SYNTHESIS AND CYTOKININ-AGONISTIC AND ANTAGONISTIC ACTIVITIES OF SUBSTITUTED PYRROLO[2,3-d]PYRIMIDINES: DEVELOPMENT OF ANTICYTOKININS

Hajime Iwamura

Department of Food Sciences and Technology, Faculty of Agriculture,

Kyoto University, Kyoto 606, Japan

Recent studies of cytokinin antagonists were reviewed, with special attention on substituted pyrrolo[2,3-d]pyrimidines. Principles for the design of anticytokinin-active compounds, their specific antagonistic nature, agonist-antagonist descrimination and chemical structure/activity relationship in terms of steric bulk of side chain and the utilization of anticytokinins in studies of plant growth are the major objects discussed.

1. Development of Anticytokinins
   - 1.1 4-Substituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]-pyrimidines
   - 1.2 4-Substituted-2-methylpyrrolo[2,3-d]pyrimidines
2. Utilization of Anticytokinins in Studies of Plant Growth
   - 2.1 Enhancement of bud-formation(cell differentiation) of tobacco callus tissue
   - 2.2 Effects on *Amaranthus* betacyanin synthesis and lettuce seed germination

2.3 Determination of active form of cytokinins

2.4 Growth retardation of intact plants by anticytokinins

Chemical researches on cytokinins started in 1955 with the isolation of kinetin from old or heated DNA(1) which induced cell division in certain excised plant tissues in the presence of exogenously supplied auxin. Characterization of its structure as 6-furfurylaminopurine promted the extensive syntheses of related substances as well as the efforts to isolat the kinetin-like substances in plants. Naturally occurring cytokinins have N^6-substituted adenine structure and much evidence has been accumulated for their important role in the regulation of cell division, cell enlargements, cell differentiation and organogenesis in developing plants.

Earlier synthetic works on the structure/activity relationship of cytokinins have shown that an intact purine ring is necessary for high growth promoting activity, and thus most attention has been placed on the modification of N^6-side chain. Among the compounds which have been synthesized and gave a positive response in the cytokinin tests, N^6-substituted adenine derivatives with 4-6 carbon atoms in the side chain tend to be highly active. Introduction of a double bond, an aromatic nuclei or a heteroatom in the side chain modifies the cytokinin activity. Within a certain range of the modification in the side chain of N^6-substituted adenine derivatives, all of the compounds hitherto reported exhibited more or less cytokinin agonistic activity but none of the compounds of this type has been reported to be antagonistic.

Relatively few cytokinin analogs with alteration in the purine ring have been tested for cytokinin activity. Among these, 8-azakinetin and 6-benzyl-amino-8-azapurine have activity similar to kinetin(2). In general, however, the modification of the central purine ring system decreases the activity

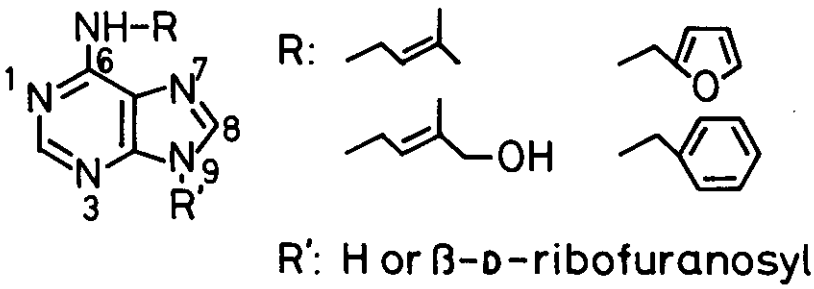
drastically. It is quite recent that the structural modification of this type yielded specific cytokinin antagonists, i.e. anticytokinins.

Development of antagonists as well as agonists is of potential value in studies of the mechanism of action of biologically active substances. Firstly, the development of antagonists itself contributes to recognition of structural differences associated with intrinsic activity and binding affinity, especially when both agonists and antagonists have a common structural basis. Secondly, antagonists extend the studies of agonist action by blocking its utilization in biological systems. The latter is of particular importance in studies of cytokinins because most plant systems grow without added exogenous cytokinins. Dwarf plants and certain plant tissues require added hormones for optimal growth and normal development. An antagonist would thus be of use in studies on the mode of action of exogenous hormone agonists.

1. Development of Anticytokinins

In view of the importance of cytokinin antagonists for the studies of action of cytokinins, several researchers have sought cytokinin-antagonistic substances among purine analogs such as 2,6-diaminopurine, 8-azaguanine and 8-azaadenine(3, 4), some of which are known as growth inhibitors. Their inhibitory activity was, however, very weak and specific antagonistic nature could not be evidenced. Later in 1971, Hecht *et al.* prepared a series of 7-substituted-3-methylpyrazolo[4,3-d]pyrimidines as structural analogy of 6-(3-methyl-2-butenylamino)purine(6-isopentenyladenine), a potent cytokinin, and tested them for cytokinin and anticytokinin activity by tobacco callus growth test(5). The principle used for the design of these compounds was reportedly that of the preparation of enzyme inhibitors; namely the

modification of a normal substrate would render it ineffective as a substrate but the modified compound may still possess enough potential to participate in the complex formation with the receptor site. Certainly the compounds with 7-alkyl substituents with 4-7 carbon atoms exhibited strong growth inhibitory activity(5, 6). Their specific anticytokinin nature has been discussed but it remained qualitative. More later in 1974, Iwamura *et al.* reported a 2nd type of anticytokinin, the 7-deaza analog of kinetin riboside, i.e. 4-furfurylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine(7). Subsequently a significant number of substituted pyrrolo[2,3-d]pyrimidine derivatives has been prepared and tested for their activity as structural analogy of cytokinin-active N^6-substituted adenines(8, 9, 10), and evidence has been being accumulated for their potential usefulness in studies of cytokinin action and of chemical structure/activity relationships. This review will thus deal with the problems associated with the pyrrolo[2,3-d]pyrimidine (7-deazapurine) analogs of N^6-substituted adenylate cytokinins.



REPRESENTATIVES OF N^6-SUBSTITUTED ADENYLATE CYTOKININS

1.1 4-Substituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidines

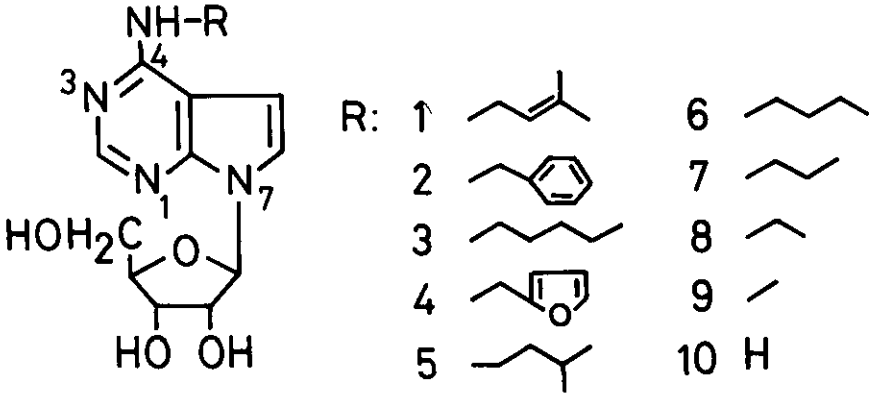
Recent development in the biochemistry of cytokinins has shown that a wide variety of plants converts cytokinins to their 7-glucosides(11, 12). Since these metabolites are themselves active as cytokinins and are highly resistant to enzymatic degradation, it has been suggested that the 7-glucosyl compounds may be the active form(11) or the storage form(12) of cytokinins. If this conversion process is indispensable to evoke the growth responce, the 7-deaza analogs of cytokinins may occur as possible cytokinin inhibitors because the structure is sufficiently similar to allow participation in the same type of receptor-substrate complex with cytokinins but lack of a nitrogen atom at the 7-position will prevent the successive glucosylation. Although the physiological significance of the 7-glucosyl metabolites are presently still unclear, the above consideration, together with the hitherto accumulated results on the heterocyclic analogs of cytokinins which drastically modifies the activity(13, 14), prompted us to prepare the 7-deaza analogs of cytokinins.

Pyrrolo[2,3-d]pyrimidine analog of cytokinins initially prepared as a potential anticytokinin was 4-furfurylamino-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidine, **4**, the 7-deaza analog of kinetin riboside(7). This compound was obtained by treatment of the known 4-chloro-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidine(15) with furfurylamine at reflux temperature in *n*-butanol.

For the test of the cytokinin and anticytokinin activity, the tobacco tissue was grown on media containing various concentrations of kinetin and the test compounds. At zero concentration of kinetin, compound **4** did not show any growth promoting activity, showing that it is ineffective as a cytokinin. It exhibited strong growth inhibitory effect, however, against kinetin when applied at concentrations of more than 3μM. At the concentrations below 3μM

of compound 4, the inhibitory effect was counteracted by kinetin(Fig. 1).

The success in preparation of the compound 4 having interesting growth inhibitory activity prompted the preparation of additional analogs in which the 4-substituent was systematically varied(8). Table 1 summarizes the compounds prepared and tested. Similar to the compound 4, none of the substances showed significant growth promoting activity in the concentration range tested(0-40μM). Compounds 1-3 showed the strongest growth inhibitory effect against 0.5μM kinetin among the compounds listed in Table 1. Because it has previously been shown that the N^6-substituted adenines which have the 3-methyl-2-butenyl(isopentenyl), benzyl and pentyl side chains are highly active cytokinins(2), it seemed reasonable to expect the above results. In line with this , the inhibitory activity of furfuryl, 4, isopentenyl, 5, butyl, 6, propyl, 7, and ethyl, 8, derivatives was expected to decrease in this order; namely, if the site of anticytokinin action of these compounds is related to the mechanism through which cytokinins themselves exert their effect, the structure/activity relationship for the adenylate cytokinins would be valid for the pyrrolo[2,3-d]pyrimidine series of anticytokinins as well. Table 1 summarizes the biological results with the reported data



of the corresponding adenylate cytokinins. The N^4-alkylated pyrrlo[2,3-d]-pyrimidine derivatives could be arranged in the order of cytokinin activity of the corresponding adenine derivatives without significant inconsistency with the order of their anticytokinin activity. The lack of anticytokinin activity of the methylamino derivative, 9, strongly supports the above idea because the corresponding methyladenine is not active as a cytokinin.

Formal removal of the side chains gives compound 10, tubercidin, which is known as antimicrobial and cytotoxic(16). Thus the apparent anticytokinin activity of this compound in Table 1 is considered to belong to that of

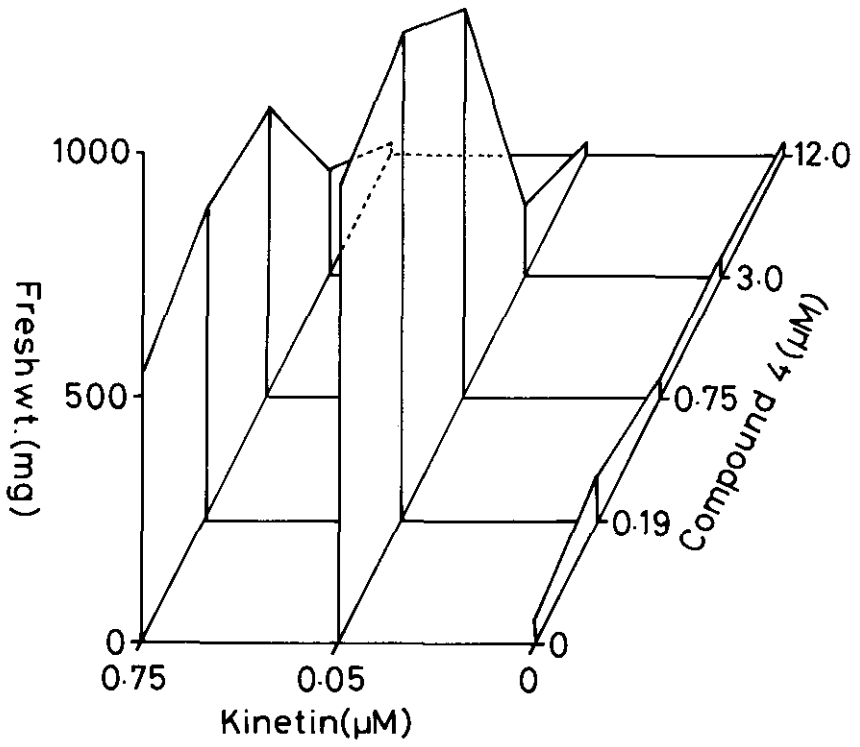
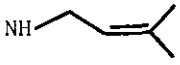
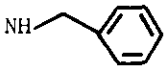
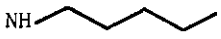
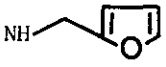
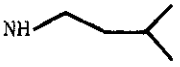
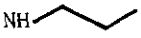


Fig. 1. Yield of tobacco callus cultured on serial combination of kinetin and compound 4.

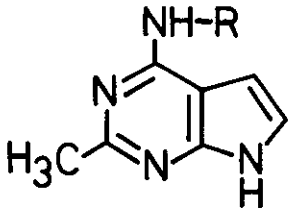
Table 1. Relation of the Structure of 4-Substituents to Anticytokinin Activity of 7-(β-D-Ribofuranosyl)pyrrolo[2,3-d]pyrimidines

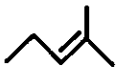
Compound No.	4-Substituent	Anticytokinin activity	Cytokinin activity of the corresponding adenine derivatives[2] Maximum yield(μM)
1		+ + +	0.02
2		+ + +	0.07
3		+ + +	0.1
4		+ +	0.1
5		+ +	0.1
6		+ +	0.1 - 0.5
7		+ +	5
8		+	12.5
9		-	∞
10	NH_2	+ + +	∞

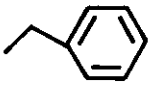
non-specific growth inhibitors. At the same time, it is also possible that the series of active 7-deaza adenine derivatives may exert their growth inhibitory activity after the degradation *in vivo* of alkyl side chain, i.e. the really active species may be tubercidin. If the side chains were removed after incorporation into the tobacco tissues, however, methyl derivative, 9, should exert as strong a growth inhibitory effect as tibercidin. Alternatively the side chains may be eliminated during autoclaving, but this possibility is also unlikely since compound 4 exhibited the activity after filter-sterilization as well as after autoclaving. All of these results means that the growth inhibitory activity of the N^4-substituted pyrrolo[2,3-d]pyrimidine ribosides is not due to the tubercidin-like cytotoxicity and suggests that the site of their action is closely related to the processes through which N^6-adenylate cytokinins express their activity.

1.2 4-Substituted-2-methylpyrrolo[2,3-d]pyrimidines

Shortly after the finding of the anticytokinin activity of the 4-substituted 7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidines, Skoog *et al.* determined the strong growth inhibitory activity of 4-substituted-2-methylthiopyrrolo-[2,3-d]pyrimidines in the tobacco callus bioassay(9). Iwamura *et al.* found that the deribosylated series of the first pyrrolo[2,3-d]pyrimidine anti-cytokinins(8), i.e. 4-substituted pyrrolo[2,3-d]pyrimidines, act as cytokinins (17). These results thus appear to indicate that the substituted pyrrolo[2,3-d]-pyrimidines, as the heterocyclic analogs of adenylate cytokinins, behave interestingly as both agonists and antagonists, and the introduction of a substituent into 2- or 7-position appears to be important for the conversion of the activity. To test this further and to obtain more information on the agonist-antagonist relationship, we prepared a series of 4-substitutedamino-



R: 11 

12 

13 

14 

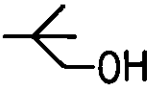
15 

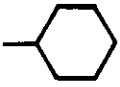
16 

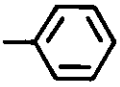
17 

18 

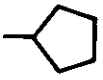
19 

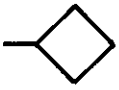
20 

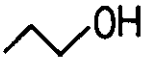
21 

22 

23 

24 

25 

26 

2-methylpyrrolo[2,3-d]pyrimidines and tested for their cytokinin and anti-cytokinin activity(10).[1]

The series of compounds was synthesized by treatment of the common intermediate, 4-chloro-2-methylpyrrolo[2,3-d]pyrimidine(19), with the appropriate amine at reflux temperature in *n*-butanol. As shown in Table 2 and Fig. 2, compounds 11, 12, 13 and 14 showed cytokinin activity in the tobacco callus growth test. Compounds 15-25 were inactive as cytokinins but inhibited the growth of callus tissue cultured on a medium containing 0.05μM kinetin(Fig. 3). Of these compounds, the cyclopentyl, 24, and cyclobutyl, 25, derivatives were most active showing detectable inhibition at 0.01-0.04μM. Characteristic of this series of comounds was the fact that the activity varied from agonistic to antagonistic with the systematic transformation of the side chains. Because the molecular structure is altered only in the N^4-side chain, the variation in the activity can be attributed to the structural modification of the substituents. Compound 14 is considered to be a borderline case and to have fairly good binding affinity at the receptor site of cytokinins but weak intrinsic activity. Even though it exerts weak cytokinin activity by itself, it hinders the binding of the agonist at the receptor site when mixed with a stronger cytokinin like kinetin.

The fact that the compounds which have a branching at α position to the exocyclic nitrogen atom(compounds 21-25) exhibited strong antagonistic activity is suggestive of the participation of a steric factor in the agonist-antagonist

---

[1] Preliminarily, Iwamura *et al.* observed anticytokinin activity in the tobacco callus bioassay of 4-substituted-7-methylpyrrolo[2,3-d]pyrimidines, where the ribofuranosyl moiety of the 4-substituted-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidines(8) had been replaced by methyl(18).

discrimination as well as the structure/activity relationship. Thus, we surveyed steric parameters currently used for analyses of quantitative structure/activity relationships and found that the maximum width, W_{max}, of substituents from the bond-axis between the exocyclic nitrogen atom and its α carbon atom illustrates the variation of the activity.

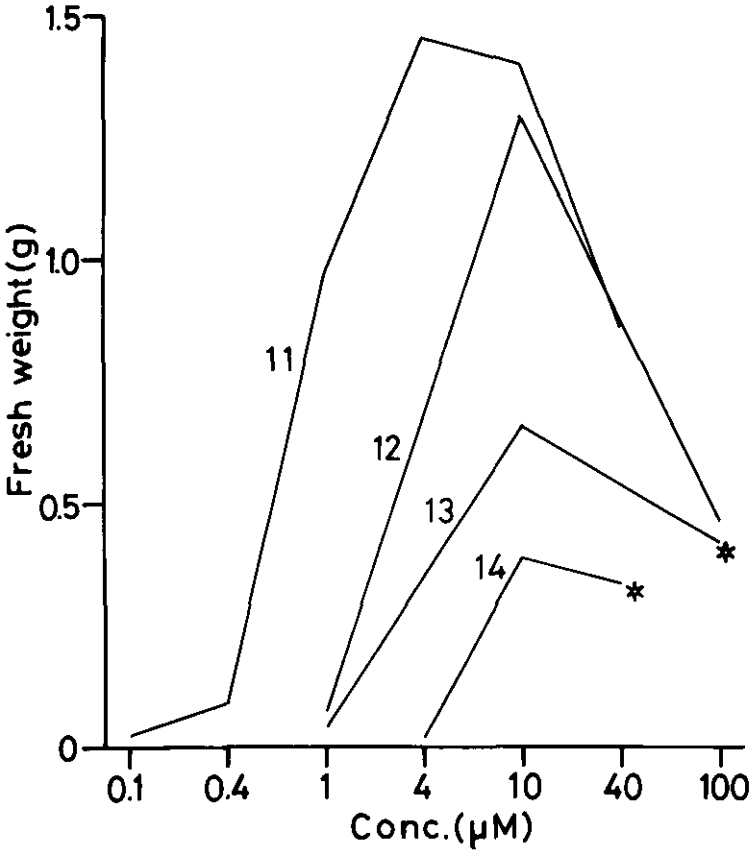


Fig. 2. Effects of compounds 11, 12, 13 and 14 on the fresh weight yield of tobacco callus. *Treatment which buds formed.

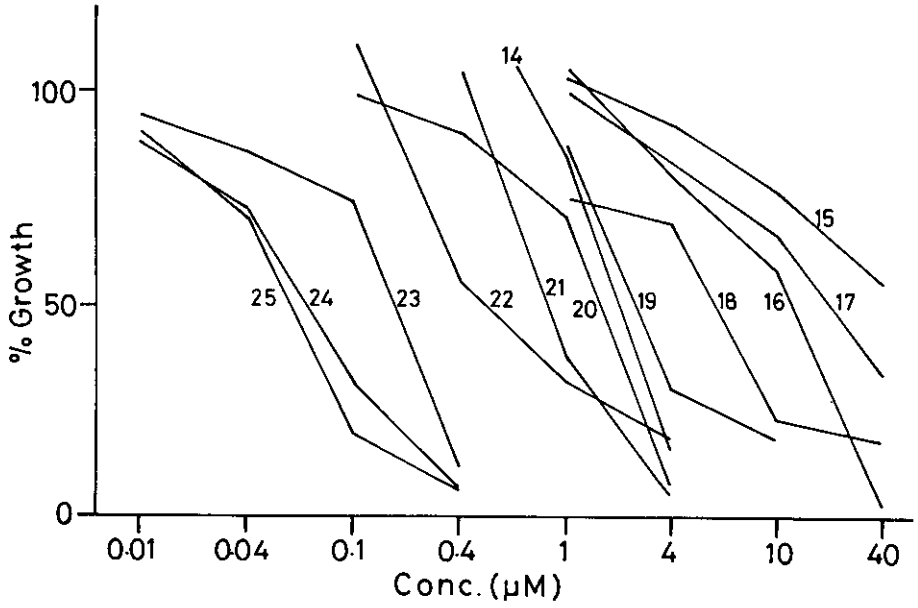


Fig. 3. Effects of compounds 14-25 on the fresh weight yield of tobacco callus cultured on medium containing 0.5μM kinetin.

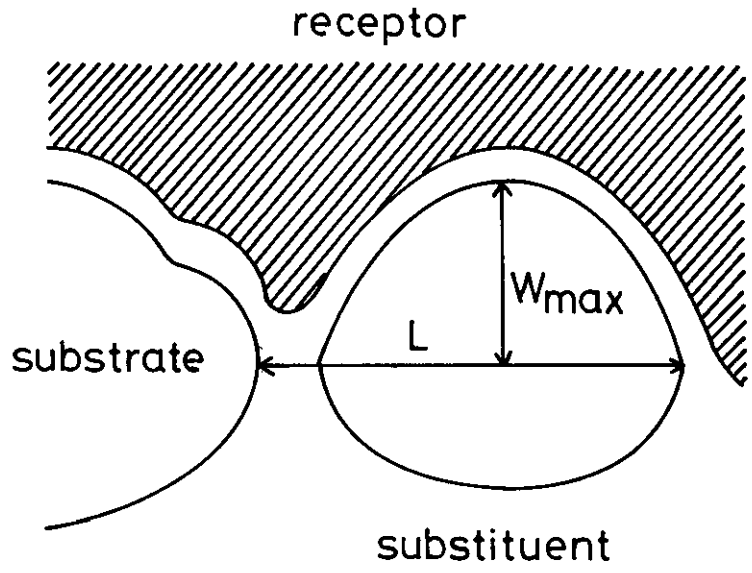
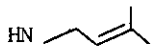
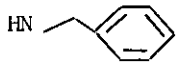
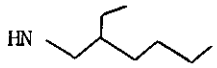
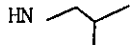
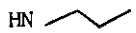
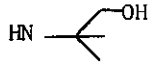
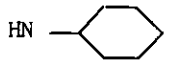
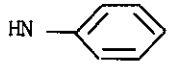
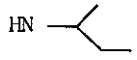
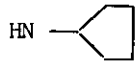
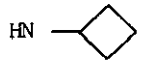


Table 2. Effect of 4-Substituted-2-methylpyrrolo[2,3-d]pyrimidines on Tobacco Callus Growth.

Compound No.	N^4-Substituent	Cytokinin activity Maximum responce (μM)	Anticytokinin activity I_{50}(μM) against 0.05μM kinetin	Maximum width(W_{max}) of N^4-substituent[a] (Å)
11		4.0		4.72
12		10.0		6.02
13		10.0		4.92
14		10.0	2.0	5.43
15			40.0	8.78
16			12.5	7.33
17			20.9	4.21
18			6.0	4.15
19			3.0	3.49
20			2.0	3.38
21			0.9	3.49
22			0.5	3.11
23			0.3	3.49
24			0.07	3.98
25			0.06	3.83

[a]Calculation was based on the fully extended conformation.

Table 2 indicates that the W_{max}[1] values of the cytokinin agonists 11, 12, 13 and 14 are within the range of 4.7-6.0. Those in which the W_{max} values are smaller than this range, e.g. compounds 17-25, and larger than 7.0, e.g. compounds 15 and 16, are cytokinin antagonists. The range of W_{max} values of the former nine compounds is 3.0-4.5 and the value for the most active, cyclopentyl derivative, 25, is 3.83. Thus there seems to exist an activity maximum depending upon the steric bulk of N^4-substituents. The *n*-decyl derivative, 15, is weaker in antagonistic activity than the *n*-octyl derivative, 16, suggesting that their W_{max} values exceed the depth of the hypothetical receptor cavity in which the N^4-substituents are engulfed.

Substituent parameters other than a steric factor, e.g lipohydrophilic ans electronic factors, may participate in the structure/activity relationship, although their contribution is considered to be less important and the exact characterization remains for future study. In general, however, it can be said from the above results that the compounds whose W_{max} values are smaller than 4.21 of compound 17 or larger than 7.33 of compound 16 do not possess intrinsic activity.

An introduction of a quantitative measure is common for the analyses of biologically active substances. However, auxins are the only class of plant hormones hitherto analyzed in this sense(21, 22, 23). The finding of the applicability of the steric parameter W_{max} would enable a quantitative approach also in the analysis of cytokinin activity.

---

[1]The W_{max} values is equivalent to the B_4 parameter recently developed by Verloop, Hoogenstraaten and Tipker based on the Corey-Pauling-Koltan models (20). The values were calculated by the STERIMOL program(20) kindly sent by Dr. Hoogenstraaten.

2. Utilization of Anticytokinins in Studies of Plant Growth

2.1 Enhancement of bud-formation(cell differentiation) of tobacco callus tissue

Cytokinins and other plant hormones generally have multiple function in intact plants and even in cultured cells. In addition to the promotion of cell division and growth, cytokinins cause differentiation of cultured cells, i.e. bud formation and rooting under appropriate conditions. When the cytokinin/auxin ratio is increased buds are formed, and when the ratio is decreased roots are initiated. These effects are thus strongly suggestive of an important role for cytokinins in the control of plant morphorogy as well as cell division.

As shown in Fig. 2, two 4-substituted-2-methylpyrrolo[2,3-d]pyrimidines, compounds 13 and 14, caused bud formation at 100μM and 40μM respectively, at the auxin(indole-3-acetic acid) concentration of 11.4μM which is generally utilized in the tobacco callus growth test(10). The fact that, despite of their very weak cytokinin activity, compounds 13 and 14 caused marked budding under conditions where buds are usually not formed strongly suggests the different cytokinin receptor sites between the two functions.

Skoog *et al.* have described the enhancement of cytokinin-promoted budding by anticytokinin derivatives, 4-cyclopentylamino- and 4-cyclohexylamino-2-methylthiopyrrolo[2,3-d]pyrimidines, at lower(1-10μ) concentrations but at high levels of added cytokinins(9). Thus the mode of action appears to differ from that of compounds 13 and 14 despite the similarity of the structure. At any rate, these results seem to indicate the possibility of differentiating the two functions of cytokinins, callus growth promotion and bud formation, in terms of chemical structure.

2.2 Effects on *Amaranthus* betacyanin synthesis and lettuce seed germination

N^6-Substituted adenylate cytokinins induce the formation of betacyanin pigments in the hypocotyls of *Amaranthus* when incubated at 25° for 18hr in the dark(24). All of the 4-substituted-2-methylpyrrolo[2,3-d]pyrimidines including the strong anticytokinins in the tobacco bioassay showed more or less agonistic activity except for compounds 20 and 26 . These results are considered to indicate species difference of receptor molecules(10).

In the lettuce seed germination test, four compounds of 4-substituted-2-methylpyrrolo[2,3-d]pyrimidine series, i.e. 3-methyl-2-butenyl, 11, benzyl, 12, *n*-amyl, 13, and phenyl, 22, derivatives, exerted significant agonist activity and the other compounds were little active as both agonists and antagonists, indicating again the species difference. Striking were the results obtained with the phenyl derivative, 22, in the three bioassay systems described above, i.e. it exhibited anticytokinin activity in the tobacco callus bioassay, slight activity in the *Amaranthus* betacyanin synthesis test and cytokinin activity in the lettuce germination test. This indicates the possibility of designing and synthesizing compounds having high selective activity between species(10).

2.3 Determination of active form of cytokinins

As to the question of the necessity and/or role of the ribosyl moiety of the 9-position of exogenously added cytokinins, Hecht *et al.* prepared 8-aza-9-deaza derivatives of isopentenyladenine(7-(3-methyl-2-butenylamino)pyrazolo[4,3-d]-pyrimidine) and its 9-methyl and 9-β-D-ribofuranosyl derivatives, and they identified their cytokinin activity by the tobacco callus assay(25). Since these derivatives possess a carbon rather than a nitrogen atom at 9-position of purine ring and thus ribosylation or deribosylation is unlikely, they suggested that the cytokinins function without ribosylation, i.e. the active form of cytokinin is the base itself. Their conclusion does not, however, exclude the possible

interaction of ribosylated form of cytokinins at the receptor site although their intrinsic activity may be weak. In fact, the 8-aza-9-deaza derivative of isopentenyladenosine was active although very weak at 1μM level as a cytokinin despite the unlike scission of the C-C glycosyl bond.

Iwamura *et al.* prepared a series of 4-substituted pyrrolo[2,3-d]pyrimidines and identified their cytokinin activity by the tobacco callus bioassay(Fig. 4) (17). The results show straightforwardly that the series of compounds exerted the cytokinin activity without ribosylation at the 7-position which is analogus to the 9-position in adenine. If glycosylation occurred at the 7-nitrogen atom, the resulting compounds should behave as anticytokinins.

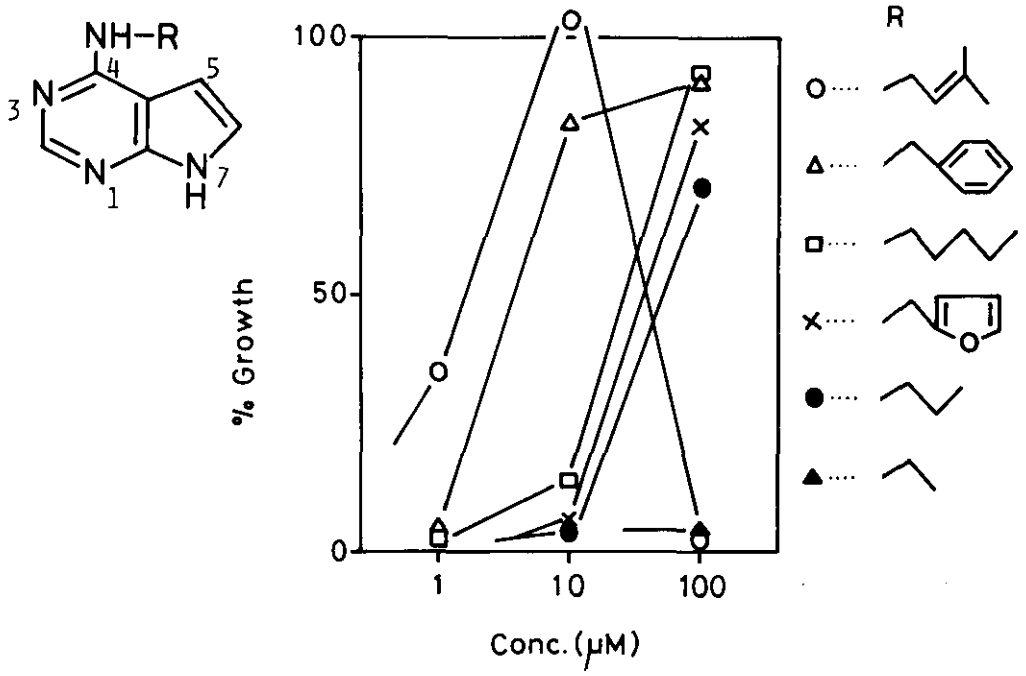


Fig. 4. Effects of 4-substituted pyrrolo[2,3-d]pyrimidines on the fresh weight yield of tobacco callus.

The corresponding ribosylated series of the compounds (1-8) were anticytokinins. Because they are considered to possess a fairly good binding affinity at the receptor site of cytokinins, the active form of cytokinins is not necessarily considered to be the deribosylated base.

Table 3. Herbicidal Activity of Anticytokinins by the Pre-emergence Test[a]

Compound			Rice		Sawa millet		Cucumber	
No.	4-Substituent	ppm	100	30	100	30	100	30
2	NH-Bz[b]		5	4	5	3	3	3
3	NH-*n*-Am		4	4	4	3	4	3
4	NH-Fu		4	2	4	2	3	2
5	NH-*i*-Am		5	4	5	3-4	4	2
6	NH-*n*-Bu		5	4	5	4	4-5	3
7	NH-*n*-Pr		4-5	4	5	4	3	2
8	NH-Et		3-4	1	3-4	1-2	4	2

[a]Method: Test solution (6ml) was pipetted into a Petri dish (φ 7cm) in which 5 germinated rice (*Oryzae sativa* L. var. "Nihonbare"), 10 germinated sawa millet (*Panicum crus-galli* L. var. *frumentaceum* Hook, *f*) grains or 3 cucumber (*Cucumis sativa* L. var. "Tokiwa") seeds were placed and incubated at 27° under fluorescent light for 5 days. The degree of growth inhibition was expressed as follows; 0, no inhibition; 1, 10% or less; 2, ~30%; 3, ~60%; 4, ~90%; 5, ~100% inhibition, respectively.

[b]Abbreviations: Bz, benzyl; Am, amyl; Fu, furfuryl; Bu, butyl; Pr, propyl; Et, ethyl.

2.4 Growth retardation of intact plants by anticytokinins

Developments in the synthesis of anticytokinins immediately suggest their usage as plant growth regulators or herbicides. As a part of the investigations on the practical usage as well as physiological properties of anticytokinin derivatives, the herbicidal activity of 4-substituted-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine anticytokinins was preliminarily examined(26).

In the pre-emergence test, as shown in Table 3, the series of the anticytokinins showed significant growth retarding activity at 100ppm. At 30ppm, however, the activity of ethyl and furfuryl derivatives was inferior to the others. Similar results were obtained in the submerged pot test as well(data not shown) where the inhibitory activity of *n*-butyl, *n*-amyl, *i*-amyl and benzyl derivatives were conspicuous. On the other hand, all of the anticytokinins tested exhibited very weak or little growth retarding activity in the foliar treatment test. This result appears to be physiologically interesting. Considering the fact that recent advances in the studies of anticytokinins have revealed the particular nature of pyrrolo[2,3-d]pyrimidine derivatives as a structural analogy of N^6-substituted adenines, continued studies on the syntheses of the derivatives as well as the examination of their physiological properties will bring about new anticytokinins which are useful in horticulture.

ACKNOWLEDGMENT The author wishes to express his sincere thanks to Professor Koichi Koshimizu for his support of this work. A part of the results described in this article was obtained by collaboration with Professor Zenzaburo Kumazawa, and most of the plant bioassays were carried out in the laboratories of Professor Satoshi Matsubara and Professor Yukiyoshi Ogawa.

REFERENCES

1 C. O. Miler, F. Skoog, M. H. von Saltza, and F. M. Strong, J. Amer. Chem. Soc., 1955, 77, 1392.

2 F. Skoog, H. Q. Hamzi, A. M. Szweykowska, N. J. Leonard, K. L. Carraway, T. Fujii, J. P. Helgeson, and R. N. Loepky, Phytochemistry, 1967, 6, 1169.

3 D. F. Blaydes, Physiol. Plant., 1966, 19, 748.

4 F. Moewas, Science, 1959, 130, 921.

5 S. M. Hecht, R. M. Bock, R. Y. Schmitz, F. Skoog, and N. J. Leonard, Proc. Natl. Acad. Sci. USA, 1971, 68, 2608.

6 F. Skoog, R. Y. Schmitz, R. M. Bock, and S. M. Hecht, Phytochemistry, 1973, 12, 25.

7 H. Iwamura, T. Ito, Z. Kumazawa, and Y. Ogawa, Biochem. Biophys. Res. Commun., 1974, 57, 412.

8 H. Iwamura, T. Ito, Z. Kumazawa, and Y. Ogawa, Phytochemistry, 1975, 14, 2317.

9 F. Skoog, R. Y. Schmitz, S. M. Hecht, and F. B. Frye, Proc. Natl. Acad. Sci. USA, 1975, 72, 3508.

10 H. Iwamura, N. Masuda, K. Koshimizu, and S. Matsubara, Phytochemistry, in press.

11 J. E. Fox, J. Cornette, G. Deleuze, W. Dyson, C. Giersak, P. Niu, J. Zapata, and J. McChesney, Plant Physiol., 1973, 52, 627.

12 C. W. Parker, and D. S. Letham, Planta, 1973, 114, 199.

13 J. H. Rogozinska, C. Kroon, and C. A. Salemiuk, Phytochemistry, 1973, 12, 2087.

14 Y. Trigoe, M. Akiyama, M. Hirobe, T. Okamoto and Y. Isogai, Phytochemistry, 1972, 11, 1623.

15 R. L. Tolman, R. K. Robins, and L. B. Townsend, J. Amer. Chem. Soc., 1969,

91, 2102.

16 R. J. Suhadolnik, 'Nucleoside Antibiotics', Wiley-Interscience, New York, 1970, pp. 315-323.

17 H. Iwamura, M. Kurimoto, S. Matsubara, Y. Ogawa, K. Koshimizu, and Z. Kumazawa, Agric. Biol. Chem., 1976, 40, 1885.

18 H. Iwamura *et al.* unpublished data.

19 R. A. West, and L. Beauchamp, J. Org. Chem., 1961, 26, 3809; Brit. Pat. 915,303(Welcome Foundation Ltd, 1963); Chem. Abstr., 1963, 59, 3937g.

20 A. Verloop, W. Hoogenstraaten, and J. Tipker, Drug Design, 1976, 7, 165.

21 C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, F. Geiger, and M. Streich, J. Amer. Chem. Soc., 1963, 85, 2817.

22 R. M. Muir, T. Fujita, and C. Hansch, Plant Physiol., 1967, 42, 1519.

23 W. L. Porter, and K. V. Thimann, Phytochemistry, 1965, 4, 229.

24 N. L. Biddington, and T. H. Thomas, Planta, 1973, 111, 183.

25 S. M. Hecht, R. M. Bock, R. Y. Schmitz, F. Skoog, N. J. Leonard, and J. L. Occolowitz, Biochemistry, 1971, 10, 4224.

26 H. Iwamura, Z. Kumazawa, S. Nagato, and S. Okuda, Agric. Biol. Chem., 1976, 40, 1653.

Received, 22nd September, 1978