Control of Seedling Growth by Pseudopurine and Pyrimidine Derivatives

Maekawa, Kazuyuki
Laboratory of Pesticide Chemistry, Faculty of Agriculture, Kyushu University

Takeya, Ryuko
Laboratory of Pesticide Chemistry, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/23490

出版情報：九州大学大学院農学研究院紀要. 21 (2/3), pp.99-105, 1977-03. Kyushu University

J. Fac. Agr., Kyushu Univ., 21, 99-105 (1977)

Control of Seedling Growth by Pseudopurine and Pyrimidine Derivatives

Kazuyuki Maekawa and Ryuko Takeya

Laboratory of Pesticide Chemistry, Faculty of Agriculture,
Kyushu University 46-02, Fukuoka 812

(Received November 15, 1976)

About 60 pseudopurine and guanidino-pyrimidine derivatives were scrutinized for the effects on the seedling growth of rice, radish, barnyard grass, and *Atriplex gmelinii*. It was found that 5-thiocyano and 5-methylthio derivatives of triazolo [1, 5-*a*] pyrimidine derivatives (A) inhibited strongly the growth of the root of the rice seedling. As compounds repressing leaves and stalks, some derivatives of thiadiazolo [3,2-a] pyrimidine(B) were found. 2-Thioacetic acid derivative of (A), 2-thiobenzyl, and alkanesulfinyl derivatives of (B) inhibited markedly barnyard grass, but not rice. On the other hand, compound 15 and 48 inhibited only the germination of radish.

In order to develop some artificial controllers for seedling growth, a number of base analogs of nucleic acid was examined for their biological effects on certain plants.

Guanidine derivatives were considered at first, because it was thought that they probably combined electrostatically with electronegative constituents of plant cells. Namely, about 20 pyrimidine derivatives and related compounds which contain a guanidine group and other reactive side chains were adopted.

On the other hand, about 40 pseudopurine derivatives prepared as analogous substances of kinetin and indoleacetic acid were also applied. The effects of these compounds upon rice, radish, barnyard grass, and a kind of goosefoot ***(Atriplex gmelinii)*** were investigated in detail,

EXPERIMENTAL

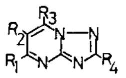
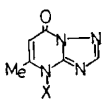
Methods and Materials

Twenty seeds were used after immersing them in water for 1 day (3 days for barnyard grass). These seeds were kept at 25°C for a certain time interval in a dark room and seedlings were measured to evaluate inhibiting or stimulating effects. The incubating duration period was 4 days for radishes, 11 days for rice, and 7 days for barnyard grass and *Atriplex* ***gmelinii.*** Seeds of barnyard grass and another plant were used after they had been dormant by keeping them for 2 weeks at 3-5°C.

Compounds applied were synthesized by the authors and collaborators (Okabe ***et al., 1972,*** 1973; Shuto ***et al.,*** 1974). Since these preparations were not so soluble in water, they were adopted as an acetone (or ethanol) solution. Namely, each

5 mg of the compound was dissolved in 50 ml of acetone (or ethanol), 10 ml of which was poured on a sheet of filter paper placed on the bottom of a Petri dish. After evaporating the solvent, 10 ml of water was added to the dish (corresponds to 100 ppm), followed by adding seeds. The experiments were repeated with the solutions of variant concentrations.

Table 1. Derivatives of triazolo [1, *5-a]* pyrimidine.

No		Compound R_1 R_2 R_3 R_4	Conc. (ppm)	Growth Rate (%) Rice Roots	Rice Stalks	*Atriplex gmelinii* Roots	*Atriplex gmelinii* Stalks	Barnyard grass Roots	Barnyard grass Stalks
1		Me H Cl H	500	120	70	100	100		
			10	320	70	100	100		
			’	70	70	100	100		
2		Me H SCN H	500	10	75				
			100	90	85	20	40	75	90
			10			120			
3		Me H SMe H	500	40	55				
			100	70		120	100		
			1	80					
4		Me H Me $SCH_2\phi Cl$	100					85	70
5		Me H Me $S(CH_2)_2OH$	100	90	130			150	70
6		Me H Me $S(CH_2)_2COOH$	100	120	120	145[1]	95[1]	100	95
7		Me H Me $SCH_2\phi$	100	60	85	90[1]	100[1]	75	100
8		Me H Me SCH_2COOH	100						50
9		Me H OH $SCH_2\phi Cl$	100	100	190	115[1]	105[1]	30	60
10		Me H OH $SCH_2\phi NO_2$	100						75
11		Me H ϕ $SCH_2\phi Cl$	100	145	130			100	80
12		Me H ϕ $SCH_2\phi$	300	90	105	115[1]	90[1]	60	95
13		Me H ϕ $S(CH_2)_2OH$	100	90	110			60	75
14		Me H ϕ SH	100	175	100	145[1]	110[1]	65	95
15		H H H H	50	85	100	0[1]	0[1]	100	100
16		$X=CH_2\phi$	500	35	65	100	100		
			10	80	65	100	100		
			1	80	65	100	100		
17		$X=CH_2CH=CH_2$	500	55	70	100	100		
			100	75		100	100		

[1] against radish.

RESULTS

Experimental results were summarized in Tables 1, 2, and 3.[1] In general, triazolo [1,5-a] pyrimidine derivatives among the pseudopurine derivatives, as seen in Table 1, showed the inhibitory effect on rice seedlings, but had no effect on *Atriplex gmelinii*. On the other hand, the compounds belonging to thiadiazolo [3,2-*a*] pyrimidine are generally more effective to barnyard grass than to

[1] The herbicidal activity of some compounds listed in these Tables was already partly reported in connection with other biological activities.

Table 2. Derivatives of thiadiazolo [3, 2-*a*] pyrimidine and related compounds.

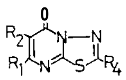
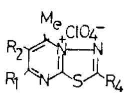
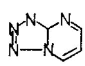
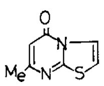
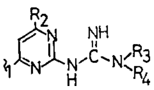
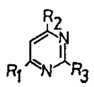
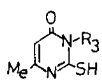
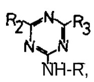
No		Compound R_1 R_2 R_4	Conc. (ppm)	Growth Rate (%) Rice oots	Rice Stalks	Radish Roots	Radish Stalks	Barnyard grass Roots	Barnyard grass Stalks
18		Me H $SCH_2\phi$	100						25
19		Me H Me	100	190	195	80	90	120	95
20		Me H ϕ	100	70	100				85
21		Me Cl Me	100	70	65			60	
22		Me Cl SOMe	100	100	95	30	50	80 45	75 75
23		Me H SOMe	100	110	90	60	105	110	100
			50					90	100
			20					115	105
24		Me Cl H	100	25	20	50	55	95	85
25		Me Cl Et	100	155	45	75	80	110	95
			50					115	120
			20					90	115
26		Me Cl iso-Pr	100	8	15	20	45		
27		Me Cl SMe	100	105	90	85	80	145	65
			50					150	120
28		Me H H	100	165	65	80	80	115	75
29		Me H Et	100	75	95	35	50	70	105
30		Me H SMe	100	95	90	105	95	105	100
			50					90	115
			20					110	95
31		Me H SEt	100	75	90	85	95	60	85
32		Me H Cl	100	0	40	2	20	0	25
			50	45	75	50	65	3	50
			20	105	95	100	100	9	85
33		ϕ H Et	100	70				50	70
34		Me Br Me	100	80	105 90	80 15	80 55	95	95
35		Me Et H	100	70	75	135	95	95	100
			50					90	95
			20					70	55
36		H CO_2Et H	100						75
37		H CO_2Et Et	100	90	100	80	110	95	90
38		Me H SO_2-Et	100	65			95	0	55
			50	105	80 90	65	95	90	120
			20	100	90			95	85
39		Me H SOiso-Pr	100	110	105	55	70	0	60
			50	100	85			35	105
			20	120	115			70	130
40		Me H Me	100	65	50	115	110	50	55
41		Me H H	100	120	100	110	95	80	55
42		H H Me	100	150	70			75	50
43			100	90	105	70	80	45	80
44			100	90	95	40	75	95	95

Table 3. Derivatives of pyrimidine and related compounds.

No	Compound	R_1	R_2	R_3	R_4	Conc. (ppm)	Growth Rate (%) Rice Roots	Rice Stalks	Radish Roots	Radish Stalks	Barnyard grass Roots	Barnyard grass Stalks
45		Me	OH	$(CH_2)_2\phi$	H	50			115	110		
46		Me	OH	Me	H	50			100	90		
47		Me	OH			100	20	90	125	100	55	95
									20			
48		Me	Me	ϕ	H	100	100	105	45	45	75	a5
						50	115	95		55		
						10			1.50	110		
49		Me	Me	$(CH_2)_2\phi$	H	100	10	75		50	5	58
						50	45	85	¯8	50	20	57
						10			170	125		
50		Me	Me	Me	H	100	105	80	35	95	45	50
51		Me	Me			50			a5	90		
52		ϕ	OH	Me	H	50			110	95		
53		ϕ	OH	Me	Me	100				65	110	105
54		Me	Me	OH	H	100	90	100	105	80	lo	45
55		Me	OH	SMe		500	30					
						100	20	40	40	90	60	a5
						10	1'					
						1	1:20	130				
56				N = C(Et)NHEt		100	80	95	90	100	65	55
57				NHCOEt		100	135	115	a5	100	50	70
58		Et	NH-Et	-S(O)Me		100	50	70	75	95	50	a5
59		H	ϕ	-SMe		100	150	105	75	80	90	55
60		H	ϕ	-S(O)Me		100	1'				20	50
						50	1:15	196	50	80	55	110
						20					80	95

rice. The typical examples were shown in compounds 18, 22, 42, especially 38 and 39.

However, a compound 32 inhibited every seedling of plants indiscriminately and strongly (Table 2). Furthermore, among some derivatives substituted with halogen, 2-substituted derivatives were more effective than 6-substituted ones.

4, 6-Dimethylpyrimidine derivatives, as shown in Table 3, had inhibitory effect on the germination of radish. Especially, 2-(β-phenethyl)-guanidino-4, 6-dimethylpyrimidine showed the strongest inhibitory effect on a radish seedling. However, the effect on rice seedlings was weak. 2-Phenylguanidino-4, 6-dimethylpyrimidine also inhibited only radish seedlings. Therefore, it was concluded that these compounds had the specificity against dicotyledonous plants.

Since a root appears at first followed by a sprout in germination, it is reasonable to conclude that when the growth of the root is bad, growth of the sprout consequently becomes bad. However, in some cases, when the growth of the root was very poor, growth of the leaves was not so bad, and was rather stimulated in an early stage of the germination. To such a group, (a), belong compounds 2-5, 7, 9, 17, 20, 39, 49, 53, and 54. Especially, compound 2 had remarkable effect on the growth of roots.

As the activity of other compounds was generally weak, and yet a great part of them consisted of lipophilic substituent, the action of these compounds might not be due to covalent combination with a biological active center.

On the other hand, it was elucidated that markedly inhibitory compounds against roots have $-NH-C(=NH)-NH-CH_2-CH_2-Ø$ or -NH-C(=NH)-NHOH group at 2-position of the pyrimidine ring. This series of compounds might combine with the anionic site of the target.

On the contrary, there are a number of compounds which stimulate roots, and inhibit leaves and stalks. To this group, (b), belong compounds 1, 10, 14, 25, 28, 41, and 42. By application of compound 14, roots of radishes became quite elongated, but leaves and stalks changed little.

The compounds involved in the feed back control mechanism were compounds 32, 38, and 55.

As for the different responses among plants, very clear results were obtained. To the group (c), which does not inhibit rice plants too much, but inhibits strongly other plants, belong compounds 4, 8, 10-14, 18, 22, 41, and 42. Compounds 38 and 39 inhibited strongly roots, especially that of barnyard grass. In general, the compounds belonging to (c) were a great many.

To the group (d), which stimulates barnyard grass, belong compounds 5 and 27. However, the compounds belonging to (d) were generally few.

It was deduced from the comparison of compounds 5 and 8 that rice seedlings have no ability oxidizing $-S-(CH_2)_2-OH$ of R_4 in compound 5, while barnyard grass has its ability. Due to this ability, barnyard grass oxidizes $-S-(CH_2)_2OH$ of 5 and is killed by its metabolite.

On the other hand, among pyrimidine derivatives there are many compounds which act on radish more strongly than on rice. Namely, the compounds having the phenylguanidine side chain such as 48 and 49 showed a strong inhibitory effect on radish seedlings.

Furthermore, to the group (e) (inactive to ***Atriplex gmelinii:*** repress rice) belong compounds 1, 3, 16, and 17. Triazolopyrimidines in which R_3 is Cl or $S-CH_3$, are not effective on ***Atriplex gmelinii,*** different from the effect on a rice seedling. N^8-substituted derivatives such as compounds 16 and 17 showed also no effect on ***Atriplex gmelinii.***

DISCUSSION

Present knowledge of the entity of seed germination is rather unsatisfactory. Information is very incomplete, scattered among work on a limited number of plant species (Mayer and Shain, 1974). However, it is apparent that protein synthesis and RNA synthesis occur quite early in germination and that DNA replication is rather late (Black and Richardson, 1965; Fowden, 1963; Shain and

Mayer, 1965 ; Yatsu and Jacks, 1968).

The activation of protein synthesis upon imbibition may be due to the formation of polysomes from preexisting ribosomes and m-RNA, resulting from a series of reactions requiring ATP (Marcus, 1969) or a ribosomal dissociation into sub-units before that attachment of the m-RNA (App *et al.,* 1971).

There is general agreement that at least ribosomal RNA synthesis is resumed quite early after imbibition and as early as *2* hours after the onset of imbibition in isolated wheat embryos (Chakravorty, 1969; Frankland *et al.,* 1971; Julien *et al.,* 1970).

Proteolytic enzymes might function in releasing long-lived, possibly masked, RNA during the early stages of germination (Mano and Nagaro, 1970). Early increase in cytochrome c reductase has been shown in lettuce and other seeds (Eldan and Mayer, 1972).

It was concluded that the axis possibly may secrete a cytokinin (Penner and Ashton, 1967b) that regulates the formation of proteolytic enzymes in the cotyledon (Penner and Ashton, 1967a).

The onset of proteolytic activity seems to be controlled in some cases by hormone action, by cytokinin in the embryo in the case of *Cucurbita maxima* and by gibberellic acid in the aleurone of barley (Jacobsen and Varner, 1967; Wiley and Ashton, 1967).

A large number of hydrolytic and phosphorylatic enzymes were secreted well before the appearance of a-amylase (Pollard, 1969). Some of these secretions could also be evoked by the application of cyclic AMP (Duffus and Duffus, 1969 ; Kamisaka and Masuda, 1971; Pollard, 1970 ; Pollard and Venere, 1970).

It is well known that 5-phosphoribosyl-1-pyrophosphate-amidotransferase prepared from adenocarcinoma 755 cells is inhibited strongly by 6-thiomethylpurine or 6-benzylthiopurine, and its mechanism is ascribed to a feed back inhibition (Hill and Bennett, 1969). The effectiveness of compound *2 or* 3 in the germination of plants might be due to the inhibition of their amido-transferase.

Since the mechanism of seed germination in the early stages is still obscure, how these pseudopurines and pyrimidines act and at what stage and what kind of inhibition, also remains unexplained. However, some of these derivatives, such as for repressing roots or, on the contrary, enormously stimulating them, or showing different responses on wild plants and closely related cultivated species, might be useful tools for biochemistry of plants and for control techniques.

Lastly, a more detailed study of the large variety of related compounds may provide clues to the understanding of the control of seed germination.

REFERENCES

App, A. A., M. G. Bulis and W. J. McCarthy 1971 Dissociation of ribosomes and seed germination. *Plant Physiol., 47: 81-86*

Black, M. and M. Richardson 1965 Promotion of germination in light-requiring seed by chloramphenicol. *Nature, 208 : 1114-1115*

Chakravorty, A. K. 1969 Ribosomal RNA synthesis in the germinating black eye pea (*Vigna* *unguiculate). Biochem. Biophys. Acta, 179: 67-82*

Duffus, C. M. and J. H. Duffus 1969 A possible role for cyclic AMP in gibberellic acid triggered release of a-amylase in barley endosperm slices. *Experientia, 25: 581*

Eldan, M. and A. M. Mayer 1972 Evidence for the activation of NADH-cytochrome c reductase during germination of lettuce. *Physiol. Plant., 26: 67-72*

Fowden, L. 1963 Amino acid analogs and the growth of seedlings. *J. Exptl. Botany, 14:* 387-398

Frankland, B., B. C. Jarvis and J. H. Cherry 1971 RNA synthesis and the germination of light-sensitive lettuce seeds. *Planta, 97* : 39-49

Hill, D. L. and L. L. Bennett 1969 Purification and properties of 5-phosphoribosyl pyrophosphate amidotransferase from adenocarcinoma 755 cells. *Biochemistry, 8: 122-130*

Jacobsen, J. V. and J. E. Varner 1967 Gibberellic acid-induced synthesis of protease by isolated aleurone layers of barley. *Plant Physiol., 42:* 1596-1600

Julien, R., F. Grellet and Y. Guitton 1970 Synthèse des RNA au cours de la germination du *radis. Physiol. Plant., 23: 323-334*

Kamisaka, S. and Y. Masuda 1971 Stimulation of gibberellin-induced germination in lettuce seeds by cyclic 3′, 5′-adenosine monophosphate. *Plant Cell Physiol., 12:* 1003-1005

Mano, Y. and H. Nagaro 1970 Mechanism of release of maternal messenger RNA induced by fertilization in sea urchin eggs. *J. Biochem. (Tokyo), 67: 611-628*

Marcus, A. 1969 Seed germination and the capacity for protein synthesis. *Symp. Soc. Exp. Biol., 23:* 143-160

Mayer, A. M. and Y. Shain 1974 Control of seed germination. *In* "Annual Review of Plant Physiology," Vol. 25, ed. by W. R. Briggs, P. B. Green and R. L. Jones, Ann. Rev. Inc., Palo Alto, California, pp. 167-193

Okabe, T., K. Maekawa and E. Taniguchi 1973 Syntheses of thiadiazolopyrimidine and related compounds. *Agr. Biol. Chem., 37:* 1197-1201

Okabe, T., E. Taniguchi and K. Maekawa 3972 Syntheses of triazolopyrimidine derivatives from amitrole and their biological activity. *Sci. Bull. Fac. Agr., Kyushu Univ.,* 26 : 105-115

Okabe, T., E. Taniguchi and K. Maekawa 1973 Reaction of 2-amino-5-substituted-1, 3, 4-thiadiazole with 1, 3-dicarbonyl compound. *J. Fac. Agr., Kyushu Univ.,* 17 : 195-202

Okabe, T., E. Taniguchi and K. Maekawa 1973 Syntheses of s-triazolo [4,3-a] pyrimidine derivatives. *Agr. Biol. Chem., 37:* 441-443

Penner, D. and F. M. Ashton 1967a Hormonal control of isocitrate lyase synthesis. *Biochem. Biophys. Acta, 148: 481-485*

Penner, D. and F. M. Ashton 1967b Hormonal control of proteinase activity in squash cotyledons. *Plant Physiol., 42* : 791-796

Pollard, C. J. 1969 A survey of the sequence of some effects of gibberellic acid in the metabolism of cereal grains. *Plant Physiol., 44:* 1227-1232

Pollard, C. J. 1970 Influence of gibberellic acid on the incorporation of 8-^{14}C adenine into adenosine 3′, 5′-cyclic phosphate in barley aleurone layers. *Biochem. Biophys. Acta, 201: 511-512*

Pollard, C. J. and K. J. Venere 1970 Gibberellin action and 3′, 5′-cyclic AMP metabolism in aleurone layers. *Fed. Proc., 29:* 670 Abs. No. 2386

Shain, Y. and A. M. Mayer 1965 Proteolytic enzymes and endogenous trypsin inhibitor in germinating lettuce seeds. *Physiol. Plant.,* 18: 853-859

Shuto, Y., E. Taniguchi and K. Maekawa 1974 Synthesis of guanidinopyrimidine derivatives and their biological activities. *J. Fac. Agr., Kyushu Univ.,* 18: 221-237

Wiley, L. and F. M. Ashton 1967 Influence of the embryonic axis on protein hydrolysis in cotyledons of *Cucurbita maxima. Physiol. Plant., 20: 688-696*

Yatsu, L. Y. and T. J. Jacks 1968 Association of lysosomal activity with aleurone grains in plant seeds. *Arch. Biochem. Biophys., 124: 466-471*