition of mycelial growth (%) A[a]	B[b]	C[c]
500	24	29	38
1000	41	34	43

[a] *F. oxysporum* f. sp. *raphani*.
[b] *F. oxysporum* f. sp. *cucumerinum*.
[c] *F. oxysporum* f. sp. *lycopersici*.

Benomyl [methyl 1-(*n*-butylcarbamoyl)-2-benzimidazolecarbamate] is a systemic fungicide which inhibits the growth of *Fusarium*.[11] When plants were treated with benomyl, the

TABLE V. PREVENTIVE ACTIVITY OF *n*-DODECYL *N*-ALLYLSARCOSINATE AND *N*-ALLYLAMINOACETONITRILE HYDROCHLORIDE AGAINST FUSARIUM WILT OF TOMATO IN THE BED TEST

No	Structure[a]	Concentration (ppm)	Preventive value (%) Race J_1	Race J_3
30	$CH_2=CHCH_2\underset{\displaystyle CH_3}{\overset{|}{N}}CH_2COOC_{12}H_{25}(n)$	1000	38	46
33	$CH_2=CHCH_2NHCH_2CN\cdot HCl$[b]	500	38	70
34		500	75	43

[a] *n* denotes normal.
[b] Ref. 1.
[c] Benomyl.

fungicide was detectable in the stems.[12] However, after tomato plants were treated with *n*-dodecyl *N*-allylsarcosinate and incubated for a few days, no substance toxic to *Fusarium* was detected in extracts of the roots, stems or leaves. This indicates that the compound is not transformed into fungitoxic principles in the plant.

As shown in Table V, both *n*-dodecyl *N*-allylsarcosinate(30) and *N*-allylaminoacetonitrile hydrochloride(33) exhibit the activity against Fusarium wilt of tomato (both the J_1 and J_3 races of *F. oxysporum* f. sp. *lycopersici*) in the bed test. The preventive activity of *n*-dodecyl *N*-allylsarcosinate against the disease caused by race J_3 was higher than that caused by race J_1. However, an opposite result was observed in the case of benomyl. *N*-Allylaminoacetonitrile hydrochloride showed a property similar to that of *n*-dodecyl *N*-allylsarcosinate. The above difference suggests a difference in the mode of action between *n*-dodecyl *N*-allylsarcosinate and benomyl. Race J_1 shows systemic infection and race J_3 only attacks the roots.

The preventive activity of *n*-dodecyl *N*-allylsarcosinate against Fusarium wilt of tomato varies with the application time. The highest activity was obtained by application 2 days before inoculation (Fig. 2). Davis and Dimond reported[13] that plant growth regulators which reduce Fusarium wilt of tomato are relatively poor fungitoxicants *in vitro* and these compounds induce modifications in the metabolism of the host. Furthermore, they showed that the chemotherapeutic activity of the plant growth regulators is directly related to the interval between inoculation and treatment. The longer the interval, the greater the potency of the compounds. On the contrary, the preventive activity of *n*-dodecyl *N*-allylsarcosinate decreases when the interval is longer than 2 days. Degradation of the compound in the plant may possibly take place during that time.

Retig reported[6] that ethephon (2-chloroethylphosphonic acid) treatment increases the resistance of susceptible plants such as tomatoes to *Fusarium* and enhances the peroxidase activity. Furthermore, Retig and Chet reported[8] that the treatment of susceptible plants such as tomato with catechol prevents Fusarium wilt and that the accumulation of total phenols is observed in the catechol-treated plants. When *n*-dodecyl *N*-allylsarcosinate was applied to tomato plants at a concentration of 1000 ppm, the peroxidase activity of the roots was found to be 1.3 times higher than that of the untreated control 4 days after application. The electrophoretic pattern of peroxidase isozymes of the treated plants was different from that of the untreated control. The content of total phenols was found to be 2.0 times greater than that of the untreated control 2 days after application. The enhancement of the peroxidase activity and the accumulation of total phenols may be related to the prevention of Fusarium diseases.

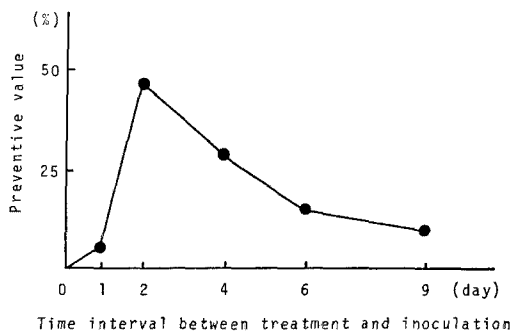


FIG. 2. Relationship between Contact Duration and Preventive Activity of *n*-Dodecyl *N*-Allylsarcosinate against Fusarium Wilt of Tomato.

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