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QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP OF CYTOKININ-ACTIVE ADENINE AND UREA DERIVATIVES

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Key Word Index—Cytokinins; N^6-substituted adenines; diphenylureas; phenylureas; structure–activity relationship.

Abstract—The substituent effect of N^6-alkyl and -aralkyl adenines on the promotion of the growth of tobacco callus was analysed quantitatively using physico-chemical substituent parameters and regression analysis. The results indicated an optimum steric condition for activity in terms of the maximum width of the N^6-substituents from the bond-axis connecting the N^6-atom with its α carbon atom. The electron withdrawing effect of the N^6-substituent enhances the activity. The substituent effect on the cytokinin activity of phenyl- and diphenylurea derivatives determined by Bruce and Zwar using the tobacco pith-block assay was also analysed. The results suggest that position-specific steric and hydrophobic effects of aromatic substituents participate in the variation in activity rationalizing the general trend of the activity; *meta* > *para* > *ortho* derivatives, for both series of compounds. The electronic effect is significant for the activity of diphenylureas but not for that of phenylureas which show somewhat different modes of interaction between the two series at the site of action. Based on inferences made from the correlations, hypothetical maps for the mode of interaction of these three sets of compounds at the site of action have been proposed.

INTRODUCTION

Earlier structure–activity studies of N^6-substituted adenines and related compounds have shown that an intact purine ring is necessary for high cytokinin activity [1]; thus, most attention has been paid to modification of the N^6-side-chain. Among the compounds giving a positive response in the cytokinin tests, N^6-substituted adenines with 4–7 carbon atoms in the side chain tend to be highly active [1]. Introduction of a double bond, an aromatic nucleus or a heteroatom in the side chain modifies the activity further [1, 2]. The challenge of synthesizing cytokinins more active than zeatin, the most active cytokinin, was not met by modification of the N^6-side-chain, but by ring substitution. 2-Chlorozeatin is reported to be as active, or slightly more active than zeatin itself [3]. Later the effect of 2-, 8- and 2,8-substituents on the purine ring has been discussed [4] and more recently, modification of the purine ring has led to the development of cytokinin antagonists [5–11]. The effect of aromatic substituents on the activity of benzyladenine in the expansion of radish leaf discs was examined but none of the substituted benzyladenines showed higher activity than the parent compound [12]. To our knowledge no similar investigation has been reported that utilizes the tobacco callus growth test.

Bruce and Zwar [13] prepared some 500 *N*-phenyl- and *N,N'*-diphenyl-urea derivatives and found that *ca* 50% are as active as cytokinins in the tobacco pith assay. The gross structural similarity of *N,N'*-diphenylurea to 6-(3-methyl-2-butenylamino) purine has been noted but the activity of diphenylureas is lower than that of the corresponding 6-phenylureidopurines [14]. Recently, some *N*-phenyl-*N'*-(4-pyridyl)urea derivatives have been reported to be as highly active as cytokinins [15]. The comparative structure–activity relationship for both the adenine and urea types of cytokinins is, however, obscure.

We classified semi-quantitatively the cytokinin-agonistic and antagonistic activities of 4-substituted-2-methylpyrrolo[2,3-*d*]pyrimidines in terms of variations in the steric dimension of the 4-substituents using a steric substituent parameter, W_{max}, which represents the maximum width of the substituent from the bond-axis that connects the N-6 with its α carbon atom [10]. More recently, we correlated quantitatively variations in the anticytokinin activity of 4-substituted-2-methylthiopyrido[2,3-*d*]pyrimidines with the steric and hydrophobic parameters of the 4-substituents using regression analysis [11]. The results strongly indicate the potentiality of the stereochemical parameter for rationalization of cytokinin activity, since both cytokinins and anticytokinins interact with the same receptor molecules.

Here we report the quantitative analysis of the cytokinin activity of N^6-substituted adenines in the tobacco callus bioassay using physico-chemical substituent parameters and regression analysis. To compare the quantitative structure–activity relationship

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with those for phenyl- and diphenyl-urea derivatives, we also analysed the substituent effect on their cytokinin activity as determined by Bruce and Zwar [13].

RESULTS

Substituent parameters

We used the STERIMOL parameters developed recently by Verloop *et al.* [16] to evaluate the steric effect of substituents. The W_{max} parameter is equivalent to Verloop's B_4 and represents in ångström units (10^{-1} nm) the maximum width of the substituents from the bond-axis: the L parameter expresses the substituent length along the bond-axis. The parameters were calculated using the STERIMOL program based on the fully extended (staggered) conformation of the substituents. The hydrophobic parameter, π, and the electronic parameter, σ^*, of the N^6-substituents in adenines were taken from or estimated according to the literature [17, 18]. The σ^* value of 3-methyl-2-butenyl was approximated by that of 2-butenyl. The value of 4-hydroxy-3-methyl-butyl was estimated as $\sigma^*(CH_2OH) \times 0.34$[3], where 0.34 is the transmission factor [19]. The sum of these two σ^* values was taken as that of 4-hydroxy-3-methyl-2-butenyl, the side-chain of zeatin. Values for N^6-substituted benzyl groups were estimated as

$$\sigma^*(CH_2C_6H_5\text{-X}) = \sigma^*(C_6H_5\text{-X}) \times \sigma^*(CH_2C_6H_5)/\sigma^*(C_6H_5)$$

and

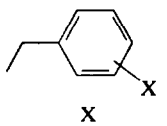
$$\sigma^*(CH_2C_6H_5\text{-}ortho\text{-X}) = \sigma^*(CH_2C_6H_5\text{-}para\text{-X})$$

where X denotes aromatic substituents. The $\sigma^*(C_6H_5\text{-X})$ values were taken from the literature [20]. The Hammett σ value [21] and the π value from substituted acetanilide (Fujita, T., unpublished results) were used for the correlations as electronic and hydrophobic parameters of substituents in phenyl- and diphenyl-ureas. The σ value of the *ortho*-substituents was taken as that of the corresponding *para*-substituents [22].

Quantitative structure–activity relationship

N^6-Substituted adenines. First we analysed the biological data for the 12 compounds in Table 1 (**1a–12a**) that have alkyl and aralkyl substituents of varying dimensions in order to examine the significance of their steric effects. Of the various combinations of steric and other substituent parameters as independent variables, the maximum width parameter

Table 1. Activity and physico-chemical parameters for tobacco callus growth promotion by N^6-substituted adenines

No.	N^6-Substituent	log $1/E_{50}$†(10^{-7}M) Obs.	Calc.†	$\Delta\log 1/E_{50}$	W_{max}	$W_{o,m}$	σ^*
1a	$CH_2CH_2CH(Me)_2$	0.23	−0.16	0.39	4.43		−0.13
2a	$CH_2CH_2CH_2CH(Me)_2$	0.26	0.02	0.24	5.66		−0.13
3a	$CH_2(CH_2)_3CH(Me)_2$	−0.35	−0.05	−0.30	5.88		−0.13
4a	$CH_2CH_2CH(CH_2OH)Me$	0.39	0.31	0.08	4.83		0.02
5a	$CH_2CH{=}C(Me)_2$	0.42	0.50	−0.08	4.72		0.13
6a	$CH_2CH{=}C(CH_2OH)Me$	0.58	0.63	−0.05	5.12		0.15
7a	$CH_2CH_2CH_2Me$	−0.30	−0.16	−0.14	4.42		−0.13
8a	$CH_2(CH_2)_3Me$	0.23	0.04	0.20	4.94		−0.13
9a	$CH_2(CH_2)_4Me$	−0.12	−0.05	−0.07	5.88		−0.13
10a	$CH_2(CH_2)_5Me$	−0.63	−0.32	−0.31	6.39		−0.13
11a	CH_2CH_2Ph	−0.27	−0.02	−0.25	4.00		0.08
	 X						
12a	H	0.56	0.61	−0.05	6.02	0.0	0.22
13a	*m*-OH	0.55	0.04	0.51	6.02	0.93	0.24
14a	*m*-Cl	0.28	0.25	0.03	6.02	0.80	0.30
15a	*m*-Me	−0.37	−0.13	−0.24	6.02	1.04	0.19
16a	*m*-OMe	−0.78	−0.57	−0.21	6.02	1.87	0.24
17a	*m*-CF_3	−0.43	−0.24	−0.19	6.02	1.61	0.32
18a	*o*-Cl	0.42	0.19	0.23	6.02	0.80	0.27
19a	*o*-OMe	−0.46	−0.78	0.32	6.02	1.87	0.14
20a	*o*-CF_3	−0.39	−0.18	−0.21	6.02	1.61	0.35
21a	*p*-Cl	−0.44	−0.60	0.16	7.44	0.0	0.27
22a	*p*-Me	−0.46	−0.41	−0.05	7.11	0.0	0.16

†Concentration at which the 50% callus yield of the maximum response is given.
‡Calculated using equation 3.

W_{max} and the Taft σ^* value as shown in equation 1 are best for correlating the activity.

$$\log(1/E_{50}) = -0.56\ W_{max}{}^2 + 5.84\ W_{max} + 1.66\ \sigma^* - 14.67 \quad (1)$$
$$(\pm 0.29) \quad (\pm 3.04) \quad (\pm 1.02) \quad (\pm 7.81)$$

where

$$n = 12, \quad r = 0.91, \quad s = 0.20.$$

In equation 1 and the following equations, n is the number of compounds included in the analysis, r is the multiple-correlation coefficient and s is the standard deviation. The figures in parentheses are the 95% confidence interval. Examples of the calculation of the W_{max} values are shown schematically in Fig. 1.

Equation 1 shows that there is an optimum steric condition for activity in terms of the maximum width of the N^6-substituents and that the electron-withdrawing substituents favour this activity.

To examine the effect of the ring substituents of substituted benzyladenines, 11 compounds (**12a–22a**) were used to derive equation 2.

$$\log(1/E_{50}) = -0.94 W_{max} - 0.71 W_{o,m} + 6.41 \quad (2)$$
$$(\pm 0.50) \quad (\pm 0.35) \quad (\pm 3.32)$$

where

$$n = 11, \quad r = 0.87, \quad s = 0.27.$$

In this equation, the W_{max} value is defined as in Fig. 1. The value is not changed by introduction of substituents into the *ortho* and *meta* positions. However, the effect of the substituents is specific to the substituent positions, and this position-specific effect is rationalized by the $W_{o,m}$ term. The $W_{o,m}$ value expresses the maximum width or the thickness of the *ortho* and *meta* substituents. The reference of the $W_{o,m}$ value is shifted to that of hydrogen so that

$$W_{o,m}(X) = (\text{width of } X) - (\text{width of } H).$$

Equation 2 shows that the thicker the *ortho* and *meta* substituents, the lower the activity. No particular width effect was observed for *para* substituents. The benzene ring of the substituted benzyl moiety may be twisted around the C_α–C_ϕ axis in various degrees depending on the *ortho* substituents. However, the twisting effect seems to be insignificant for determining the variation in activity. Additions of the W_{max}^2 and σ^* terms singly or together to equation 2 do not improve the correlation. In this set of benzyladenines, the W_{max} value is always higher than the optimum W_{max} value estimated from equation 1. Thus, the W_{max} term in equation 2 may reflect only the supraoptimal 'linear' part of the probable parabolic relation. The variation in the σ^* value is less remarkable than that for substituents in equation 1.

The two sets of compounds were combined, and analysis was performed with the same independent variables as used in equations 1 and 2 to give equation 3.

$$\log(1/E_{50}) = -0.32 W_{max}^2 + 3.35 W_{max} - 0.65 W_{o,m} + 2.03\sigma^* - 8.50 \quad (3)$$
$$(\pm 0.15) \quad (\pm 1.71) \quad (\pm 0.26) \quad (\pm 0.98) \quad (\pm 4.74)$$

where

$$n = 22, \quad r = 0.85, \quad s = 0.26.$$

Figure 2 shows the parabolic dependence of the activity on the W_{max} value for the whole set of compounds. Table 2 shows that any pair of N^6-substituent parameters including the hydrophobicity value π does not show significant colinearity. Thus, the variation in hydrophobicity of the N^6-substituents in this series of compounds does not seem to contribute to the variation in activity. As expected, the optimum W_{max} value, estimated as 5.2 from equation 3, coincides with that estimated from equation 1. The positive coefficient, 2.03, of the σ^* term suggests that the electron withdrawing effect of the substituents in the vicinity of the

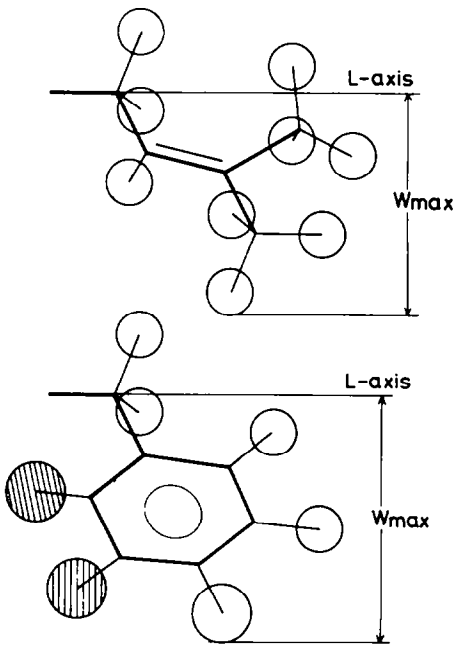


Fig. 1. Schematic representation along the L-axis of 3-methyl-2-butenyl (upper) and benzyl (lower) substituents showing the W_{max} parameter. Maximum width of the shadowed substituents on the benzene ring entered into equations 2 and 3 as $W_{o,m}$.

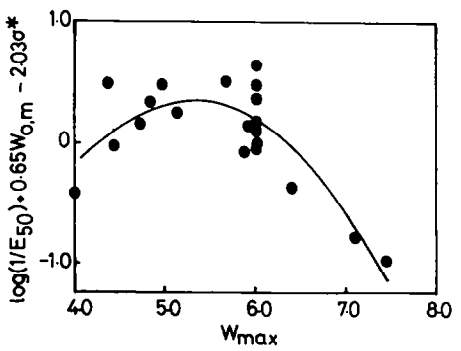


Fig. 2. Parabolic relation of the cytokinin activity of N^6-substituted adenines to W_{max} as expressed by equation 3.

Table 2. Squared correlation matrix for variables of equation 3

	W_{max}	σ^*	$W_{o,m}$
σ^*	0.19	1.0	
$W_{o,m}$	0.08	0.39	1.0
π	0.17	0	0.01

bridge nitrogen atom, perhaps on the hydrogen bond formation at the NH hydrogen with electron donors, enhances activity.

Diphenylureas. The cytokinin activity of diphenylureas, in which one of the benzene rings is unsubstituted, is listed in Table 3 with substituent parameters. As the *meta* substituted compounds were noted to be generally more active than the *ortho* and *para* isomers [13], the substituent effect seems position-specific. Thus, first we analysed the activity data for each of the monosubstituted isomers. Preliminary results indicated that the electronic effect of the substituents is significant but that the steric and hydrophobic effects operate separately, depending on the substituent positions. In elaborating the analyses, we found that the activity data for the di- and tri-substituted compounds can be incorporated into relationships found for monosubstituted derivatives according to the following principles: (i) Polysubstituted compounds having at least one substituent at the *ortho* position are assigned to be the '*ortho*' derivatives regardless of the substituents at the other positions. (ii) Those having substituents at the

Table 3. Activity and physico-chemical parameters for cell-division promotion of tobacco pith-blocks by substituted diphenylureas

No.	Compound	log 1/*C*(mM)		Δlog 1/*C*	σ	L_o	L_p	π_m
		Obs.	Calc†.					
1b	H	2.40	2.00	0.40	0.0	0.0	0.0	0.0
2b	*o*-CF_3	1.44	1.44	0.00	0.54	1.24	0.0	0.0
3b	*o*-OH	1.36	1.09	0.27	−0.37	0.68	0.0	0.0
4b	*o*-Br	0.95	0.71	0.24	0.23	1.77	0.0	0.0
5b	*o*-COOH	0.89	0.84	0.05	0.45	1.85	0.0	0.0
6b	*o*-Cl	0.87	0.97	−0.10	0.23	1.46	0.0	0.0
7b	*o*-F	0.85	1.55	−0.70	0.06	0.59	0.0	0.0
8b	*o*-Me	0.84	1.05	−0.21	−0.17	0.94	0.0	0.0
9b	*o*-I	0.70	0.33	0.37	0.18	2.17	0.0	0.0
10b	*o*-Et	0.55	0.13	0.42	−0.15	2.05	0.0	0.0
11b	*o*-CN	0.38	0.76	−0.38	0.66	2.17	0.0	0.0
12b	*o,m*-diMe	1.38	0.99	0.39	−0.24	0.94	0.0	0.0
13b	*o,p*-diMe	0.86	0.90	−0.04	−0.34	0.94	0.0	0.0
14b	*o,o'*-diMe	0.86	0.90	−0.04	−0.34	0.94	0.0	0.0
15b	*o,p,m'*-triMe	0.77	0.84	−0.06	−0.41	0.94	0.0	0.0
16b	*o,m*-*di*Cl	0.62	1.30	−0.68	0.60	1.46	0.0	0.0
17b	*o,m'*-diCl	0.93	1.30	−0.37	0.60	1.46	0.0	0.0
18b	*o*-Me, *p*-Cl	1.42	1.74	−0.32	0.60	0.94	0.0	0.0
19b	*o*-Me, *p*-NO_2	1.43	1.75	−0.32	0.61	0.94	0.0	0.0
20b	*o*-Me, *m'*-NO_2	1.96	1.69	0.27	0.54	0.94	0.0	0.0
21b	*o*-NO_2, *p*-OMe	1.46	1.29	0.17	0.51	1.38	0.0	0.0
22b	*o*-COOH, *p*-vinyl	1.15	0.82	0.33	0.43	1.85	0.0	0.0
23b	*p*-NO_2	3.10	2.32	0.78	0.78	0.0	1.38	0.0
24b	*p*-COOH	2.40	1.90	0.50	0.45	0.0	1.85	0.0
25	*p*-OMe	1.92	1.23	0.69	−0.27	0.0	1.92	0.0
26b	*p*-COMe	1.92	1.90	0.02	0.50	0.0	2.00	0.0
27b	*p*-OH	1.36	1.48	−0.12	−0.37	0.0	0.68	0.0
28b	*p*-I	1.01	1.57	−0.56	0.18	0.0	2.17	0.0
29b	*p*-OEt	0.71	1.00	−0.29	−0.24	0.0	2.86	0.0
20b	*p*-$N(Me)_2$	0.58	0.85	−0.27	−0.83	0.0	1.47	0.0
31b	*m,p*-diMe	1.89	1.96	−0.07	0.24	0.0	0.94	0.0
32b	*m,p*-diCl	1.96	2.14	−0.18	0.60	0.0	1.46	0.0
33b	*p*-Me	1.06	1.59	−0.53	−0.17	0.0	0.94	0.0
34b	*m*-Br	3.52	3.65	−0.13	0.39	0.0	0.0	1.20
35b	*m*-Cl	3.40	3.47	−0.07	0.37	0.0	0.0	1.05
36b	*m*-NO_2	3.40	3.04	0.36	0.71	0.0	0.0	0.37
37b	*m*-Me	2.52	2.48	0.04	−0.07	0.0	0.0	0.50
38b	*m*-OEt	2.40	2.54	−0.14	0.10	0.0	0.0	0.42

Table 3. (*continued*)

No.	Compound	log 1/C(mM) Obs.	log 1/C(mM) Calcd.	Δlog 1/C	σ	L_o	L_p	π_m
39b	*m,m'*-diMe	2.70	2.42	0.28	−0.14	0.0	0.0	0.50
40b*	*o*-OMe, *p*-NO_2	2.52	0.83	1.69	0.51	1.92	0.0	0.0
41b*	*o*-NO_2	2.40	1.53	0.87	0.78	1.38	0.0	0.0
42b*	*o*-OMe	2.40	0.13	2.27	−0.27	1.92	0.0	0.0
43b*	*o*-NO_2, *p*-Me	>2.4	1.38		0.61	1.38	0.0	0.0
44b*	*o*-OMe, *m'*-NO_2	>2.4	0.77		0.44	1.92	0.0	0.0
45b*	*p*-Cl	3.40	1.81	1.59	0.23	0.0	1.46	0.0
46b*	*p*-Br	0.95	1.72	−0.77	0.23	0.0	1.77	0.0
47b*	*p*-F	>2.4	1.89		0.06	0.0	0.59	0.0
48b*	*m*-OH	2.40	1.43	0.97	0.12	0.0	0.0	−0.62
49b*	*m,m'*-diNO_2	2.52	3.68	−1.16	1.42	0.0	0.0	0.37
50b*	*m,m'*-diCl	>2.4	3.80		0.74	0.0	0.0	1.05
51b*	*o*-Ph	inactive	−1.58		−0.01	4.22	0.0	0.0
52b*	*o,p*-diCl	inactive	1.18		0.46	1.46	0.0	0.0
53b*	*o*-COMe	inactive	0.76		0.50	2.00	0.0	0.0
54b*	*m*-Cl, *p*-Me	>2.4	1.92		0.20	0.0	0.94	0.0
55b*	*m*-NO_2, *p*-Cl	>2.4	2.45		0.94	0.0	1.46	0.0
56b*	*m*-NO_2, *p*-Me	>2.4	2.23		0.54	0.0	0.94	0.0
57b*	*m*-CF_3, *p*-NO_2	>2.4	2.63		1.12	0.0	1.38	0.0
58b*	*m*-F	>2.4	2.80		0.34	0.0	0.0	0.46
59b*	*m*-OMe	>2.4	2.15		0.12	0.0	0.0	0.04
60b*	*m*-CF_3	>2.4	3.77		0.43	0.0	0.0	1.27
61b*	*m*-Et	>1.1	2.97		−0.07	0.0	0.0	0.95
62b*	*o*-COPh	2.00	0.26	1.74	0.43	2.51	0.0	0.0
63b*	*p*-Ph	0.94	0.84	0.10	−0.01	0.0	4.22	0.0
64b*	*m*-Ph	0.94	4.23	−3.29	0.06	0.0	0.0	2.00
65b*	*o*-COOEt	0.94	−0.89	1.83	0.45	3.90	0.0	0.0
66b*	*o*-OEt	0.90	−0.64	1.54	−0.24	2.86	0.0	0.0
67b*	*p*-COOEt	2.45	1.34	1.11	0.45	0.0	3.90	0.0
68b*	*m*-COOEt	0.94	3.24	−2.30	0.37	0.0	0.0	0.84
69b*	*m*-vinyl	1.89	2.98	−1.09	0.05	0.0	0.0	0.86

*Compounds not included in the analysis.
†Calculated using equation 7.
‡*o'*- and *m'*- denote substitutions at 6- and 5-positions, respectively.

meta and *para* positions are classified as '*para*' compounds. (iii) The rest, those having substituents only at one or two of the *meta* positions, are classed as the '*meta*' series. (iv) The position-specific steric and hydrophobic effects are considered only for the single substituent located at each assigned position.

Among the equations with various combinations of parameters, equations 4–6 gave the best correlations. For *ortho* compounds (**1b–22b**):

$$\log(1/C) = 0.51\sigma - 0.69L_o + 1.86 \quad (4)$$
$$(\pm 0.43) \quad (\pm 0.29) \quad (\pm 0.37)$$

where

$$n = 22, \quad r = 0.75, \quad s = 0.33.$$

For *para* compounds (**1b, 23b–33b**):

$$\log(1/C) = 1.25\sigma - 0.37L_p + 2.15 \quad (5)$$
$$(\pm 0.67) \quad (\pm 0.42) \quad (\pm 0.71)$$

where

$$n = 12, \quad r = 0.84, \quad s = 0.46.$$

For *meta* compounds (**1b, 34b–39b**):

$$\log(1/C) = 1.08\sigma + 0.62\pi_m + 2.34 \quad (6)$$
$$(\pm 0.76) \quad (\pm 0.56) \quad (\pm 0.37)$$

where

$$n = 7, \quad r = 0.95, \quad s = 0.19.$$

The L_o and L_p parameters are the length of the substituents at the *ortho* and *para* positions, respectively, along the 'principal' bond axis as shown in Fig. 3. The reference of the L parameter is taken as H so that $L(H) = 0$. The Hammett σ constant of the substituents is selected so that the electronic effect is directed to the side chain. The $\sum\sigma$ value is used for the electronic effect of polysubstituted compounds. Unsubstituted diphenylurea is included in each of the three series as the reference. The biological data for

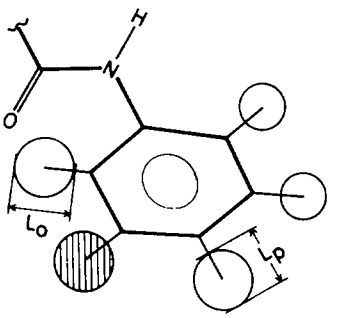


Fig. 3. Schematic representation of the substituted phenyl moiety of diphenylureas showing the parameters L_o and L_p. π of the shadowed *m*-substituent entered into equation 6 as π_m.

this compound used in the correlations are not the original log (1/*C*) value but log (1/*C*) − log 2, which takes the symmetry factor into account. For the unsubstituted compounds, a suitable orientation of the unsubstituted benzene ring at the site of action is assumed to be twice that of the substituted compounds.

Since the coefficient of the σ term, ρ value, in equations 4–6 overlap within the 95% confidence interval, we can combine the 3 sets of compounds as equation 7.

$$\log(1/C) = \underset{(\pm 0.33)}{0.90\sigma} - \underset{(\pm 0.25)}{0.85L_o} - \underset{(\pm 0.22)}{0.27L_p} + \underset{(\pm 0.58)}{1.04\pi_m} + \underset{(\pm 0.32)}{2.00} \quad (7)$$

where

$$n = 39, \quad r = 0.91, \quad s = 0.38.$$

The fact that the ρ value is positive indicates that the electron-withdrawing substituents favour the activity. The magnitude of the value, 0.90 (±0.33), suggests that an interaction with electron donors or nucleophiles at either the N_α—H hydrogen or the C=O carbon is important for enhancing the activity.

The position-specific steric and hydrophobic effects of the substituents are expressed by the L_o, L_p and π_m terms. At the *ortho* and *para* positions, substituent length is unfavourable. At the *meta* position, the high hydrophobicity of the substituents enhances activity. Except for the hydrophilic substituents, the *L* and π values are generally parallel. Therefore, equation 7 rationalizes the general trend of activity among the positional isomers; *meta* > *para* > *ortho*. No significant colinearity was observed at each position or for the whole set of compounds for any pair of variables as shown in Tables 4 and 5. The slope of the L_o term is more negative than that of the L_p term. Thus, the steric demand for *o*-substituents is more strict than that for *p*-substituents. The steric effect of the *m*-substituents is insignificant. The rules for incorporation of the polysubstituted derivatives indicate that the activity is governed by a substituent located at a position where the steric effect is most significant.

Bruce and Zwar [13] defined an index for cell-division activity; the sum of the 3 determinations of judgement of the amount of division per tobacco pith-block using a scale of 1–10. The 11 compounds (**40b–50b**) with indices less than 8, *ca* 25% of the maximum index 36, are not included in the analyses even though they are reported to be detectably active at concentrations equal to or less than 1 ppm. The activities estimated according to equation 7 are less than those reported for 8 (**40b–45b, 47–48b**) of the compounds. The reported activity for these poor cell-division promotive compounds may have been overestimated.

Table 4. Squared correlation matrices for variables used in derivation of equations 4–6

	o-series		*m*-series		*p*-series	
	L	π	*L*	π	*L*	π
σ	0.15	0.04	0.01	0.13	0.01	0.25
L	1.0	0.15	1.0	0.15	1.0	0.07

Table 5. Squared correlation matrix for variables used in derivation of equation 7

	σ	L_o	L_p
L_o	0.05	1.0	
L_p	0.01	0.30	1.0
π_m	0.01	0.13	0.05

The 11 compounds (**51b–61b**) whose activity was not definitely determined were also excluded from the correlations, but their activity data were calculated using the correlation. Two inactive compounds (**51b** and **53b**) show calculated log (1/*C*) values less than 1. The activity values for 8 compounds (**54b–61b**) seem to be compatible with the calculated values.

The 2-benzoyl (**62b**) and 3- and 4-phenyl (**64b** and **63b**) derivatives were omitted from the correlation. The dimension of the aryl substituents is so large that the derivatives are not accommodated well at the site of action. Also excluded from the analysis were the *o*-, *m*- and *p*-ethoxycarbonyl (**65b, 68b** and **67b**) and the *o*-ethoxy (**66b**) and *m*-vinyl (**69b**) derivatives. The ester group may be hydrolysed during autoclaving or in the tissue. Further studies are required for these poorly predicted derivatives. However, for the majority of compounds, the activity data are rationalized by equation 7.

Monophenylureas

As noted, activity decreases in general in the order of the *meta*, *para* and *ortho* isomers in this series of compounds [13]. Separate calculations for the *ortho*, *meta* and *para* derivatives suggested that both the steric and hydrophobic effects are important for activity. However, the number of compounds per number of independent variables included in each correlation was not so great that it was difficult to select the most reliable combination of variables. Thus, we analysed the combined set of derivatives. During the analyses, we found that the polysubstituted compounds can be included in the 'mono' substituted series according to the same principles as used for the polysubstituted diphenylureas.

Equation 8, for compounds **1c–31c**, was derived

from data in Table 6. Other combinations of variables gave poorer results.

$$\log(1/C) = -0.34L^2 + 1.87L - 0.98W_{max} \quad (\pm 0.24)\ (\pm 0.81)\ (\pm 0.40)$$
$$+ 0.63\pi_m + 0.25 \quad (8) \quad (\pm 0.63)\ (\pm 0.64)$$

where

$n = 31, \quad r = 0.82, \quad s = 0.37.$

The steric effect of the substituents on the activity is represented by both the length and width parameters. Both types of steric effect are expressed in common terms irrespective of the substituent positions. Although the position-specific steric effect of the substituents is in terms of assignment of the polysubstituted compounds into each of the monosubstituted series, the specificity seems to be much less in phenylureas than in diphenylureas. Activity varies parabolically with respect to the length of the substituent, the optimum value of L being *ca* 2.8. The

Table 6. Activity and physico-chemical parameters for cell-division promotion of tobacco pith-blocks by substituted monophenylureas

No.	Compound	log 1/C (mM)		Δlog 1/C	L	W_{max}	π_m
		Obs.	Calcd.				
1c	H	0.26	0.25	0.01	0.0	0.0	0.0
2c	*o*-Br	1.85	1.56	0.29	1.77	0.95	0.0
3c	*o*-Et	1.22	0.73	0.49	2.05	1.97	0.0
4c	*o*-Cl	0.93	1.47	−0.54	1.46	0.80	0.0
5c	*o*-CF_3	0.48	0.47	0.01	1.24	1.61	0.0
6c	*o*-NO_2	0.43	0.77	−0.34	1.38	1.44	0.0
7c	*o*-Et	0.43	0.51	−0.08	2.86	2.36	0.0
8c	*o*-OH	0.36	0.45	−0.09	0.68	0.93	0.0
9c	*o*-COOH	0.43	0.92	−0.49	1.85	1.66	0.0
10c	*o,m*-diCl	1.82	1.48	0.34	1.46	0.80	0.0
11c	*o*-Me, *m'*-NO_2‡	0.77	0.68	0.09	0.94	1.04	0.0
12c	*o*-OMe, *m'*-NO_2	0.63	0.75	−0.12	1.92	1.87	0.0
13c	*o,m'*-diCl	0.79	1.47	−0.68	1.46	0.80	0.0
14c	*o*-Me, *p*-Cl	1.04	0.68	0.36	0.94	1.04	0.0
15c	*o*-OMe, *p*-NO_2	0.81	0.75	0.06	1.92	1.87	0.0
16c	*o*-NO_2, *p*-OMe	0.81	0.77	0.04	1.38	1.44	0.0
17c	*p*-Br	2.30	1.56	0.74	1.77	0.95	0.0
18c	*p*-I	1.64	1.58	0.06	2.17	1.15	0.0
19c	*p*-NO_2	1.26	0.77	0.49	1.38	1.44	0.0
20c	*p*-Me	0.18	0.68	−0.50	0.94	1.04	0.0
21c	*m*-NO_2, *p*-Cl	1.34	1.47	−0.13	1.46	0.80	0.0
22c	*m*-CF_3, *p*-NO_2	1.10	0.77	0.33	1.38	1.44	0.0
23c	*m*-Cl, *p*-Me	0.75	0.68	0.07	0.94	1.04	0.0
24c	*m*-Br	2.52	2.30	0.22	1.77	0.95	1.20
25c	*m*-Cl	1.75	2.11	−0.36	1.46	0.80	1.05
26c	*m*-CF_3	1.31	1.25	0.06	1.24	1.61	1.27
27c	*m*-Me	0.88	0.99	−0.11	0.94	1.04	0.50
28c	*m*-NO_2	0.74	1.00	−0.26	1.38	1.44	0.37
29c	*m*-OEt	0.74	0.77	−0.03	2.86	2.36	0.42
30c	*m*-OMe	0.40	0.78	−0.38	1.92	1.87	0.04
31c	*m,m'*-diMe	1.43	0.99	0.44	0.94	1.04	0.50
32c*	*m m'*-diNO_2	inactive	1.00		1.38	1.44	0.37
33c*	*m*-OH	inactive	0.07		0.68	0.93	−0.62
34c*	*m*-Et	inactive	1.31		2.05	1.97	0.95
35c*	*o*-Me	inactive	0.68		0.94	1.04	0.0
36c*	*o*-F	inactive	0.89		0.59	0.35	0.0
37c*	*o*-I	inactive	1.58		2.17	1.15	0.0
38c*	*o*-OMe	inactive	0.75		1.92	1.87	0.0
39c*	*o,o'*-diMe	inactive	0.68		0.94	1.04	0.0
40c*	*o,m*-diMe	inactive	0.68		0.94	1.04	0.0
41c*	*o,m'*-diMe	inactive	0.68		0.94	1.04	0.0
42c*	*o,p*-diMe	inactive	0.68		0.94	1.04	0.0
43c*	*o,p*-diCl	inactive	1.47		1.46	0.80	0.0

Table 6. (*Continued*)

No.	Compound	log 1/C (mM) Obs.	log 1/C (mM) Calcd.	Δlog 1/C	L	W_{max}	π_m
44c*	*o*-Me, *p*-NO_2	inactive	0.68		0.94	1.04	0.0
45c*	*o*-NO_2, *p*-Me	inactive	0.77		1.38	1.44	0.0
46c*	*o*-COOH, *p*-vinyl	inactive	0.92		1.85	1.66	0.0
47c*	*p*-OH	inactive	0.45		0.68	0.93	0.0
48c*	*p*-OMe	inactive	0.75		1.92	1.87	0.0
49c*	p-OEt	inactive	0.51		2.86	2.36	0.0
50c*	*p*-COOH	inactive	0.92		1.85	1.66	0.0
51c*	*p*-COOEt	inactive	−0.83		3.90	3.29	0.0
52c*	*p*-COMe	inactive	0.74		2.00	1.93	0.0
53c*	*p*-phenylazo	inactive	−4.80		6.37	3.31	0.0
54c*	*p*-F	inactive	0.89		0.59	0.35	0.0
55c*	*m*,*p*-diMe	inactive	0.68		0.94	1.04	0.0
56c*	*m*-F	>2.0	1.17		0.59	0.35	0.46
57c*	*p*-Cl	>2.0	1.47		1.46	0.80	0.0
58c*	*m*,*p*-diCl	>2.0	1.47		1.46	0.80	0.0
59c*	*m*-Ph	1.85	1.28	0.57	4.42	2.11	2.00
60c*	*o*-Ph	0.63	0.05	0.58	4.22	2.11	0.0
61c*	*o*-COPh	0.66	−2.07	2.73	2.51	4.98	0.0
62c*	*p*-Ph	1.03	0.05	0.98	4.22	2.11	0.0
63c*	*m*-NO_2, *p*-Me	2.30	0.68	1.62	0.94	1.04	0.0

*Compounds not included in the analysis.
†Calculated using equation 8.
‡*o*'- and *m*'- denote substitution at the 6- and 5-positions, respectively.

W_{max} term shows that the shorter the width of the substituent at each position, the higher the activity. The position-specific hydrophobic effect is indicated by the π_m term which rationalizes the general trend in activity; *meta* > *para* ≃ *ortho*. No σ term is significant in equation 8 in contrast to equation 7. Table 7 shows the degree of independence of the variables used in equation 8.

Compounds reported inactive (**32c**–**55c**) are not included in the analyses. Of these compounds, most were predicted to have log (1/*C*) values of less than 1, except for the *o*-iodo (**37c**), *m*-ethyl (**34c**) and *o*, *p*-dichloro (**43c**) derivatives. For each of the *m*-fluoro (**56c**), *p*-chloro (**57c**) and *m*,*p*-dichloro (**58c**) derivatives where the log (1/*C*) values were not definitely determined but have been reported to be higher than 2.0, the calculated values are around 1.2–1.5. *o*-, *m*- and *p*-phenyl (**60c**, **59c** and **62c**) derivatives are not included in the correlation since they may be poorly accommodated at the action site as are the corresponding diphenylurea derivatives.

Table 7. Squared correlation matrix for variables used in derivation of equation 8

	L	W_{max}	π_m
W_{max}	0.54	1.0	
π_m	0	0	1.0
π	0.03	0.03	0.1

DISCUSSION

Our analyses indicate that variations in cytokinin activity is governed mainly by variations in the interaction with the target site of action. If the effect of physico-chemical properties on the transport process(es) participates in the activity, it should be much less specific to the substituent position, and the hydrophobicity of the whole molecule should be involved in the structure–activity relationships. The importance of the steric dimension of the N^6-substituents for the interaction of N^6-substituted adenines with the action site is evident and conforms with results of our previous studies on anticytokinins. The optimum width of the N^6-substituents, 5.2 Å, predicted from equation 3, for cytokinin activity agrees very well with that of the N^4-substituents, 4.5 Å, estimated for the anticytokinin activity of N^4-substituted 2-methylthiopyrido[2,3-*d*]pyrimidines, and was to be expected for the mode of interaction of both cytokinins and their antagonists [11].

The known high activity of N^6-substituted adenines with C_4–C_7 saturated alkyls [1] can be rationalized by equation 1 or 3. Below or above this range of carbons the steric condition of the N^6-substituents is less suitable for the activity. Equations 1 and 3 do not explain,

however, the reportedly high activity of N^6-farnesyladenine [1], the W_{max} of which is obviously far from the optimum in the fully extended form. It may be possible that such a long chain would attain a more compact conformation in the receptor cavity to lower the energy of interaction. Alternatively the isopentenyl units in the farnesyl side-chain may be degraded stepwise in the tissue to afford a more active species. The generally recognized enhancement of activity by the unsaturation of the N^6-side-chain can be also delineated by equation 1. The higher activity for zeatin (**6a**) and its deoxy analogue (**5a**) than the corresponding saturated derivatives (**4a** and **1a**, respectively) is mainly due to the W_{max} value of the unsaturated side chain being closer to the optimum.

Hecht *et al.* [23] attributed the higher activity of the *trans* than the *cis* isomers of zeatin and 6-(3-chloro-2-butenylamino)purine to the co-planarity of the side-chain. They considered that the disturbance of co-planarity due to steric interference of the 3-substituents with α-methylene in the *cis* forms is less suitable to the activity. The W_{max} value for the chlorobutenyl side-chain of the *trans* isomer, 4.72, is further apart from the optimum than that of the *cis* form, 5.20. These W_{max} values do not correspond to the activity difference. A distortion due to the possible steric interaction within the side chain in the *cis* isomer may change the W_{max} from 5.20 to a value less suitable than that of the *trans* isomer. The difference in W_{max} between 5.12 for *trans* and 4.88 for *cis* zeatin implies the higher activity of *trans* than *cis* isomer according to equation 1. The calculated activity difference, *ca* 0.13 in the log unit, does not conform, however, to the reported large activity difference of more than 10 times [24]. Thus it may be due to the deformation of the *cis* side-chain structure, i.e. the actual W_{max} value may be much lower than the value calculated by means of the Corey–Pauling–Koltan atomic models with normal sp^2 and sp^3 carbons on which the STERIMOL program was based. Before drawing any conclusions, however, further studies with more compounds having variety of crowded side-chains are necessary, since factor(s) other than width effect might participate in determining the variations in activity.

The steric effect of aromatic substituents in the phenyl- and diphenyl-ureas seems to be determined mainly by the substituent length and not by the width. Equation 7 for the diphenylureas indicates that the activity decreases with an increase in substituent length at the *otho* and *para* positions. This implies that the effect of substituent length is supra-optimum. Thus the substituted benzene ring is considered to be located closer to the spatial wall of the receptor cavity than it is in the phenylureas. Equation 8 for the phenylureas reveals a parabolic relationship between substituent length and activity. The substituted benzene ring of this series of compounds is not so tightly bound with the receptor site so that activity increases first with the increase in substituent length, then decreases beyond the optimum. For substituted diphenylureas, the unsubstituted benzene ring may participate in binding in such a way that it assists the substituted benzene ring to locate closer to the receptor. Why the steric effects are more position-specific in the diphenylureas can be understood from this line of reasoning. The closer the substituted benzene ring to the receptor site, the more specific should be the substituent effects.

The π_m term is significant in equation 7 as well as in equation 8 and suggests a common type of interaction corresponding to the similarity in the structures of the phenyl- and diphenyl-ureas. The higher coefficient of this term in equation 7 means that a higher degree of desolvation occurs in interaction with the receptor site. It is not incompatible with the closer fit with the receptor site in the diphenylureas.

The electronic effect of the substituents is significant for diphenylureas but not for phenylureas. This effect of the substituents would operate on the NH group so as to form a hydrogen bond with electron donors on the receptor site. If such hydrogen bonding participates in enhancing the activity, it should be very sensitive to the geometry of the binding. Since the fit of the phenylureas with the receptor is looser than the diphenylureas, the lack of significance of the σ term in equation 8 is understandable. The significance of the σ^* in equation 3 for the N^6-substituted adenines also suggests an analogous electronic effect on the NH bridge, probably a hydrogen bonding interaction with electron donors.

Since the hydrophobic substituent effect is insignificant in determining the activity of adenylate cytokinins, as shown in equation 3, the site of interaction for N^6-substituents of the adenine derivatives probably differs from that for the aromatic substituents of phenyl and diphenyl-ureas. In summation Fig. 4 shows schematically the mode of binding for both the adenine and urea cytokinins.

In equation 7, no activity data for diphenylureas, where both the benzene rings are substituted, are included. The assignments of one of the benzene rings to the base and the other to the side-chain were not at all straightforward. The *N*-phenyl-*N'*-alkylureas are also not included since they are only very slightly active. However, the activity data reported for these compounds [13] suggest an optimum steric condition in terms of W_{max} values of the *N'*-substituents similar to those for the N^6-substituents of the adenines. In Table 8, the cytokinin activity of *N*-phenyl-*N'*-substituted ureas is compared with the W_{max} values of

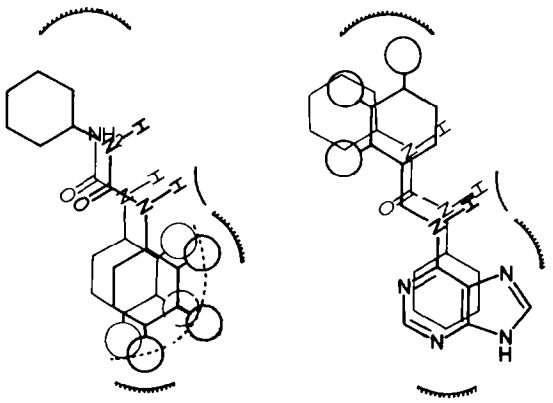


Fig. 4. Schematic cytokinin–receptor complex where a hexagon represents a benzene ring. Stippled lines show the spatial walls, smooth lines the electron-donating site and the dotted square the hydrophobic region.

Table 8. Relationship between activity and W_{max} of *N*-phenyl-*N'*-substituted ureas

N'-Substituent	log 1/*C* (mM)	W_{max}
H	0.26	1.0
Cyclopropyl	0.73	2.88
Ethyl	1.22	2.97
Phenyl	2.40	3.11
Propyl	0.73	3.49
Allyl	0.73	3.78
Butyl	0.46	4.42

N'-substituents estimated from the *N'*-C axis. The activity peak seems to occur when W_{max} is *ca* 3, which suggests that there is a fundamentally similar but somewhat different steric demand required for the *N'*-substituents from the N^6-substituents of adenylate cytokinins.

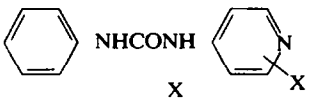
Recently, Takahashi *et al.* [15] studied the cytokinin activity of *N*-phenyl-*N'*-(4-pyridyl)urea derivatives by the tobacco callus bioassay. We examined whether their activity data for compounds with intact benzene rings are in accord with equation 7 for diphenylureas, where one of the rings is unsubstituted. Their data are listed in Table 9 with the substituent parameters. Since the '*p*'-aza(—N=) function of the pyridine ring is highly electron-withdrawing, $\sigma = 0.99$ [25], the activity which is higher than that of the corresponding diphenylureas can be understood. The overall correspondence between their data and the data predicted by equation 7 is very good, the simple correlation coefficient being 0.87.

EXPERIMENTAL

Test substances. Synthesis of the following compounds has been reported previously: 6-*n*-butyl-, 6-*n*-amyl-, 6-*n*-hexyl- and 6-*n*-heptyl-aminopurines (**7a**, **8a**, **9a** and **10a**) [12]; 6-(3-methyl-2-butenylamino)purine (**5a**) [26]; *trans*-zeatin (**6a**) [27]; dihydrozeatin (**4a**) [28]; 6-isoamylaminopurine (**1a**) [1]; 6-phenethylaminopurine (**11a**) [29]; 6-benzylaminopurine (**12a**) [30]; 6-*m*-hydroxy-, 6-*m*-chloro-, 6-*m*-methoxy-, 6-*o*-chloro-, 6-*p*-chloro- and 6-*p*-methyl-benzylaminopurines (**13a**, **14a**, **16a**, **18a**, **21a** and **22a**) [31]; 6-*m*-methylbenzylaminopurine (**15a**) [30]. 6-Isohexylamino- and 6-isoheptylaminopurines (**2a** and **3a**), 6-*m*-trifluoromethyl-, 6-*o*-methoxy- and 6-*o*-trifluoromethyl-benzylaminopurines (**17a**, **19a** and **20a**) were prepared conventionally by refluxing 6-chloropurine and appropriate amines in *n*-BuOH. Experimental details will be reported elsewhere.

Cytokinin activity. The cytokinin activity of N^6-substituted adenines was measured in terms of fr. wt yield of tobacco callus derived from *Nicotiana tabacum* var. Wisconsin No. 38. The tobacco callus was grown at 28° for 4 weeks on the standard medium specified in ref. [32] to which the test compounds was added in different concns. The activity was expressed by E_{50} which is the concn at which the 50% callus yield of the maximum response is given. Activities of diphenyl- and phenyl-ureas given originally in ppm [13] were converted to molar concn which is reportedly the minimum concn that gives a detectable response in the tobacco pith assay.

Table 9. Cytokinin activity and physico-chemical parameters of *N*-phenyl-*N'*-(4-phridyl)ureas



	log 1/*C* (mM)	$\Sigma\sigma$	π_m	L_o	log 1/*C* (mM) of the corresponding diphenylureas
2-Chloro	5.40	1.36	1.05	0	3.40
2-Methyl	3.40	0.92	0.50	0	2.52
H	3.33	0.99	0.00	0	2.40
2,6-Dimethyl	2.40	0.85	0.50	0	2.70
3-Methyl	1.38	0.82	0	0.94	0.84

*2- and 3-positions correspond to the *m*- and *o*-positions in diphenylureas, respectively.

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