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## Studies on Biologically Active Guanidinopyrimidines Part II\*. Effect of Guanidinopyrimidines on Phytopathogens

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Fifteen new guanidinopyrimidine derivatives were prepared and their fungicidal and antiviral activities were examined. The quantitative structure-activity relationship was also examined. At a relatively high concentration 2-benzylguanidino-4-phenyl-6-methylpyrimidine was as effective as validamycin against *Pellicularia sasakii* and as edifenphos against *Pyricularia oryzae*. 2-Phenylpropylguanidino-4-phenyl-6-methylpyrimidine effectively blocked the invasion by *Pseudoperonospora cubensis*. In the antiviral test against TMV, some compounds were fairly effective at 100 ppm level.

### INTRODUCTION

Pyrimidine derivatives have attracted particular interest as fungicides (Woodcock, 1972), since Elias *et al.* (1968) reported a systemic fungicide dime-thirimol (5-butyl-2-dimethylamino-4-hydroxy-6-methylpyrimidine), and its analogue ethirimol (5-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine) (Beb-bington *et al.*, 1969). While, guanidinopyrimidine derivatives are known to be potent antimalarial drugs (Curd and Rose, 1946; Urbanski and Serafin, 1967; Elslager *et al.*, 1974).

In a previous paper, we reported the syntheses of guanidinopyrimidine derivatives and their biological activities against plants, insect and cultured cells (Shuto *et al.*, 1974). 2-Phenethylguanidino-4,6-dimethylpyrimidine almost inhibited the growth of radish roots at 50 ppm, while at lower concentrations it stimulated their growth remarkably. In the case of the moulting process in a housefly strain, 4,6-dimethylpyrimidine derivatives delayed its pupation. On the other hand, 4-hydroxy-6-methyl derivatives seemed to accelerate it.

This paper describes the preparation of 15 new guanidinopyrimidines and their biological activities against fungi and tobacco mosaic virus.

### MATERIALS AND METHODS

#### Chemicals

The new compounds prepared were enumerated in Tables 1 and 2. Melting points were uncorrected. NMR spectra were recorded on a JEOL nuclear

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\* For Part I, see Reference Shuto *et al.*, 1974.

**Table 1.** Analytical data of guanidinopyrimidines.

Compd. No.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	Yield (%)	M. p. (°C)	Elemental Analysis (Found/Calcd.)		
								C	H	N
1	i-Pr	H	OH	H	CH <sub>3</sub>	30	290	51.22 51.66	7.19 7.23	32.90 33.47
2	PhCH <sub>2</sub>	H	OH	H	CH <sub>3</sub>	57	305	60.70 60.68	5.84 5.88	27.34 27.22
3	Ph(CH <sub>2</sub> ) <sub>3</sub>	H	OH	H	CH <sub>3</sub>	49	284	63.23 63.14	6.77 6.71	24.68 24.55
4	Ph(CH <sub>2</sub> ) <sub>3</sub>	H	CH <sub>3</sub>	H	CH <sub>3</sub>	36	195-7	67.87 67.81	7.52 7.47	24.85 24.72
5	PhCH <sub>2</sub>	H	OH	H	Ph	46	305-6	66.73 67.69	5.49 5.37	22.00 21.93
6	Ph(CH <sub>2</sub> ) <sub>3</sub>	H	OH	H	Ph	17	275-6	68.64 69.14	6.11 6.09	20.40 20.16
7	Ph(CH <sub>2</sub> ) <sub>2</sub>	H	Ph	H	CH <sub>3</sub>	20	150-1	72.18 72.48	6.44 6.39	21.11 21.13
8	Ph(CH <sub>2</sub> ) <sub>3</sub>	H	Ph	H	CH <sub>3</sub>	39	144	73.08 73.01	6.55 6.71	20.15 20.28
9	CH <sub>3</sub>	H	Ph	H	CH <sub>3</sub>	21	197-8	64.68 64.71	6.36 6.27	28.96 29.03
10	CH <sub>3</sub>	CH <sub>3</sub>	Ph	H	CH <sub>3</sub>	28	208	65.90 65.86	6.77 6.71	27.54 27.43
11	PhCH <sub>2</sub>	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	30	211	66.99 66.89	6.96 7.11	26.04 26.00
12	Ph(CH <sub>2</sub> ) <sub>2</sub>	H	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	64	196	67.12 67.81	7.27 7.47	24.44 24.72
13	Ph(CH <sub>2</sub> ) <sub>2</sub>	H	OH	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	50	194	64.19 64.19	7.14 7.07	23.36 23.40

**Table 2.** Analytical data of guanidinopyrimidine analogues.

Compd. No.	R <sub>1</sub>	R <sub>2</sub>	Yield (%)	M. p. (°C)	Elemental Analysis (Found/Calcd.)		
					C	H	N
14	PhCH <sub>2</sub>	H	31	190	58.63 58.76	6.15 6.16	28.39 28.56
15	Ph(CH <sub>2</sub> ) <sub>2</sub>	H	33	225	60.39 60.21	6.67 6.61	26.97 27.01

magnetic resonance spectrometer MH-100.

**2-Phenylpropylguanidino-4-hydroxy-6-methylpyrimidine (3)**

Phenylpropylbiguanide hydrochloride (0.02 mole) was added in the ethanolic solution of sodium ethoxide (0.02 mole) and stirred for a while, then the precipitate was filtered off. After the solvent was evaporated, ethyl acetoacetate (0.04 mole) was added and heated at 120–130°C for an hour. The precipitate was collected and thoroughly washed with methanol. The crude product was recrystallized from dimethylformamide to furnish white crystals. Yield 2.8 g (49 %). M.p. 284°C. NMR  $\delta_{\text{Me}_4\text{Si}}^{\text{DMSO}-d_6}$ ; 2.08 (3H, s), 5.60 (1H, s), 7.40 (5H), 10.81 (1H, broad).

2-Isopropylguanidino- and 2-benzylguanidino-4-hydroxy-6-methylpyrimidines (1 and 2) were prepared in the same way. Similarly, 4-hydroxy-6-phenylpyrimidine **derivatives (5, 6)** were synthesized using ethyl benzoylacetate instead of ethyl acetoacetate.

**2-Phenylpropylguanidino-4,6-dimethylpyrimidine (4)**

To the ethanolic solution of phenylpropylbiguanide (0.02 mole), acetylacetone (0.04 mole) was added and heated under reflux for 2 hr. After cooling, the product was collected and recrystallized from ethanol, giving white needles. Yield 2.1 g (36 %). M.p. 195–7°C. NMR  $\delta_{\text{Me}_4\text{Si}}^{\text{CDCl}_3}$ ; 1.99 (2H, quartet), 2.39 (3H, s), 2.80 (2H, t), 3.33 (2H, t), 6.57 (1H, s), 7.39 (5H).

**2-Methylguanidino-4-phenyl-6-methylpyrimidine (9)**

To the ethanolic solution of methylbiguanide (0.02 mole), benzoylacetone (0.02 mole) was added and heated under reflux for 2 hr. Then, the solvent was evaporated to a small portion and ether was added to the residue. The product precipitated was collected and recrystallized from aqueous ethanol. Yield 1.0 g (21%). M.p. 197–8°C. NMR  $\delta_{\text{Me}_4\text{Si}}^{\text{CDCl}_3}$ ; 2.51 (3H, s), 3.03 (3H, s), 7.13 (1H, s), 7.63 (3H), 8.17 (2H).

Other 4-phenyl-6-methylpyrimidine **derivatives (7, 8, 10)** were also obtained in the same way.

**2-Phenethylguanidino-4-hydroxy-5-ethyl-6-methylpyrimidine (13)**

To the ethanolic solution of phenethylbiguanide (0.02 mole), ethyl 2-ethylacetoacetate was added and heated for an hour. The precipitate was collected and thoroughly washed with methanol. The crude product was recrystallized from ethanol. Yield 3.0 g (50 %). M.p. 194°C. NMR  $\delta_{\text{Me}_4\text{Si}}^{\text{DMSO}-d_6}$ ; 1.05 (3H, t), 2.11 (3H, s), 2.47 (2H, quartet), 3.03 (2H, t), 3.54 (2H, quartet), 7.42 (5H, s).

**2-Benzylguanidino-6-oxo-3,4,5,6-tetrahydropyrimidine (14)**

To the solution of benzylbiguanide (0.02 mole) in 5ml of dimethylformamide was added with stirring 2 g (0.02 mole) of ethyl acrylate at room temperature and the mixture was stood for 2 days. The crystals separated were collected by filtration, washed with ethanol and recrystallized from ethanol. Yield 1.5 g (31%). M.p. 190°C.

Other compounds than those described were synthesized by the methods reported previously (Shuto *et al.*, 1974; Furukawa *et al.*, 1971).

### Biological test.

#### Test for *Pellicularia sasakii*

*Pellicularia sasakii* was grown in a medium containing 20 g of bran, 15g of chaff and 30 ml of a 0.5 % pepton solution, at 28°C for 7 days, then three-fold volume of chaff and 60 ml of the pepton solution were added and blended thoroughly. After standing under the condition of 28°C and 97 % relative humidity for 24 hr, the mixture was supplied for inoculation. The rice seedlings at the 5-leaf stage were transplanted in a pot (1/5000 are), and were grown in a green house for 60 days. In a preventive test, a test solution (10 ml per pot) in a concentration of 500ppm level was applied to the plant by spraying. One day after, a 3 g lot of the *Pellicularia sasakii* mixture was applied at the foot of the plant. In a curative test, one day after inoculation of *P. sasakii*, a test solution (10 ml) was sprayed onto the plants. The treated plants were grown under the condition of 28°C and 97 % relative humidity. After 4 days, the length of the diseased spots was measured and then the relative preventive or curative activities were calculated by the following equation.

$$\text{Relative activity} = \left( 1 - \frac{\text{Total length of the diseased spots for the chemical-treated plants}}{\text{Total length of the diseased spots for control}} \right) \times 100$$

#### Test for *Pyricularia oryzae*

After culturing 10 days on a slant straw-extract agar medium, spores of *Pyricularia oryzae* were collected. The suspension of the spores was supplied for inoculation. In a preventive test, 15ml of a test solution was applied by a sprinkler onto the 30-day rice plant seedlings in a pot. The following day, the spore suspension was sprayed on the plants to inoculate the pathogen. In a curative test, 15 ml of a test solution was applied for each pot one day after inoculation of *P. oryzae*. After 10 days in a green house at 28°C, the number of diseased spots was counted and the relative activity was calculated by the following equation:

$$\text{Relative activity} = \left( 1 - \frac{\text{Total number of diseased spots for the chemical-treated plants}}{\text{Total number of diseased spots for control}} \right) \times 100$$

#### Test for *Pseudoperonospora cubensis*

Seeds of cucumber were immersed in distilled water for 24 hr at 28°C and then a seed was allowed to germinate in a cup ( $\phi = 9$  cm). After 18 days, 5 ml of a test solution was sprayed on the plant, then the plant was kept under the condition of 25~26°C and 80% relative humidity. The following day, the spore suspension of *Pseudoperonospora cubensis* was inoculated on 3 points of the second leaf of the plant. After 10 days under the condition of 20°C and 95 % relative humidity, the degree of infection was calculated out as the protective value by the similar equation described above. The results are shown as the

minimum inhibiting concentration (MIC) which was defined as the lowest concentration with the protective value of more than 90 % (Table 4).

#### Antiviral test against tobacco mosaic virus

The ordinary strain of tobacco mosaic virus (TMV) was supplied by the Institute for Plant Virus Research.

The test plant was *Nicotiana tabacum* L. cv. Xanthi-nc.

1) Inhibition of local lesion formation; TMV (8 µg/ml) was inoculated on a tobacco leaf. After 30 min, a solution of a test compound was applied to one half of the leaf. The other half was treated with deionized water in a similar manner. After 4 days, the number of local lesions formed was counted. For each test 8 leaves from 2 plants were used. The inhibition rate of lesion formation was calculated by the following equation:

$$\text{Inhibition rate} = \left(1 - \frac{\text{Total number of local lesion for the chemical-treated leaves}}{\text{Total number of local lesion for control}}\right) \times 100$$

When the inhibition rate was below 30 %, the result was indicated with a minus sign, (—), in Tables 5 and 6.

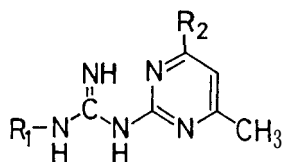
2) Inhibition of multiplication ; TMV (2 µg/ml) was inoculated on tobacco leaves and allowed to stand under fluorescent lamps (1000 lux) at 31°C for 24 hr. Then each leaf was divided into two parts from the midrib. One part was floated on a solution of a test compound and the other half on deionized water. They were kept for 24 hr under the same conditions as mentioned above. Then, during lesion formation they were kept at 21°C. The size of local lesion formed was measured. The effect of a chemical on virus multiplication was shown as follows :—, the size of lesion is corresponding to that of control ; +, the size of lesion is smaller than that of control; ++, the size of lesion is extremely smaller than that of control.

## RESULTS AND DISCUSSION

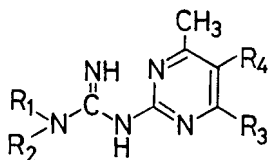
Table 3 shows the fungicidal activities of some guanidinopyrimidines. In the fungicidal test to *Pellicularia sasakii*, the average length of the diseased spots of the plants treated with 2-benzylguanidino-4-phenyl-6-methylpyrimidine (17) was 0.4 mm and the relative protective rate was 97.1 %. In the curative test the length of the diseased spots was 0.6 mm and the relative activity was 93.9 %. Therefore, at a relatively high concentration the compound was as effective as validamycin against *Pellicularia sasakii*. The sporecidal activity of the same compound against *Pyricularia oryzae* was almost equal to that of edifenphos, but the curative activity was less effective than the latter.

Against *Pseudoperonospora cubensis*, 4,6-dimethylpyrimidine (4 and 16) and 4-phenyl-6-methylpyrimidine derivatives (8, 10 and 17) showed relatively strong activities. In general, 4-phenyl-6-methylpyrimidines were more effective. Especially, 2-phenylpropylguanidino-4-phenyl-6-methylpyrimidine (8) completely prevented the invasion of the pathogen at a level of 60 ppm. The compound containing a hydroxy group in the pyrimidine ring (2 and 13) showed

Table 3. Fungicidal activities of guanidinopyrimidines.



No.	R <sub>1</sub>	R <sub>2</sub>	Conc. (ppm)	<i>Pellicularia sasakii</i>		<i>Pyricularia oryzae</i>	
				Protective Effect (%)	Curative Effect (%)	Protective Effect (%)	Curative Effect (%)
4	Ph(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	500	13.2	0	84.55	68.79
16	Ph(CH <sub>2</sub> ) <sub>3</sub>	CH <sub>3</sub>	500	97.1	93.9	97.5	43.6
17	PhCH <sub>2</sub>	Ph	300	—	—	98.3	97.8
	Edifenphos		30	100	91.8	—	—
	Validamycin		30	—	—	—	—

Table 4. Fungicidal activity against *Pseudoperonospora cubensis* and regression analysis of structure-activity relationship.

No.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	MIC (ppm)	$\pi_T$	log 1/MIC	
							Obs.	Calcd.*
2	PhCH <sub>2</sub>	H	OH	H	>500	—	—	—
4	Ph(CH <sub>2</sub> ) <sub>3</sub>	H	CH <sub>3</sub>	H	>500	—	—	—
10	Ph(CH <sub>2</sub> ) <sub>3</sub>	H	Ph	H	500	4.63	-2.398	-2.355
13	CH <sub>3</sub>	CH <sub>3</sub>	Ph	CH <sub>2</sub> CH <sub>3</sub>	>500	3.63	-2.699	-2.704
	Ph(CH <sub>2</sub> ) <sub>2</sub>	H	CH <sub>3</sub>	H	500	—	—	—
16	PhCH <sub>2</sub>	H	Ph	H	—	3.63	-2.699	-2.704
17	PhCH <sub>2</sub>	H	—	—	125	5.26	-2.097	-2.135

$$*\log 1/\text{MIC} = 0.349(\pi_T) - 3.971$$

no activity. This fact is contrary to the results obtained from the investigation of dimethirimol and its analogues, in which case the hydroxy group plays an important role in its activity (Woodcock, 1972).

Table 4 shows values of MIC of the compounds tested, together with the hydrophobic parameters,  $\pi_T$ , which were obtained as the sum of each substituent-constant of every substituent of a guanidinopyrimidine based on the data of Leo *et al.* (1971). A regression analysis gave a good correlation as shown in the following equation.

$$\log 1/\text{MIC} = 0.349(\pi_T) - 3.971$$

$$\begin{array}{ccc} n & r & s \\ 5 & 0.997 & 0.034 \end{array}$$




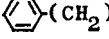


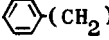
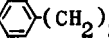


On the other hand, the relation between the activity and the ionization con-

Table 5. Antiviral activity of guanidinopyrimidines against tobacco mosaic virus.

No.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Inhibitory Effect (%)			
				Local Lesion		Multiplication	
				Conc. (ppm)		Conc. (ppm)	
				250	25	500	50
1	i-Pr	H	OH	84*			—
2	PhCH <sub>2</sub>	H	OH	84*	—		—
3		H	OH	77*			
4	PhPh(CH <sub>2</sub> ) <sub>4</sub> (CH <sub>2</sub> ) <sub>1</sub>	H	CH <sub>3</sub>	67			—
7		H	Ph	83	—	+	±
9	Ph(CH <sub>2</sub> ) <sub>3</sub>	H	Ph				±
10	PhCH <sub>2</sub>	CH <sub>3</sub>	Ph				—
16		H	CH <sub>3</sub>				—
17	PhCH <sub>2</sub>	H	Ph				—
18	i-Pr	H	CH <sub>3</sub>			+	—

\* Emulsifier was used.

Table 6. Antiviral activity of guanidinopyrimidines to tobacco mosaic virus.

No.	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	Inhibitory Effect (%)			
					Local Lesion		Multiplication	
					Conc. (ppm)		Conc. (ppm)	
					500*	100	500	100
19		H	OH	CH <sub>3</sub>	67	—	+	—
20		H	OH	CH <sub>3</sub>	—	—	—	—
21		H	OH	CH <sub>3</sub>	43	47	—	—
22		H	OH	CH <sub>3</sub>		—	+	—
23	CH <sub>3</sub>	CH <sub>3</sub>	OH	CH <sub>3</sub>	—	—		—
24	C		OH	CH <sub>3</sub>	73	35		
25		H	CH <sub>3</sub>	CH <sub>3</sub>	76	61	+	+
26		H	CH <sub>3</sub>	CH <sub>3</sub>	40	57	+	—
27		H	CH <sub>3</sub>	CH <sub>3</sub>	41	—	+	—
28	CH <sub>3</sub>	H	CH <sub>3</sub>	CH <sub>3</sub>	56	—		—
29		Li	OH		72	—	+	+
30	CH <sub>3</sub>	CH <sub>3</sub>	OH		40	66	—	—
31	OH	H	CH <sub>3</sub>	CH <sub>3</sub>	66		—	—

\* All of the chemicals tested were phytotoxic at this concentration.



stants (electronic parameter) was not observed. In general, it is known that the permeability of a drug through biological membranes correlates to the hydrophobic parameters, which were based on the partition coefficients (Hansch, 1971). Therefore, the results suggested that the activity against *Pseudoperonospora cubensis* might primarily correlate to the ability of penetration to the site of action.

In the antiviral test against TMV, most of the compounds inhibited the formation of local lesion at 500ppm as shown in Tables 5 and 6. However, they were also phytotoxic at that concentration. At a level of 250ppm 2-phenethylguanidino-4-phenyl-6-methylpyrimidine (7) and 2-phenylpropylguanidino-4,6-dimethylpyrimidine (4) were considerably inhibitory against TMV. 2-Dimethylguanidino-4-hydroxy-6-phenylpyrimidine (30) and 2-phenylguanidino-4, 6-dimethylpyrimidine (25) were fairly effective at a level of 100 ppm; they inhibited lesion formation up to 66 and 61 %, respectively. As to the effect on multiplication, 2-phenylguanidino- (25) and 2-(*p*-chlorophenyl)guanidino-4, 6-dimethylpyrimidines (26) were effective.

#### ACKNOWLEDGEMENTS

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