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Synthesis of Guanidinopyrimidine Derivatives and Their Biological Activities

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Present paper deals with the synthesis of guanidinopyrimidine derivatives from substituted-biguanides, and with thier biological activities. 2-Arylguanidino-4,6-dimethylpyrimidine derivatives had stronger herbicidal activity than 2-alkylguanidines. Especially, 2-phenethylguanidino-4,6-dimethylpyrimidine inhibited almost the growth of radish roots at the concentration of 50 ppm, while at the concentration of 10 ppm it stimulated the growth of radish roots remarkably. For the molting of housefly there were no strikingly effective compounds. However, 4,6-dimethylpyrimidine derivatives made the pupation delay, except 2-morpholinoguanidino-4,6-dimethylpyrimidine. On the other hand, 4-hydroxy-6-methyl-derivatives seemed to accelerate it.

Until now a variety of compounds have been screening against plant virus. They include pyrimidine and purine derivatives, antibiotics, organic dyes, many compounds with high molecular weights, like nucleic acids, and so on (Hirai and Noguchi, 1965; Suzuki, 1970; Yuki, 1968). The available substances which are able to use in practice, however, are not developed yet.

Present paper deals with the combination of pyrimidine derivatives, as the antimetabolites of bases of nucleic acids; and guanidine derivatives which were reported about their antiviral effects (LaColla et al., 1970; Melander, 1960; Yuki, 1968). The guanidino group is one of the most basic groups, therefore it is thought that it binds electrostatically with the electronegative surfaces of bacteria, fungi, tumor cells and so forth. While in the pyrimidine derivatives, there are many compounds which have an antiviral activity ***in vitro*** and ***in vivo,*** such as 5-iodo-2'-deoxyuridine (IDU), arabinosyl cytidine, azacytidine. Even more simple compounds, like 2-amino-4-methyl-6-hydroxypyrimidine, 2,6-dimethyl-3-amino-4-hydroxypyrimidine, 2,4,6-triamino-5-phenylazopyrimidine and 2-thiouracil, have some degrees of antiviral effect. Generally, in these compounds the more substituents, such as -NH,, -Cl, -NO,, -OH, -COOH, there are in each position of pyrimidine ring, the more the activity increases.

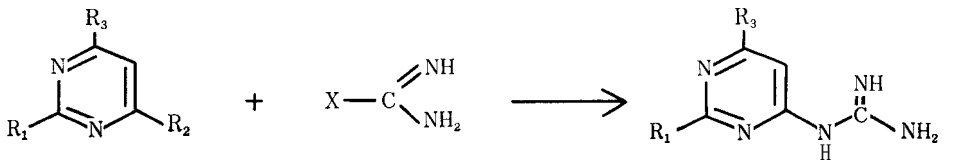
Besides such an antiviral activity, the authors were interested in the biological effects of guanidinopyrimidines on plant and insect. So the authors synthesized some new pyrimidine derivatives which carry a guanidino group in whether free or substituted state, and tested their biological activities.

I. SYNTHESES

Until now there are several reports about the synthesis of guanidinopyrimidine derivatives. Rackmann (1910) reported the synthesis of 4,6-dihydroxy-2-guanidinopyrimidine from biguanide and diethyl malonate at first. Curd and Rose (1946) described the synthesis of the titled compounds from arylbiguanides and ethyl acetoacetate and their antimalarial activity. King and King (1947) synthesized 2,6-dichloro-4-guanidinopyrimidine by the substitution reaction of guanidine to a chlorine atom attached to a pyrimidine ring. Takagi and Hubert-Habart (1970) prepared 2-morpholinoguanidino-pyrimidine derivatives from N-(guanidinoiminomethyl)morpholine and benzofurans. Recently, Portnyagina and Danilenko (1971) reported the reaction of amino group on a pyrimidine ring with S-methylisothiourea to give guanidinopyrimidine derivatives.

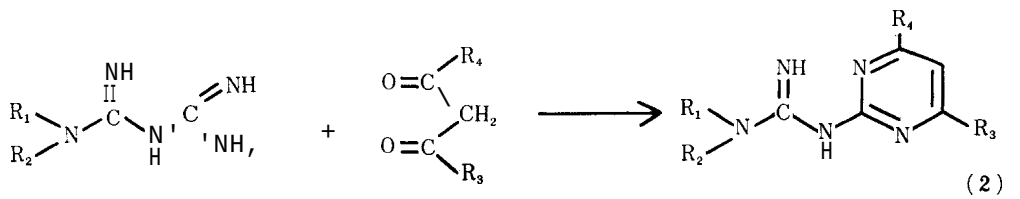
The synthetic methods of guanidinopyrimidines, mentioned above, are classified in two types as follows :

1. By the substitution reaction of -NH, or -Cl attached to a pyrimidine ring with S-methylisothiourea or guanidine, respectively (Bühler and Pfleiderer, 1966 ; King and King, 1947; Portnyagina and Danilenko, 1971).



R_2 : NH, , *Cl* *X :* CH_3S, NH, (1)

2. By the condensation reaction of various biguanides with 1,3-dicarbonyl derivatives (Curd and Rose, 1946; Curd and Rose, 1947; Rackmann, 1910; Ridi *et. al.*, 1958 ; Urbanski et al., 1967) or benzofuran derivatives (Takagi and Hubert-Habart, 1971).



(2)

So the authors tried to synthesize some new guanidinopyrimidines by applying the following methods.

(1) By the substitution reaction

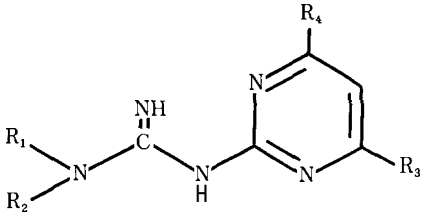
According to the method of King (1947), 2,6-dichloro-4-guanidinopyrimdine was easily prepared from 2,4,6-trichloropyrimidine and guanidine in aqueous acetone at room temperature. Then the authors tried to prepare diguanidinopyrimidine derivatives, but did not succeed by heating under reflux in the ethanol solution. Other chloropyrimidines also were synthesized and heated with guanidine in ethanol for a few hours, but in all cases the polyguanidinopyrimi-

dines could not be obtained.

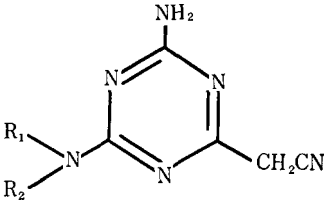
Furthermore the authors attempted the reaction of aminopyrimidine and S-methylisothiourea by heating under reflux in aqueous solution. Afer 10 hours under reflux a little spot which was positive for the Sakaguchi reaction was appeared on TLC, but the product could not be isolated.

(2) **By the condensation reaction**

Various N-substituted-biguanides were prepared and condensed with ethyl acetoacetate, acetylacetone and ethyl benzoylacetate. In these reactions 2-substituted-guanidino-pyrimidine derivatives (I) were produced. The biguanide derivatives used were N-phenylbiguanide, N-(β-phenethyl) biguanide, N-(p-methylphenyl) biguanide, N-(p-chlorophenyl) biguanide, N-methylbiguanide, N,N-dimethylbiguanide, morpholinobiguanide and piperidinobiguanide. These compounds were obtained as follows (Bamberger and Seeberger, 1891; Cohn, 1911; Sugino and Izumi, 1944) : Arylbiguanide·HCl were prepared from dicyandiamide and corresponding amine·HCl by heating under reflux in aqueous solution for an hour, Dimethylbiguanide·HCl and piperidinobiguanide. HCl were obtained from dicyandiamide and dimethylamine·HCl or piperidine ·HCl by fusion. Methylbiguanide · 2HCl was produced from dicyandiamide and methylamine ·HCl. Morpholinobiguanide ·HCl and β-phenethylbiguanide·HCl used were commercial materials. The former is known as ABOB, one of the clinical antiviral drugs. The latter is used to cure diabetes.



(I)



(II)

When diethyl malonate was used, the product could not be obtained, in disaccord with the earlier paper (Rackmann, 1910). Furthermore, when ethyl cyanoacetate was used, only s-triaiine derivative (II) was obtained.

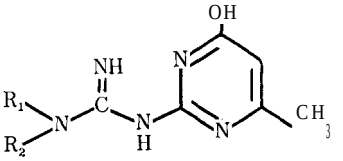
(I) *The reaction with ethyl acetoacetate*

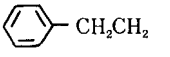
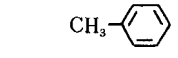
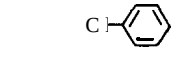
The products from the reaction of biguanides with ethyl acetoacetate were 2-substituted-guanidino-4-hydroxy-6-methylpyrimidine derivatives and by-products, s-triazine derivatives. Both pyrimidine and triazine derivatives show the same molecular formula, but different melting point ; they are structural isomers each other. In these isomers, the compound shown higher melting point was a pyrimidine and other was a triazine derivative. This fact was confirmed by comparison with compounds prepared by the other synthetic route by Curd and Rose **(1946).**

Products which had aryl-substituents on a guanidino group were generally obtained in considerable yields and hardly soluble in water and alcohol. Other

products which carried alkyl substituents, except dimethylguanidino derivative, were obtained not in good yield. The analytical data of the compounds obtained are shown in Table 1.

Table 1. The analytical data of 2-substituted-guanidino-4-hydroxy-6-methylpyrimidine derivatives.



No.	R_1	R_2	M. P. (°C)	Yield (%)	Recryst. Solvent	Elemental Analysis (Found / Calcd.) C	H	N
1		H	225	87	Pyridine	59.28 59.25	5.61 5.39	***2%. 54*** ***2%. 79***
2		H	296-'	45	DMF	61.44 61.97	6.41 6.32	***25.40*** ***25.81***
3		H	275	54	DMF	60.48 60.68	5. a5 5.88	***27.13*** 27.22
4		H	284	46	DMF	***52.14*** ***51. a9***	***4.36*** ***4.32***	25.23 25.23
5	CH_3	H	269	21	EtOH	***46.30*** ***46.40***	***6.36*** ***6.12***	37. a3 38.65
6	CH_3	CH_3	234	67	EtOH	49.21 49.22	6. a3 6.71	35.73 35.88
7			262	20	H_2O	50.43 50.62	6.54 6.37	29.28 29.52
a			233	15	EtOH-H_2O	55.70 56.15	7.31 7.2%	***29.53*** ***29.77***

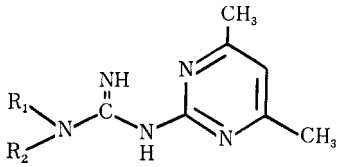
(2) *The reaction with acetylacetone*

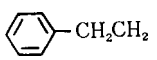
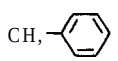
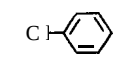
The products of the reaction of biguanides with acetylacetone were Z-substituted-guanidino-4,6-dimethylpyrimidines. In these reactions there was no by-product. The solubility of the products was increased by the introduction of methyl group in stead of hydroxy group in I-position of the ring and these preparations were mostly recrystallized from alcohol or water. The analytical data of the compounds are shown in Table *2.*

(3) The reaction with ethyl benzoylacetate

The products of the reaction of biguanides with ethyl benzoylacetate were 2-substituted-guanidino-4-hydroxy-6-phenylpyrimidines. The analytical data of these compounds are shown in Table 3.

Table 2. The analytical data of 2-substituted-guanidino-4,6-dimethylpyrimidine derivatives.

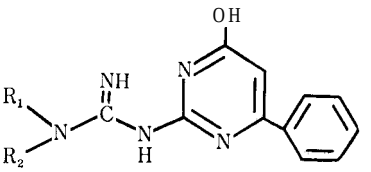


No.	R_1	R_2	M. P. (°C)	Yield (%)	Recryst. Solvent	Elemental Analysis (Found / Calcd.) C	H	N
9		H	204	84	EtOH	64.86 64.71	6.48 6.27	29.04 29.03
10		H	177	82	EtOH	66.53 66.89	7.11 7.11	25.91 26.00
11		H	195	53	EtOH	65.96 65.86	6.82 6.71	27.50 27.43
12		H	206	93	EtOH	56.75 56.62	5.11 5.08	25.54 25.41
13	CH_3	H	231	26	EtOH	53.58 53.61	7.36 7.31	38.82 39.08
14	CH_3	CH_3	178	37	H_2O	56.13 55.93	7.90 7.82	36.36 36.24
15			198	64	H_2O	56.00 56.15	7.32 7.28	29.76 29.77
16			175–1	57	MeOH-Ether	61.88 61.77	8.28 8.21	29.98 30.02

(4) Production of the s-triazine derivatives

Curd and Rose (1946) had already reported the by-production of s-triazine derivatives during the reaction of biguanides and ethyl acetoacetate, but they did not investigate in detail about that. So the authors tried to estimate the quantitative changes of the both products in various conditions. It was finally found that by the change of the substituent of biguanides the yield of products changed remarkably as shown in Table 1. In this experiment *p*-chlorophenylbiguanide and ethyl acetoacetate or methyl acetoacetate were used as materials (3). The result is shown in Table 4. When ethanol was added as the solvent in the reaction mixture, the yield of pyrimidine derivatives was better at a higher reaction temperature than at a lower one. The same result was obtained in case that alkali was not added. However when alkali or ethanol was added, the yield was better at room temperature than in a heated condition.

Table 3. The analytical data of 2-substituted-guanidino-4-hydroxy-6-phenylpyrimidine derivatives.



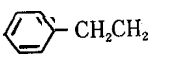
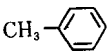
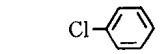
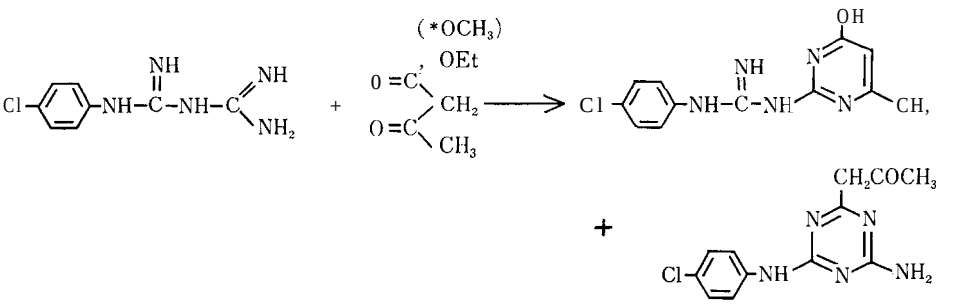
No.	R_1	R_2	M. P. (°C)	Yield (%)	Recryst. Solvent	Elemental Analysis (Found / Calcd.) C	H	N
17		H	282-4	24	DMF	68.05 68.45	5.89 5.74	21.31 21.01
18		H	264-6	46	DMF	67.61 67.69	5.55 5.37	22.00 21.93
19		H	264-6	21	DMF	59.73 60.08	4.20 4.12	20.72 20.62
20	CH_3	H	294-6	27	DMF	58.58 59.25	5.46 5.39	29.77 28.79
21	CH_3	CH_3	211	35	AcOEt-EtOH	60.60 60.68	5.88 5.88	27.10 27.22

Table 4. Yields of pyrimidine and triazine derivatives in various conditions.

Reaction Conditions				Yield		Ref.
Solvent	Alkali	Temp.	Time	Pyrimidine	Triazine	
		90°C	1 hr.	58	26	
		130°C	50 min.	67	24	
—		130°C	1 hr.	66	—	17
EtOH		room t.	5 days	60	22	
EtOH	—	90°C	1.5hrs.	46	32	
EtOH	NaOH	room t.	20 hrs.	68	—	8
EtOH	NaOH	room t.	4 days	79	12	
EtOH	NaOH	room t.	7 days	79	—	17
EcOH	NaOEt	90°C	1 hr.	58	30	
AcOH	—	130°C	24 hrs.	—	—	
*EtOH	—	90°C	1.5hrs.	49	29	

* Methyl acetoacetate was used instead of ethyl acetoacetate.



(3) Miscellaneous methods

For the synthesis of guanidinopyrimidines another method was also applied. For example, according to the method described by Bee and Rose (1966) 2-hydroxyguanidino-4,6-dimethylpyrimidine (III) was prepared from hydroxylamine·HCl and 2-cyanoamino-4,6-dimethylpyrimidine (IV) (Birtwell, 1953). The melting point of the obtained compound was 209-210°C in a sealed tube, but Bee and Rose (1966) had reported it as 196-197°C (decomp.).

Furthermore, using 2-cyanoaminopyrimidine and several alkylamine . HCl (Kraus, 1966) the reactions were carried out, but the products were not obtained in all cases.

Experimental

2-(β-Phenethyl) guanidino-4-hydroxy-6-methylpyrimidine (1) and 2-(β-phenethyl) guanidino-4-hydroxy-6-phenylpyrimidine (17)

β-Phenethylbiguanide·HCl (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 alcohol was evaporated, ethyl acetoacetate or benzoylacetate (0.04 mole) was added and heated at 120-130°C for an hour. The precipitate was collected and washed with alcohol, then boiled with methanol to remove ethyl acetoacetate or benzoyl acetate. The crude product was recrystallized from pyridine or dimethylformamide.

2-Methylguanidino-4-hydroxy-6- methylpyrimidine (5), 2-dimethylguanidino-4- hydroxy- 6-methylpyrimidine (6) and 2-methylguanidino-4-hydroxy-6-phenylpyrimidine (20)

These compounds were also obtained by the same way as (1) and (17).

2-(β-Phenethyl) guanidino-4,6-dimethylpyrimidine (10)

β-Phenethylbiguanide·HCl (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. To the filtrate acetylacetone (0.04 mole) was added and heated under reflux for one to three hours in water-bath. After cooling, the product was collected and recrystallized from alcohol.

2-Methylguanidino-4,6-dimethylpyrimidine (13)

This compound was synthesized by the above method.

2-Dimethylguanidino-4,6-dimethylpyrimidine (14)

To the methanolic solution of dimethylbiguanide·HCl (0.01 mole), 0.5 ml of 10N NaOH aqueous solution and acetylacetone (0.02 mole) were added under ice-cooling and stirring. The mixture was stirred for 36 hours at room temperature. After the solution was evaporated to a small portion, water was added to it. The precipitate obtained was collected and recrystallized from water.

2-Morpholino- or 2-piperidinoguanidinopyrimidine derivatives (7, 8, 15,16)

Morpholinobiguanide. HCl or piperidinobiguanide·HCl (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. To the filtrate ethyl acetoacetate or acetyl-

acetone was added and heated under reflux for a few hours. Then the solvent was evaporated to a small portion and to the residue ether or water was added to precipitate the product, which was then recrystallized from solvents shown in Tables 1 and 2.

2-Dimethylguanidino-4-hydroxy-6-phenylpyrimidine (21)

Dimethylbiguanide·HCl *(0.02* mole) was added in the ethanolic solution of sodium ethoxide. After removing NaCl, ethyl benzoylacetate *(0.04* mole) was added to the ethanolic solution of dimethylbiguanide and heated under reflux for 4 hours. After cooling, precipitate was collected and resuspended in hot AcOEt, then insoluble material was collected again. The product was recrystallized from AcOEt-EtOH.

2-Hydroxyguanidino-4,6-dimethylpyrimidine (22)

2-Cyanoamino-4,6-dimethylpyrimidine obtained according to the method of Birtwell (1953), and hydroxylamine ·HCl were stirred under reflux in n-butanol for 2 hours. After cooling, the precipitate was collected and washed with n-butanol, and dissolved in hot water. Addition of ammonia water gave the product. The product was recrystallized from ethanol. The melting point, 209–210°C (sealed tube).

Elemental analysis,

		C	H	N
$C_7H_{11}ON_5$	Found	46.55	6.03	38.69
	Calcd.	46.40	6.12	38.65

II. PHYSICAL AND CHEMICAL PROPERTIES

(1) Infrared spectrometry

The IR spectra were measured by the method of KBr disk. For pyrimidine derivatives, bands in the 3300-3000 cm^{-1} region are not easily identifiable, because there are a number of overlapping absorptions due to NH group and also, probably, due to some combination bands. In general, it is known that the hydroxy group exists as a keto-form on the pyrimidine ring. The C=O group usually gives a strong absoption band at **1720** cm-'. When this group is connected with a nitrogen atom, however, as in the case of uracil, cytosine, its frequency is lowered to 1700-1620 cm-' (Tsuboi and Kyogoku, 1973). This fact was applicable to the spectra of the compounds shown in Table 1; the absorption corresponding to C=O group was not seen in the region above 1700 cm-'. The intrinsic frequencies of the C=N and C=C bonds are considered to be at -1600 cm-'. Therefore, the C=O, C=N streching vibrations often couple with one another, resulting in a few complicated vibrations. Besides, the absorption of guanidino group exists in this region. In any case, the appearance of a few broad strong absorption bands in the 1650-1450 cm-' region may be regarded as a characteristic of these compounds. As examples, the spectra of 2-morpholinoguanidinopyrimidine derivatives are shown in Fig. 1.

(2) Ultraviolet absorption spectrometry

Maximum absorptions (λ_{max}) and molar extinction coefficients (ε_{max}) of com-

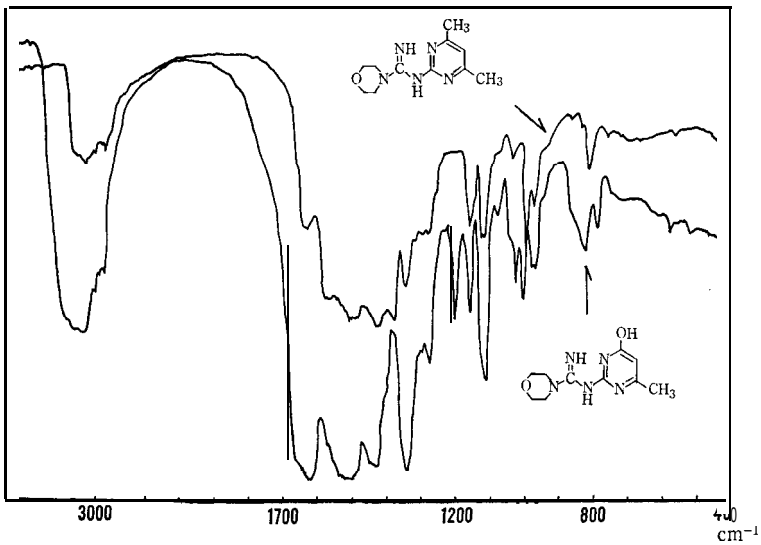


Fig. 1. IR spectra of 2-morpholinoguanidino-4-hydroxy-6-methylpyrimidine and 2-morpholinoguanidino-4,6-dimethylpyrimidine. (KBr-tablet)

pounds synthesized are shown in Table 5. The absorption patterns were different according to the sort of substituents attached to 4- and 6-position of the pyrimidine ring. If the substituents were OH and CH_3, four maximum absorption bands were observed. While in case of CH, and CH_3 two bands were observed,

Table 5. Maximum absorptions and molar extinction coefficients of guanidinopyrimidine derivatives (methanolic solution).

No.	λ_{max} (nm)				$\varepsilon_{max} \times 10^{-4}$			
1	*230*	271(s)	*277*	290(s)	0.a5		1.35	
2	*231*	259	267(s)	297	0.97	1.34		0.73
3	*232*		278	292(s)	1.11		2.11	
4	*233*	273(s)	282	299(s)	1.21		2.08	
5	*233*	261	268(s)	297	0.43	0.80		0.36
6	*232*	*262*	270(s)	301	0.90	1.40	1.30	0.76
7	*233*	*263*	272	301	1.17		1.85	1.05
8	*235*	*265*	*273*	302	0.29	0.70	0.70	0.36
9	*241*		*275*		1.36		1.57	
10	*233*		268		1.70		0.64	
11	*239*		267		1.56		1.27	
12	*244*		277		1.68		1.75	
13	*233*		265		1.84		0.49	
14	*235*		272		1.16		1.86	
15	240(s)		273		0.73		2.97	
16	*240*		274		1.54		1.49	
17		260(s)		315			1.96	0.63
18		260(s)	264 272	319			2.75	1.28
19		261(s)	276	319			2.41	1.07
20		252	260(s)	309			1.67	0.49
21			268	322			3.26	1.04

* *(s);* shoulder

in case of OH and phenyl, three bands were observed. And each band shifted by the change of substituents attached to the guanidino group. Generally, if the substituents of the guanidino group were alkyl ones, the bands of maximum absorption of disubstituted-guanidinopyrimidines, such as 2-dimethylguanidino- and 2-piperidinoguanidinopyrimidine, shifted to longer wave length than that of monosubstituted-guanidinopyrimidine (Albert, 1973). As examples, the absorption spectra of 2-dimethylguanidino derivatives are shown in Fig. 2.

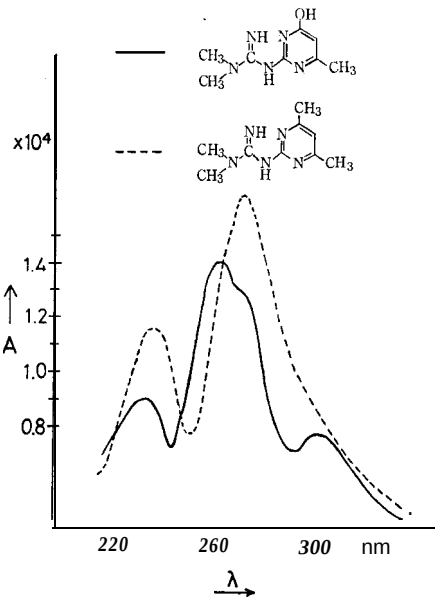
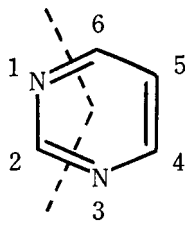


Fig. 2. UV absorption spectra of 2-dimethylguanidino-4-hydroxy-6-methylpyrimidine and 2-dimethylguanidino-4,6-dimethylpyrimidine. (methanolic solution)

(3) Mass spectrometry

As to the mass spectrometry, only one sample, 2-dimethylguanidino-4-hydroxy-6-methylpyrimidine, was measured. Therefore the mechanism of the fragmentation could not be elucidated conclusively.

Generally, the major fragmentation path of the molecular ion of pyrimidine is the sequential loss of two HCN molecules, that is, at first the cleavage occurs at a loss of the fragment consisted of l-N, 2-C. At the same way uracil expels HNCO and guanine expels CH_2N_2 at first (DeJongh, 1973). In the present sample the same fragmentation took place to separate the fragment containing l-N, 2-C,



but it seemed that the fragment containing l-N, 2-C is more stable than the other fragment because of the bulky side chain or the presumable resonance. While the remaining fragment is more stable than the fragment containing 1-N, 2-C, in case of attaching no or small substituent to the 2-position of pyrimidine ring, as the case mentioned above. In this case the latter fragment is stabilized by protonation to form the ion at m/e 84. The observation of metastable ion at m/e 64.3 is assigned to the fragmentation ; m/e 195 (M^+)→m/e 112, shown in Fig. 3, In most cases, the amino group directs the fragmentation. If functional

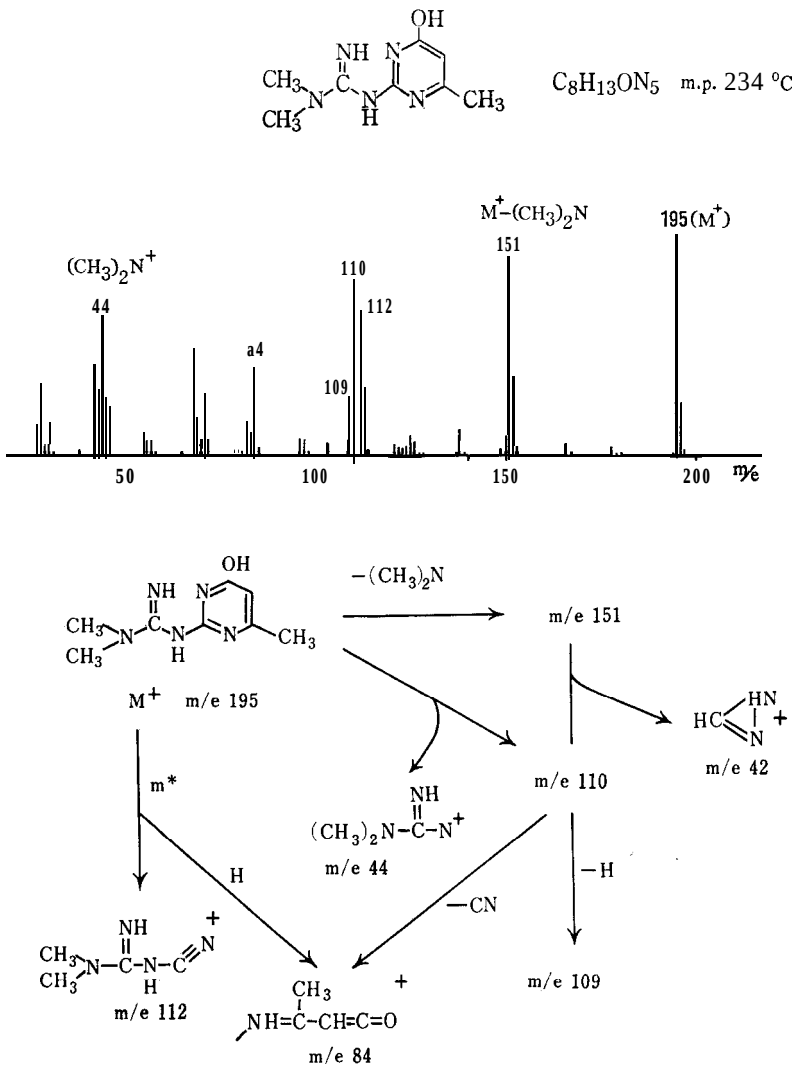


Fig. 3. Mass spectrum and the fragmentation path of 2-dimethylguanidino-4-hydroxy-6-methylpyrimidine.

groups more complex than 2-amino are attached to the ring, as in 2-piperidinopyrimidine and 4-(2-pyrimidinyl) morpholine, fragmentations triggered by these groups may compete effectively with those of pyrimidine ring (DeJongh, 1973). In the present spectrum, the ions at m/e 151; M+-$(CH_3)_2N$, m/e 110 and m/e 109, which are derived from the cleavage of the side chain, are observed.

On the other hand, in the mass spectrum of the by-product, triazine derivative, the base ion peak is at m/e 180, which is produced by a loss of CH_3 from molecular ion at m/e 195. This fact is reasonable, because this compound has both acetyl and dimethylamino group, from which CH_3 is expeled easily. Further the peak at m/e 153, which is formed by protonation of ($M^+-CH_3C=O$) and/or ($M^+-CH_3N=CH_2$), and the peak at m/e 43, $CH_3C=O$ and/or $CH_3N=CH_2$, are observed in considerable intensities. An example of the spectra is shown in Fig. 4.

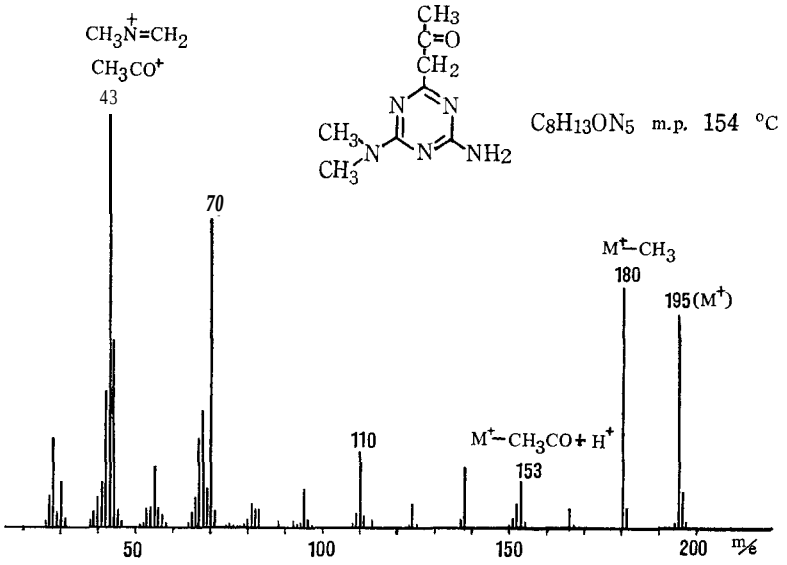


Fig. 4. Mass spectrum of 2-dimethylamino-4-amino-6-acetylmethyl-s-triazine.

III. BIOLOGICAL ACTIVITIES

The biological activities were tested for plant, housefly and cultured cell lines. As plant radish and rice were chosen. An inhibitory or stimulating effect was tested against their growth of roots and stalks. For housefly effects on pupation and emergence were tested. Furthermore, cytotoxicities of several compounds for cultured cell lines (Ehrlich ascites tumor cell and normal cell of rat) were tested ***in vitro.*** As to the antiviral activity it will be reported elsewhere. As a result a few of these compounds had shown some degree of biological activities.

(1) Herbicidal and plant regulatory effect

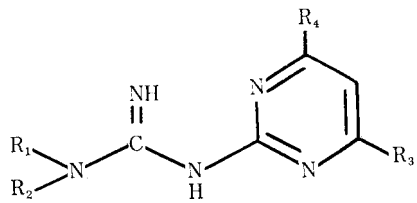
(a) Materials and method

10 ml of test solution was poured in a petri-dish placed a filter paper and added 20 radish seeds or rice grains, which had been steeped in advance in water overnight as a pre-treatment. They were kept at 25°C in a dark chamber. After 4 days the length of a primary root and stalk was measured and the growth rate was calculated against control.

(b) Results and discussion

The results are shown in Table 6. It was found that 4,6-dimethylpyrimidine derivatives had considerable activities among the tested compounds. Especially, 2-(β-phenethyl)guanidino-4,6-dimethylpyrimidine (No. **10)** was the most effective one. This compound inhibited almost the growth of radish roots and changed

Table *6.* The herbicidal and plant regulatory effects of the guanidinopyrimidines.



Sample No.	R_1	R_2	R_3	R_4	Conc. (ppm)	Growth Rate (%) Radish Roots	Radish Stalks	Rice Roots	Rice Stalks
2	CH_2CH_2-Ph	H	CH_3	OH		114	109	—	
5	CH_3	H	CH_3	OH	50	100	92	—	
8	C		CH_3	OH	100	124	99	92	103
9	C	H	CH_3	CH:	100	20	45	100	105
					50	46	56	114	93
					10	150	112	—	
10	CH_2CH_2-Ph	H	CH_3	CH_3	100	10	50	35	85
					50	8	51	46	85
					10	171	124		—
13	CH_3	H	CH_3	CH_3	100	36	95	—	
15	O		CH_3	CH_3	40	86	91		—
20	CH,	H	Ph	OH	50	108	93		
21	CH,	CH_3	Ph	OH	100	103	66	88	103
22	OH	H	CH_3	CH_3	100	37	81	52	101

their color brown at the concentration of 50 ppm. Against rice it showed the inhibitory effect similarly, but weaker than against radish. On the other hand, it was found that at the concentration of 10 ppm remarkable stimulating effect for the growth of radish roots and stalks came about. Similarly 2-phenylguanidino-4,6-dimethylpyrimidine (No. 9) showed the same activity for radish, but not for rice. It is thought that it might have a specificity for dicotyledonary plants. In other 4,6-dimethylpyrimidines hydroxyguanidino-(No. 22) and methylguanidino-derivative (No. 13) showed some degree of inhibitory effect, but others had a slight or no effect.

(2) **Effect on the pupation and the emergence of housefly**

(a) Materials and method

About 100 larvae of ***Musca domestica*** on the second day after hatching were placed in a beaker containing ***25 g*** of artificial diets (powdery yeast : bran = 1:1), 25 ml of water and the test compound (25 mg or 15 mg), and reared at 26 "C. When the pupation began, the pupae were counted every day. The collected pupae were kept in a beaker covered by gauze at 26°C and the emerged adults were counted.

(b) Results and discussion

The degree of the pupation was shown by the percentage against control on 4th day and final. Similarly those of the emergence were shown by the percentage on 10th day and final. These are shown in Table 7. The pupation was not inhibited finally by the tested compounds, but delayed by some compounds,

Table 7. The effects of the guanidinopyrimidines for the pupation and the emergence of housefly.

Sample* No.	Conc. (ppm)	Molting Rate (%)			
		Pupation		Emergence	
		4 days	final	10 days	final
1	500	118	100	188	111
2	500	125	100	218	111
3	500	130	300	183	108
4	500	100	99	201	105
6	500	140	104	150	114
7	500	133	104	130	117
8	500	102	105	13	86
9	500	96	100	99	90
10	500	70	98	16	99
11	500	55	97	5	101
12	500	33		18	110
13	300	63	105 101	100	114
14	500	93		54	98
15	500	160	104 104	160	112
16	300	60	104	46	70
17	500	96	99	162	109
18	500	110	99	235	105
19	500	88	99	56	94
20	300	70	105	70	lii
21	500	100	105	150	101

* ref. to Tables 1, 2 and 3

or stimulated by others. In general, it might be said that 4,6-dimethylpyrimidine derivatives made the pupation delay, except 2-morpholinoguanidino-4,6-dimethylpyrimidine (No. 15) by which the pupation seemed to be most accelerated. On the other hand, 4-hydroxy-6-methylpyrimidines seemed to accelerate it. As to the emergence it could not be discussed about delay and acceleration, because it is considered that if the pupation was delayed the emergence would be naturally delayed, too. But as to some compounds shown no effect against the pupation, such as 2-(*p*-chlorophenyl)guanidino-4-hydroxy-6-methyl-pyrimidine (No. 4) and 2-piperidinoguanidino-4-hydroxy-6-methylpyrimidine (No. 8), the effect were able to be discussed ; the former accelerated development of wings and the latter delayed. The mortal effect was not seen in general, but 2-piperidinoguanidino-derivatives (No. 8, 16) had it somewhat.

(3) **The cytotoxicity to cultured cell lines**

(a) Materials and method

(i) Cell lines: The used cell lines were EATC (Ehrlich ascites tumor cell), RRLC-11 (tumor cell of rat) and RFL (normal cell of rat).

(ii) Medium: The medium used in this test was Eagle's MEM, for which calf serum was supplemented 20 % in case of EATC or 10 % in cases of RRLC-11 and RFL.

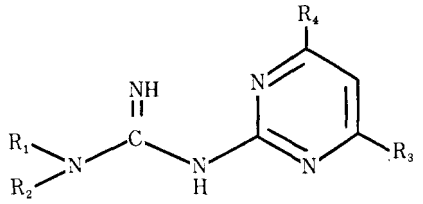
(iii) Method : The water soluble sample was dissolved in PBS (−) and added into the test tube containing cultured cells to make a certain concentration. The sample insoluble in water was dissolved in dimethylsulfoxide (DMSO). This solution was added into the two days cultured cell suspension to make a certain concentration. These were cultured at 37°C and the medium exchange was made on the second day. After 4 days from additions of tested compounds, cells were stained and counted.

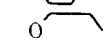
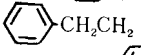
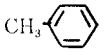
(b) Results and discussion

The results are shown by the percentage of the multiplication against the control, in Table 8. Although this experiment was mostly performed at the level of higher concentration, such as 1,000 or 10,000 ppm, some compounds, like 2-(β-phenethyl)guanidino-4,6-dimethylpyrimidine(No.10), showed the inhibitory activity to the multiplication of cells. And it might be said that in their action it seemed to be no specificity for tumor cells or normal cell.

Urbansky *et al.* (1967) reported about the values of $LD_{,,}$ for mice of several arylguanidinopyrimidine derivatives. As the result they concluded that those compounds were of low toxicity in general. This agrees with our results; tested compounds seemed to have only slight toxicity, compared with the toxicity of Mitomycin C.

Table 8. The cytotoxicity of the guanidinopyrimidines to cultured cell lines.



Sample No.	R_1	R_2	R_3	R_4	Solvent	Dose (ppm)	Growth Rate (%) Cell line EATC	RRLC-11	RFL
1		H	OH	CH_3	DMSO	10000	103	—	**110**
4		H	OH	CH_3	DMSO	10000	100	—	**88**
7			OH	CH_3	PBS(-)	1000	89	75	—
					PBS(-)	100	104	88	—
9		H	CH_3	CH_3	DMSO	10000	49	—	*104*
10		H	CH_3	CH_3	DMSO	10000	17	—	*10*
11		H	CH_3	CH_3	DMSO	10000	43	37	*47*
					DMSO	1000	100	—	—
12		H	CH_3	CH_3	DMSO	10000	33	—	14
16			CH_3	CH_3	DMSO	10000	61	—	*85*
19		H	OH		DMSO	10000	43	—	*65*
21		CH_3	OH		DMSO	10000	85	92	*84*
					DMSO	1000	107	—	—
22	OH	H	CH_3	CH_3	DMSO	10000	41	—	—
					PBS(-)	100	13	—	—
	Mitomycin C				PBS(-)	10	63	—	—

PBS(-) : Phosphate buffer saline(—Ca, —Mg)

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