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Cytokinin Activity of Pyrimidine Derivatives

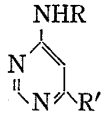
Cytokinin activity of several aminopyrimidine derivatives were tested in the tobacco callus bioassay. Substituents at N^4 and 6 positions of 4-aminopyrimidine were changed. Some derivatives were found to promote cell devision of the tobacco callus.

Keywords——cytokinin; tobacco callus; plant hormone; amino pyrimidine; structure-activity relationship; tissue culture

Cytokinin active compounds with a variety of structures have been studied in relation to the structure-activity relationship.[1,2] In the course of our study searching potent cytokinin active compounds with non-purine or modified purine structures,[3,4] we found a cytokinin active urea derivative, N-phenyl-N′-(2-chloro-4-pyridyl)urea, whose activity in the tobacco callus bioassay is more than ten times stronger than the activity of 6-benzylaminopurine (BA) or kinetin.[5] In this communication, we wish to report the biological activity of some pyrimidine derivatives in the tobacco callus bioassay. It may be quite interesting from the standpoint of structure-activity relationships that the pyrimidine moiety of purine cytokinins is suggested to be an important structural part for the emergence of the cytokinin activity.

Compounds tested were prepared from 4,6-dichloropyrimidine with amines by conventional methods. All of them satisfied spectral properties and elemental analyses. A signifi-

Table I. Cytokinin Activity of 4-Aminopyrimidine Derivatives in the Tobacco Callus Bioassay

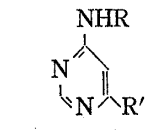


Compound	R=	R′=	Optimum conc. M (ppm)	Maximum callus yield[a]
1	n-C_4H_9	H	Inactive	
2	n-C_4H_9	Cl	5.4×10^{-5} (10)	+
3	$C_6H_5CH_2$	H	Inactive	
4	$C_6H_5CH_2$	Cl	2.3×10^{-5} (5)	+
5	$C_6H_5CH_2$	NH_2	5.0×10^{-5} (10)	++
6	$C_6H_5CH_2$	$NHCH_3$	4.7×10^{-5} (10)	+++
7	$(CH_3)_2C{=}CHCH_2$	H	6.1×10^{-5} (10)	++
8	$(CH_3)_2C{=}CHCH_2$	Cl	5.0×10^{-6} (1)	+++
9	C_6H_5CO	H	5.0×10^{-5} (10)	++
10	C_6H_5CO	Cl	4.3×10^{-5} (10)	+++
11	C_6H_5NHCO	H	4.7×10^{-6} (1)	+++
12	C_6H_5NHCO	Cl	4.0×10^{-7} (0.1)	+++
13	H	H	Inactive	
14	H	Cl	Inactive	
6-Benzylaminopurine (BA)			4.4×10^{-8} (0.01)	+++

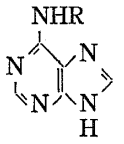
a) Number of + corresponds to the fresh weight of the callus.

1) T. Okamoto, *Chemical Regulation of Plants*, **10**, 59 (1975).
2) N. Leonard, *Recent Advance Phytochem.*, **7**, 21 (1974); N. Leonard, in "The Chemistry and Biochemistry of Plant Hormones," V. Runeckles, E. Sondheimer, and D. Walton ed., Academic Press, New York, 1973, pp. 147—184.
3) Y. Torigoe, M. Akiyama, M. Hirobe, T. Okamoto, and Y. Isogai, *Phytochem.*, **11**, 1623 (1972).
4) T. Okamoto, K. Shudo, and Y. Isogai, "Plant Growth Substances," Hirokawa Co., Tokyo, 1973, pp. 447—455.
5) S. Takahashi, K. Shudo, T. Okamoto, K. Yamada, and Y. Isogai, *Phytochem.*, in press.

cant structural relation exists between these aminopyrimidines and N^6-substituted aminopurine cytokinins such as BA and zeatin: these pyrimidine derivatives can be regarded as incomplete aminopurine derivatives lacking the imidazole ring. The cytokinin activity was tested by tobacco callus bioassay which was described earlier,[3] in the presence of 2 ppm of indole-acetic acid. The fresh weight of callus was weighed after 30 days, incubation. The experiments were repeated more than three times and quite reproducible.



R=alkyl
aralkyl
acyl
R′=H, Cl, NH_2, $NHCH_3$



R=benzyl (BA)
$CH_3(CH_2OH)C=CHCH_2$-(zeatin)

4-Butylaminopyrimidine (**1**) and 4-benzylaminopyrimidine (**3**) were completely inactive in the assay.[6] An introduction of a chlorine atom at their 6-position (compounds **2** and **4**) effected in the appearance of weak cytokinin activity. They gave a maximum callus yield at 10 ppm with lower callus yield than BA. Compounds **5** and **6** with an amino or a methylamino group, respectively, at the 6-position as the substituent showed the cytokinin activity giving good callus yield at the optimum concentration of 10 ppm. 4-Isopentenylaminopyrimidine (**7**), a compound related to zeatin, was active at the concentration of 10 ppm, and an introduction of a chlorine atom at the 6-position (compound **8**) enhanced the activity. The optimum concentration of **8** was 1 ppm and a maximum callus yield was as good as BA. 4-Benzamidopyrimidine (**9**) and its 6-chloro derivative (**10**) were also active at 10 ppm. This activity is close to that of 6-benzamidopurine.[7]

A chlorine atom or an amino group at the 6-position is not always necessary for the appearance of the activity. However, the presence of these electronegative atom or group at the position seems to enhance the cytokinin activity. This result has been observed in the case of N-(4-pyridyl)-N′-phenylurea, where the introduction of a chlorine atom at the α position to the pyridine nitrogen caused a surprising increase in activity.[5] 4-(3-Phenylureido)-pyrimidine (**11**) and its 6-chloro derivative (**12**) were active. The presence of the chlorine atom enhanced the activity similarly to the above cases.

Cytokinin active substances so far known can be classified to three structural types: purine derivatives, modified purine (aza and deaza purine) derivatives, and urea (and probably amido)[4] derivatives. Here we wish to add aminopyrimidines as the fourth groups of compounds with significant cytokinin activity. At present, we believe these groups can be structurally correlated each other. The discussion on the structure-activity relationships of various cytokinins will be summarized in future.

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6) H. Segal, C. Hedgcoth, and C. G. Skinner, *J. Med. Pharm. Chem.*, **5**, 871 (1962). They reported the inactivity of these compounds in the seed germination test, and suggested that the purine nucleus would be essential for producing analogs which possess kinetin-like activity.
7) Unpublished result.