Communications to the Editor

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4-Pyridylureas are surprisingly Potent Cytokinins. The Structure-Activity Relationship

Cytokinins initiate or promote cell division and cell differentiation of plants. N-(4-Pyridyl)-N′-phenylureas with an electronegative substituent(s) at positions 2 and 6 of the pyridine ring are the strongest cytokinins so far known. Structure-activity relationships between urea- and purine-cytokinins were discussed.

Keywords——cytokinin; pyridylurea; benzyladenine; tobacco callus; tissue culture; structure-activity relationship

Cytokinins are defined as compounds which initiate or promote cell division and cell differentiation of plants.[1] Many adenine derivatives (purine-cytokinins), such as kinetin, ^{6}N-benzyladenine (BA) and zeatin are known as cytokinins.[2] Several purine analogs also show the cytokinin activity.[2,3] On the other hand, though N,N′-diphenylurea (DP) was reported to be responsible for the cytokinin activity of coconut milk,[4] and many aromatic ureas were found to promote development of pea buds and to retard senescence,[5] these urea cytokinins have often been neglected in considering cytokinins. We confirmed the activity of DP in repeated bioassays using tobacco callus,[3] and subsequently found that N-(4-pyridly)-N′-phenylurea (4PU, **1**: X=H) has strong cytokinin activity on tobacco callus growth.[6] The effective concentration of 4PU is 10 times that of BA. More recently, we found that N-(2-chloro-4-pyridyl)-N′-phenylurea (4PU-30, **1**: X=Cl) has even higher cytokinin activity than BA and kinetin: the optimum concentration of 4PU-30 for callus production is 4×10^{-9} M,[7] while the optimum concentrations of BA and zeatin are 4×10^{-8} M and 4×10^{-9} M, respectively. Vigorous shoot formation occurred with a concentration of 10^{-7} M 4PU-30 in the absence of added auxin.[8] This paper concerning pyridylureas reports the finding of more potent derivatives, and structure-activity relationships between urea- and purine-cytokinins.

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The molecular requirements for high cytokinin activity in aromatic urea derivatives include the presence of a 4-pyridyl, but not a 2- or 3-pyridyl, moiety and an N′-phenyl group. N-Alkylations reduced the activity, and most substituents on the phenyl ring also reduced the activity, but *meta* fluorine (an electronegative atom smaller than other halogens) somewhat enhanced the activity. N-(3-Chloro-4-pyridyl)-N′-phenylurea and its derivatives showed very low activity. Substitution on position 2 of the pyridine ring, as in 4PU-30, greatly enhanced the activity. The cytokinin activities of various derivatives (**1**) having a substituent at the 2-position were tested on tobacco callus cultured on Murashige-Skoog agar medium in the presence of 10^{-5}M indoleacetic acid as an auxin.[3] Growth of the callus was very good in every case, but the optimum concentrations of the tested compounds in the medium ranged from 10^{-6} (**1**: X=NH_2 and OH) to 10^{-9} M (**1**: X=F, Cl, Br, CN, CF_3) (Table I). Compounds with a nonpolar electronegative substituent had high cytokinin activity, while polar substituents suppressed the activity. The similar activities of compounds having X=H and a larger substituent (CH_3), and the strong activity of compounds with a larger substituent (CF_3 and Br) suggested that a steric effect at this position is not important.

Quantitative structure-activity analysis by the Hansch method was applied to confirm this impression. The index of activity chosen was the reciprocal of the optimum concentration

(C, mol) for promotion of growth of callus. The correlation between log ($1/C$) and the Hammet σ_m constants for the substituents is expressed by

$$\log (1/C) = 4.62\ (\pm 1.39)\ \sigma_m + 6.36\ (\pm 0.42)$$
$$(n=11,\ s=0.440,\ r=0.930) \qquad (1)$$

In equation (1) and following equations, n is the number of samples, s is the standard deviation and r is the correlation coefficient. The figures in parentheses are 95% confidence limits. On the other hand, there was no significant correlation between the activity and Taft Es values, a relation that reflects the steric effect. A slight correlation between the activity and hydrophobic parameter π by Hansch is observed ($r=0.703$). Combination of Hammett σ_m and Hansch π improved the correlation:

$$\log (1/C) = 3.81\ (\pm 1.01)\ \sigma_m + 0.52\ (\pm 0.32)\ \pi + 6.50\ (\pm 0.28)$$
$$(n=11,\ s=0.281,\ r=0.975) \qquad (2)$$

This correlation coefficient is excellent, and it also reflects the accuracy of the bioassay used. The equation clearly shows that the major contributor is the electronic effect, and that the contribution of polarity is minor. The calculated values from equation (2) are listed in Table I.

Table I. Cytokinin Activity on Callus Production of N-(2-Substituted-4-pyridyl)-N′-phenylureas

Substituent	Observed (M)	Calculated (M)
H	4.7×10^{-7}	3.2×10^{-7}
CH_3	4.1×10^{-7}	3.2×10^{-7}
Cl	4.0×10^{-9}	4.9×10^{-9}
Br	3.4×10^{-9}	3.3×10^{-9}
F	4.3×10^{-9}	1.4×10^{-8}
CF_3	3.6×10^{-9}	2.0×10^{-9}
NH_2	4.4×10^{-6}	6.0×10^{-6}
OCH_3	4.1×10^{-8}	9.5×10^{-8}
OH	4.0×10^{-7}	2.0×10^{-7}
$NHCOCH_3$	1.9×10^{-7}	1.3×10^{-7}
CN	4.2×10^{-9}	3.3×10^{-9}

The concentrations shown are the concentrations inducing the highest tobacco callus production on Murashige-Skoog medium in the presence of 1.1×10^{-5} M of indoleacetic acid. The callus weight was measured after 30 d incubation. Values were calculated by equation 2.

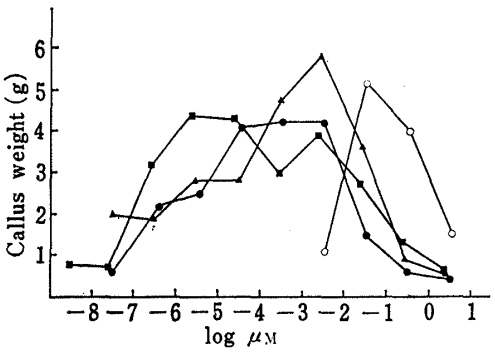

Fig. 1. Dose-response Curves of 2,6-Disubstituted-4-pyridylureas

▲: N-(2, 6-dichloro-4-pyridyl)-N′-(*m*-fluorophenyl)urea.
■: N-(2,6-dibromo-4-pyridyl)-N′-phenylurea.
●: N-(2-chloro-6-methoxy-4-pyridyl)-N′-(*m*-fluorophenyl)urea.
○: [6]N-Benzyladenine.

Since in the compounds tested the steric effect of a substituent at position 2 was very small, we prepared some 2,6-di-substituted 4-pyridylurea derivatives (**2**; X,Y=Cl, Br, OCH_3, CH_3), although we knew that the 2,6-dimethyl derivative (X=Y=CH_3) had weaker activity than 4PU or 2-monomethyl derivative (**1**; X=CH_3).[7] The biological activities, expressed as the optimum concentrations are shown in Table II. Some of

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the compounds showed surprising high activity: the optimal concentrations for promotion of

Table II. Cytokinin Activity on Callus Production of N-(2,6-Disubstituted-4-pyridyl)-N′-phenylureas

X	Y	Optimum concentration (M)
CH_3	CH_3	4.0×10^{-6}
Cl	CH_3	3.8×10^{-9}
Cl	Cl	10^{-8}—10^{-13}
Cl	OCH_3	10^{-8}—10^{-9}
OCH_3	OCH_3	3.7×10^{-8}
Br	Br	10^{-9}—10^{-13}
# Cl	Cl	10^{-9}—10^{-11}
# Cl	OCH_3	10^{-9}—10^{-11}

Values were obtained as described for Table I. Where ranges are shown under optimum concentration, good callus production was obtained throughout the range. (Examples were shown in Fig 1). Compounds with the symbol # have a *m*-fluorophenyl group.

callus growth ranged from 10^{-9} to 10^{-13} M, concentration at which the best callus production was obtained. In particular, N-(2,6-dichloro-4-pyridyl)-N′-phenylurea, N′-(2,6-dibromo-4-pyridyl)-N′-phenylurea and N-(2,6-dichloro-4-pyridyl)-N′-*m*-fluorophenylurea had high activity. The dose response curves for some compounds are shown in Fig. 1. Thus, the activity of the urea cytokinins exceeds that of the most active known cytokinins of adenine type.[8] The activities of the present urea cytokinins are so strong that they should be useful in the biochemical studies, and we are prepared to supply some urea cytokinins to anyone who is interested in using them for this purpose.

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References and Notes

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