products cleanly separated from the unreacted 8, a specific activity of 2200 Ci/mol was assumed.

For conversion into azides, each sample of [^{125}I]**9**, -**10**, or -**11** (~1 mCi) was concentrated to dryness and reconstituted in 10 μL of 6 N acetic acid and then diluted with 10 μL of H_2O. At 0 °C, $NaNO_2$ (5 μg/mL of H_2O) and NaN_3 (5 μg/1 μL of H_2O) were added (2 min interval), and the reaction was warmed at room temperature for 5 min before neutralization with NH_4OH (8 μL). The reaction mixture was analyzed, and the products were isolated by the same procedure as described in the radioiodination above. Greater than 90% yield of **12** (R_f 0.50), **6** (R_f 0.48), and **13** (R_f 0.56) was obtained from **9**, **10**, and **11**, respectively. Each isolated product was demonstrated to comigrate with the known parent compound on TLC.

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Registry No. **2**, 85135-25-7; **2** picrate, 85135-37-1; **3**, 82408-64-8; **4**, 81530-23-6; **4**·HCl, 85135-34-8; **5**, 51997-51-4; **6**, 85135-26-8; [^{125}I]**6**, 85135-45-1; **7**, 85135-27-9; **7** picrate, 85135-39-3; **8**, 82408-63-7; **8** picrate, 85150-62-5; **9**, 85135-28-0; [^{125}I]**9**, 85135-41-7; **10**, 85135-29-1; [^{125}I]**10**, 85135-42-8; **11**, 85135-30-4; [$^{125}I_2$]**11**, 85135-43-9; **12**, 85135-31-5; [^{125}I]**12**, 85135-44-0; [$^{125}I_2$]**13**, 85135-46-2; **14**, 85135-32-6; **14** picrate, 85135-40-6; **15**, 85135-33-7; 1,1-dimethyl-2-(4-aminophenyl)ethylamine, 51131-55-6; trifluoroacetic anhydride, 407-25-0; *N*-[1,1-dimethyl-2-(4-nitrophenyl)ethyl]-trifluoroacetamide, 85135-35-9; *N*-[1,1-dimethyl-2-(4-aminophenyl)ethyl]trifluoroacetamide hydrochloride, 85135-36-0; 1,1-dimethyl-2-(4-azido-3-iodophenyl)ethylamine, 81530-24-7; 2-(4-nitrophenyl)-1,1-dimethylethylamine hydrochloride, 79886-11-6; 2-(2-nitrophenyl)-1,1-dimethylethylamine hydrochloride, 79886-18-3; (±)-15-(2-nitrobenzyl)carazolol, 85135-38-2; 1,1-dimethyl-2-phenylethylamine, 122-09-8.

Supplementary Material Available: ^{1}H NMR spectra (250 MHz) of the aromatic region of all carazolol derivatives described in this paper (Figure 1), tabulated ^{1}H NMR chemical shifts (δ) of all carazolol derivatives described in this paper (Table 1), tabulated ^{13}C NMR chemical shifts (δ) of 4-(2,3-epoxypropoxy)carbazole (**5**) and 4-(2,3-epoxypropoxy)-1-iodocarbazole (**15**) (Table 2), and MS fragmentation pattern of carazolol derivatives (Scheme 1) (4 pages). Ordering information is given on any current masthead page.

Quantitative Aspects of the Receptor Binding of Cytokinin Agonists and Antagonists

Hajime Iwamura,*[†] Noboru Masuda,[‡] Koichi Koshimizu,[‡] and Satoshi Matsubara[§]

Department of Agricultural Chemistry, Faculty of Agriculture, and Department of Food Science and Technology, Kyoto University, Kyoto 606, Japan, and Laboratory of Applied Biology, Kyoto Prefectural University, Shimogamo, Kyoto 606, Japan. *Received August 23, 1982*

Congeneric 4-anilino- and 4-(alkylamino)-2-methylpyrrolo[2,3-*d*]pyrimidines showed cytokinin and anticytokinin activities, depending on the structure of their 4-substituents, and the antagonistic nature of the latter was established kinetically. The effect of the substituent on these activities was analyzed quantitatively by using physicochemical parameters and regression analysis to give a single, common equation for both the agonists and antagonists. The results indicated that the maximum width of the N^4 substituents is an important factor both for binding to the receptor, thus the extent of activity, and for the quality of activity, agonistic or antagonistic. The electron-withdrawing effect and hydrophobicity of the substituents further enhance binding and, thus, activity, irrespective of the quality of the activity. These results coincide with and/or provide evidence for the hypothesis that in hormonal action, agonist binding causes a conformational change of an otherwise inactive receptor to the active form and that antagonists are species that bind similarly to the receptor but do not cause the effective conformational change.

The agonists and antagonists of a biologically active compound play an important role in studying its bioregulatory mechanisms and its mode of action. In a field of a class of plant hormones, cytokinins, five structural classes of antagonists, anticytokinins, have been developed in the past 10 years.[1-7] All of them possess similarities in structure to naturally occurring N^6-adenylate cytokinins, like zeatin [(*E*)-6-[(4-hydroxy-3-methyl-2-butenyl)-amino]purine] and 6-(3-methyl-2-butenylamino)purine. Among these, the 4-substituted 2-methylpyrrolo[2,3-*d*]-pyrimidines[6] are interesting because their activity varies from agonistic to antagonistic with the transformation of the side chain at the 4-position. In the previous paper,[6] we have shown that a steric substituent parameter, W_{max}, which represents the maximum width of substituents from the bond axis between the exocyclic nitrogen atom and its α-carbon atom, governs the variation of the activity; i.e., the W_{max} values of the cytokinin agonists in this series are within the range of 4.7–6 Å, and those in which the W_{max} values are smaller or larger than this range are anticytokinins. It is thus obvious that the steric dimension of compounds is one of the main factors that determines the intrinsic activity, i.e., agonistic or antagonistic. The question that immediately arises is how they interact with the receptor. Quantitative structure–activity relationship

[†] Kyoto University, Department of Agricultural Chemistry.
[‡] Kyoto University, Department of Food Science and Technology.
[§] Kyoto Prefectural University.

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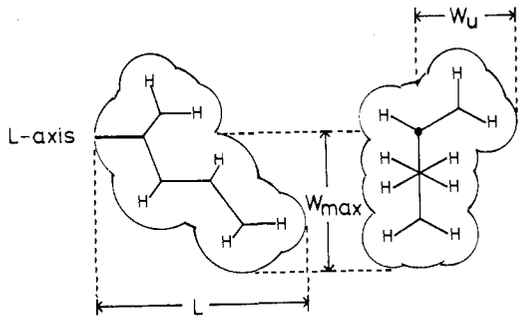

Figure 1. Schematic representation of the STERIMOL parameters for alkyl substituents.

studies have been shown to provide a useful guide in understanding the mechanism of action of biologically active compounds. The results from this method have been confined mostly to congeneric compounds having the same type of activity.

In the present work, the activities of 4-(arylamino)- and 4-(alkylamino)-2-methylpyrrolo[2,3-*d*]pyrimidines were analyzed quantitatively by regression analysis with physicochemical parameters to give a single, common equation for both the agonists and antagonists. The result was that the congeneric series of compounds exerted both agonistic and antagonistic activities with a common binding mode, which coincides with and supports the concept, common to hormonal action, that the receptor is activated by conformational change by the binding of an agonist molecule and that an antagonist is a molecule that binds similarly but does not cause the conformational change to the active form.

Test Substances. Compounds **25**, **27–31**, **34**, **36**, **38**, and **42–46** have been reported previously.[6] Other 4-substituted 2-methylpyrrolo[2,3-*d*]pyrimidines were prepared conventionally by refluxing a solution of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine[8] and appropriate amines in 1-butanol. 4-Alkoxy-2-methylpyrrolo[2,3-*d*]pyrimidines were synthesized by refluxing the 4-chloro precursor in appropriate alcohols, in which Na metal was dissolved.

Biological Parameters. The activity was measured in terms of the fresh weight yield of tobacco callus derived from *Nicotiana tabacum* var. Wisconsin No. 38. The callus was grown on the medium specified by Linsmaier and Skoog,[9] to which the compounds to be tested for cytokinin activity were added in different concentrations. Anticytokinin activity was tested similarly by adding 0.05 μM kinetin to the medium. The cytokinin activity was expressed by E_{50} value, which is the concentration at which 50% of the maximum response of callus yield is obtained. The I_{50} value for anticytokinin activity is the concentration at which is obtained 50% of the callus growth on the medium with 0.05 μM kinetin but without anticytokinin.

Substituent Parameters. The steric parameters used were calculated by the STERIMOL program developed by Verloop et al.[10] The L parameter expresses the length of N^4 substituents along the bond axis that connects the substituents to the rest of molecule. To best reflect the steric features in terms of the widths of the N^4-alkyl substituents that are responsible for the biological activity, we divided them into two parts, so that one includes the zigzag, main chain and the other includes the C-1 substituent or the bulkiness of the region around the C-1 carbon atom, as shown by Figure 1. W_{max}, then, was defined as the width in the direction to which the main chain extends in the fully extended (staggered) conformation. It thus expresses at the same time the thickest width of the molecule perpendicular to the L axis. To express the bulkiness of the right-hand side of the molecule in Figure 1, we adopted the parameter W_u, which is the width upward of that part of the side chain. The W_{max} of the phenyl substituents is the width in the direction to which ortho or meta substituents extends, as shown by Figure 2. The W_u in this series of compounds corresponds to the thickness of the benzene moiety. These steric parameters correspond nearly to Verloop's B_5 and B_3 parameters,[10] but by definition these are not necessarily rectangular to each other. For benzyl and phenethyl, the benzene ring was twisted 30° from the plane in which the skeletal N^4–C–C_1^{Ph} or N^4–C–C–C_1^{Ph} chains lies, minimizing steric constraint. The angle was taken as 90° in the literature,[10] and the length and some width parameters differ between the conformations. The norbornyl structure cannot be exactly built by normal sp^3 carbon but was approximated by it.

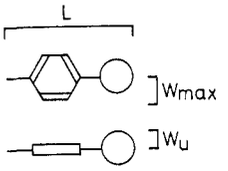

Figure 2. Schematic representation of the STERIMOL parameters for aromatic substituents.

The hydrophobic parameter, π, for the N^4 substituents was determined from experimental data according to the literature.[11,12] The electronic parameter, σ^*, was also estimated for N^4 substituents, and thus the effect is directed toward the N^4 atom. The value was determined according to the literature.[11,13,14] The values for $(CH_2)_nR$ were estimated by $\sigma^*(R) \times 0.34^n$, where 0.34 is the transmission factor,[14] and values for alkyl chains larger than n-butyl were approximated by that of n-butyl. Similarly, the σ^* value of 3-methyl-2-butenyl was approximated by 2-butenyl, and σ^* value of norbornyl was approximated by cyclohexyl. The values for substituted phenyl groups were estimated as σ^* (C_6H_5-X) = $0.72\sigma_x + 0.58$[15] and σ^* (C_6H_5-ortho-X) = σ^* (C_6H_5-para-X), where X denotes aromatic substituents.

Results

Biological Activity. Of the compounds tested and listed in Tables I and II, compounds 1–18 in the N^4-phenyl series and compounds **25–40** in the N^4-alkyl series inhibited the growth of tobacco callus cultured on a medium containing 0.05 μM kinetin, whereas compounds **20–24** and **43–48** exhibited cytokinin activity. In the previous work,[6] we suggested the participation of a steric factor in the discrimination of the growth-promoting and inhibitory activities of certain N^4-alkyl derivatives and found that the maximum width, W_{max}, of substituents correlates semiquantitatively with the variation of activity. Similar discrimination in terms of structure is also possible for the

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Table I. Activity and Physicochemical Properties of 4-Anilino-2-methylpyrrolo[2,3-*d*]pyrimidines

no.	benzene substituents	activity: log $1/I_{50}$ obsd	activity: log $1/I_{50}$ calcd[a]	activity: log $1/E_{50}$ obsd	activity: log $1/E_{50}$ calcd[a]	Δ log $1/I_{50}$ or Δ log $1/E_{50}$	physicochemical parameters[b]: L	W_{max}	W_u	π	σ^*
1	H	0.26	0.18			0.08	6.28	3.11	1.71	1.68	0.58
2	*p*-F	0.43	0.30			0.13	6.87	3.11	1.71	1.82	0.62
3	*p*-Cl	0.65	0.74			−0.09	7.74	3.11	1.80	2.39	0.74
4	*p*-Br	0.77	0.82			−0.05	8.04	3.11	1.95	2.54	0.74
5	*p*-I	0.66	0.92			−0.26	8.45	3.11	2.15	2.80	0.71
6	*p*-Me	0.12	0.31			−0.19	7.09	3.11	1.95	2.24	0.45
7	*p*-Et	0.67	0.51			0.16	8.33	3.17	1.91	2.70	0.47
8	*p*-NH_2	−0.71	−1.04			0.33	7.15	3.11	1.84	0.45	0.11
9	*p*-OH	−0.81	−0.50			−0.31	6.96	3.11	1.71	1.01	0.31
10	*p*-OMe	0.18	−0.07			0.25	8.20	3.11	2.02	1.66	0.39
11	*p*-OEt	−0.46	−0.10			−0.36	9.02	3.36	2.07	2.06	0.41
12	*p*-Ac	0.19	0.30			−0.11	8.28	3.13	2.02	1.13	0.94
13	*p*-CF_3	1.15	1.11			0.04	7.52	3.11	2.61	2.56	0.97
14	*p*-*n*-Pr	0.98	0.47			0.51	9.14	3.49	3.02	3.23	0.49
15	*p*-*i*-Pr	1.34	0.78			0.56	8.33	3.17	3.02	3.21	0.47
16	*p*-CN	0.30	0.46			−0.16	8.45	3.11	1.71	1.11	1.06
17	*m*-NH_2	−1.41	−1.69			0.28	6.28	4.09	1.82	0.45	0.46
18	*o*-Me	−1.29	−0.88			−0.41	6.28	4.20	2.06	2.24	0.45
19[c]	*p*-*n*-Bu	insol[d]	−0.38				10.39	4.54	4.03	3.81	0.49
20	*m*-Br			−0.80	−0.83	0.03	6.42	4.76	2.13	2.54	0.86
21	*m*-Cl			−0.66	−0.62	−0.04	6.28	4.48	1.87	2.39	0.85
22	*m*-I			−0.73	−1.16	0.43	6.72	5.15	2.49	2.80	0.83
23	*m*-Me			−1.12	−0.87	−0.34	6.36	4.20	2.06	2.24	0.53
24	*m*-OMe			−1.29	−0.82	−0.47	7.17	4.11	2.00	1.66	0.67

[a] Values were calculated by eq 3. [b] L, the length of N^4 substituents along the bond axis; W_{max}, the maximum width perpendicular to the L axis; W_u, the width perpendicular to the L axis and rectangular to W_{max}; π, the hydrophobic parameter for N^4 substituents; σ^*, Taft's electronic parameter for N^4 substituents. [c] Compounds not included in the analysis. [d] Insoluble.

Table II. Activity and Physicochemical Properties of 4-(Alkylamino)-2-methylpyrrolo[2,3-*d*]pyrimidines

no.	N^4 substituent	activity: log $1/I_{50}$ obsd	activity: log $1/I_{50}$ calcd[a]	activity: log $1/E_{50}$ obsd	activity: log $1/E_{50}$ calcd[a]	Δ log $1/I_{50}$ or Δ log $1/E_{50}$	physicochemical parameters[b]: L	W_{max}	W_u	π	σ^*
25	$CH_2CH_2CH_3$	−0.47	−0.84			0.37	4.92	3.49	1.91	1.50	−0.12
26	$CH(CH_3)_2$	0.26	0.16			0.10	4.11	3.17	2.98	1.30	−0.19
27	$CH(CH_3)CH_2CH_3$	0.76	0.57			0.19	4.92	3.49	3.16	1.80	−0.21
28	c-C_4H_7	1.13	0.71			0.42	4.77	3.82	3.18	1.44	−0.15
29	c-C_5H_9	1.14	1.22			−0.08	4.97	4.15	3.46	1.85	−0.20
30	c-C_6H_{11}	0.05	0.45			−0.40	6.17	3.49	3.16	2.26	−0.15
31	$CH_2CH{=}CH_2$	−0.17	−0.50			0.34	5.11	3.78	1.90	1.20	0.22
32	$CH_2CH_2CH_2OH$	−1.75	−1.66			−0.09	6.02	4.15	1.91	−0.30	0.06
33	$CH_2CH(OH)CH_3$	−1.52	−1.67			0.15	4.92	3.49	1.91	−0.50	0.07
34	$C(CH_3)_2OH$	−0.78	−0.26			−0.52	4.79	3.38	3.16	−0.20	0.00
35	$CH_2CH_2OCH_3$	−0.78	−1.30			0.52	6.03	4.44	1.92	0.02	0.18
36	$(CH_2)_7CH_3$	−1.17	−1.05			−0.11	5.82	6.85	2.17	4.00	−0.13
37	CH_2-c-C_3H_5	1.11	0.76			0.35	5.14	4.43	3.10	1.64	−0.15
38	$CH_2CH(CH_3)_2$	−1.41	−0.37			−1.04	4.92	4.45	1.91	1.80	−0.13
39	$CH_2C(CH_3)_3$	−0.70	−0.26			−0.44	4.91	4.45	1.91	2.10	−0.17
40[c]		−0.45	1.22			−1.67	5.68	4.01	3.46	2.27	−0.15
41[c]	c-C_3H_5	insol[d]	0.60				4.14	3.24	3.08	1.14	0.11
42[c]	CH_2CH_2OH	nr	−1.72				4.79	3.38	1.91	−0.80	0.19
43	$(CH_2)_4CH_3$			−0.72	−0.76	0.04	6.97	4.94	1.92	2.50	−0.13
44	$CH_2CH{=}C(CH_3)_2$			0.08	−0.48	0.56	6.39	4.82	1.92	2.00	0.13
45	$CH_2CH(CH_2CH_3)CH_2CH_2CH_2CH_3$			−0.84	−1.16	0.32	8.22	5.96	1.91	3.80	−0.13
46	CH_2-C_6H_5			−0.72	−0.61	−0.11	5.28	6.02	1.92	2.18	0.22
47	CH_2CH_2-C_6H_5			−1.37	−0.84	−0.53	8.33	4.30	1.91	2.68	0.08
48	$CH_2CH_2OCH_2CH_3$			−1.37	−1.35	−0.02	6.85	4.81	1.92	0.52	0.18

[a] Values were calculated by eq 3. [b] See footnote *b* of Table I. [c] Compounds not included in the regression analysis. [d] Abbreviations used: insol, insoluble; nr, not reached to the maximum growth within the concentration range tested.

phenyl series of compounds presented here; i.e., the growth-inhibiting activity was expressed mostly by para-substituted compounds, while meta derivatives were agonistic. Exceptional are the antagonistic *m*-amino (17) and *o*-methyl (18) derivatives, and they are borderline cases. These results suggest that the activity changes consecutively from promotive to inhibitory with the transformation of the structure and that the inhibitors are specific anticytokinins, i.e., cytokinin antagonists. To confirm this, we applied the method of Lineweaver and Burk,[16] which has been previously applied to the estab-

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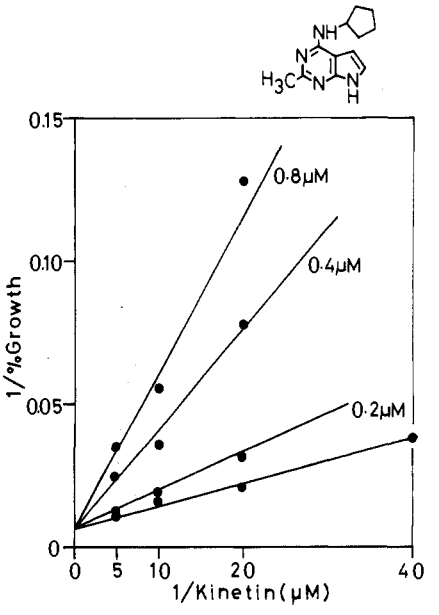

Figure 3. The reciprocal of the growth rate of tobacco callus plotted as a function of the reciprocal of the concentration of kinetin alone (bottom line) and in the presence of compound **5**.

lishment of a specific antiauxin nature[17] as well as the anticytokinin nature of pyrido[2,3-*d*]pyrimidine derivatives.[7] The results of this treatment on compound **29** is shown in Figure 3, where the reciprocal of the growth response was plotted against the reciprocal of the concentration of added kinetin. The fact that the resultant family of straight lines possesses a common intercept fulfills the requisite for a competitive inhibitor.[18] The antagonistic nature of the inhibitors, which possess a common heterocyclic moiety with the agonists and differ only in the substituent structure, provides a basis for investigating the mode of interaction of this series of compounds with a common receptor.

Quantitative Structure–Activity Relationship. Tables I and II list the activity data and substituent parameters used. First, the N^4-phenyl derivatives 1–18 with anticytokinin activity were analyzed by multiple regression analysis. Of the various combinations of variables, eq 1

$$\log A = \underset{(\pm 0.19)}{0.58\pi} - \underset{(\pm 0.48)}{1.19W_{max}} + \underset{(\pm 0.67)}{0.87\sigma^*} + \underset{(\pm 1.77)}{2.41} \quad (1)$$

$$n = 18,\ r = 0.94,\ s = 0.30$$

gave the best correlation, where A is $1/I_{50}$. In this and 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. The positive coefficients of the hydrophobic and electronic terms, π and σ^*, indicate that hydrophobic and electron-withdrawing substituents enhance activity.

Five meta-substituted derivatives, **20–24**, exhibited cytokinin activity; i.e., they promoted callus growth and did not inhibit it in the presence of 0.05 μM kinetin. The smaller number of these compounds made it difficult to analyze them quantitatively and select the most reliable combination of variables. Preliminary examination of the biological and physicochemical data suggested, however, that these agonists could be incorporated into eq 1 for the antagonists. The resultant eq 2 was conspicuous in that

$$\log A = \underset{(\pm 0.19)}{0.61\pi} - \underset{(\pm 0.23)}{1.10W_{max}} + \underset{(\pm 0.63)}{0.99\sigma^*} + \underset{(\pm 0.83)}{1.98} \quad (2)$$

$$n = 23,\ r = 0.94,\ s = 0.32$$

the activity of both the agonists and antagonists were expressed by a single equation. The activity term, log A, in the equation thus means log $1/I_{50}$ for compounds 1–18 and log $1/E_{50}$ for compounds **20–24**. Addition of the π^2 and W_{max}^2 terms to eq 2, singly or in combination, did not improve the correlation. Because of the negative coefficient of the W_{max} term, the values for this set of compounds are already supraoptimal and the term in the equation reflects only the downward, linear part of the hypothetical parabola. The W_u parameter, which expresses the width perpendicular to the benzene ring, does not enter into the correlation, probably because the values are nearly constant through the phenyl series of compounds. In contrast, it is worthy to note that the length (L) of the substituents does not affect the activity despite wide variation. Table III shows the development of eq 2, and Table IV shows the degree of independence of the variables considered.

The activity of the N^4-alkyl derivatives cannot be explained by eq 2 as it stands, indicating that the mode of their interaction somewhat differes from that of the phenyl series of compounds. Examination of the data for the alkyl series of compounds suggested that the steric mode of interactions appear different between the two series, reflecting the different molecular shape, but the significance of the other factors may be common. Thus, both series of compounds were combined, and the correlations were explored based on the result of eq 2 and considering the steric parameters separately. In eq 3 thus obtained, the

$$\log A = \underset{(\pm 0.15)}{0.53\pi} + \underset{(\pm 0.65)}{1.21\sigma^*} - \underset{(\pm 0.27)}{1.09W_{max}^{Ph}} + \underset{(\pm 1.86)}{2.98W_{max}^{R}} - \underset{(\pm 0.19)}{0.33(W_{max}^{R})^2} + \underset{(\pm 0.37)}{1.10W_u^{R}} - \underset{(\pm 0.20)}{0.35L^{R}} + \underset{(\pm 4.64)}{10.25In^{Ph}} - \underset{(\pm 4.53)}{8.27} \quad (3)$$

$$n = 44,\ r = 0.92,\ s = 0.39$$

steric parameters for the alkyl series of compounds are marked by a superscript R and those for phenyl derivatives by Ph. Table V shows the correlation matrix of the parameters used for the alkyl derivatives. The squared sim-

Table III. Development of Equation 2

const	π	W_{max}	σ^*	r	s	$F_{x,y}$[a]
3.09		−0.89		0.67	0.65	$F_{1,21}$ = 16.83
2.23	0.67	−1.03		0.90	0.39	$F_{1,20}$ = 39.71
1.89	0.61	−1.11	0.99	0.94	0.32	$F_{1,19}$ = 10.88

[a] F statistic for the significance of the addition of each variable.

Table IV. Squared Correlation Matrix for Variables Used in the Development of Equation 2

	π	W_{max}
W_{max}	0.03	
σ^*	0.06	0.05

Table V. Squared Correlation Matrix of the Variables Used for the Correlation of Alkyl Derivatives in Equation 3

	π	σ^*	W_{max}	W_u
σ^*	0.02			
W_{max}	0.10	0.10		
W_u	0.01	0.12	0.01	
L	0.16	0.37	0.06	0.08

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Table VI. Development of Equation 3

const	π	σ^*	W_{max}^{Ph}	W_{max}^{R}	$(W_{max}^{R})^2$	W_u^R	L^R	In^{Ph}	r	s	$F_{x,y}$[a]
−0.74	0.27								0.31	0.85	$F_{1,42}$ = 4.54
−0.52	0.26			−0.09					0.40	0.84	$F_{1,41}$ = 2.87
2.06	0.55		0.92	−0.77					0.74	0.62	$F_{1,40}$ = 34.07
2.65	0.54		−1.07	−0.49			−0.32		0.79	0.58	$F_{1,39}$ = 7.44
1.79	0.55		−0.85	−0.50		0.45	−0.34		0.83	0.53	$F_{1,38}$ = 7.75
1.35	0.57	1.28	−0.96	−0.49		0.64	−0.35		0.87	0.48	$F_{1,37}$ = 10.57
−0.90	0.48	1.26	−1.09	−0.30		0.99	−0.22	2.93	0.89	0.45	$F_{1,36}$ = 5.56
−8.27	0.54	1.21	−1.09	2.98	−0.33	1.10	−0.35	10.25	0.92	0.39	$F_{1,35}$ = 13.18

[a] F statistic for the significance of the addition of each variable.

ple correlation coefficient between π and σ^* in the combined set is 0.02. The σ^* term indicates that both series of compounds interact electrostatically with a common basic site on the receptor surface, probably at the N^4-imino hydrogen atom. Another common term, π, with positive coefficient, shows the importance of hydrophobicity in activity. It appears to participate in the transportation process(es). The significance of the $(W_{max}^{R})^2$ term indicates that there is an optimum steric condition for the binding of the alkyl series of compounds to the receptor. Figure 4 shows the parabolic dependence of the cytokinin agonistic and antagonistic activities of the alkyl derivatives to the W_{max}^{R} value, whereas Figure 5 shows the linear, downward relationship of the activity of phenyl derivatives to the W_{max}^{Ph}. The positive coefficient of the W_u^R term indicates that the wider the N^4-alkyl substituents in the W_u^R direction, the higher the activity; on the other hand, the negative coefficient of the L term suggests that the longer the substituents, the lower the activity. The W_u^R term reflects, above all, the bulkiness of the compounds having a branch at the C-1 region. Table VI shows the development of eq 3.

The activity of the *n*-octyl derivative (**36**) is best explained by using the steric parameters for the compact conformation reported by Verloop et al.[10] rather than those for the staggered conformation. This result appears to suggest that such a long chain would attain a more compact conformation in the receptor cavity to minimize the energy of interaction. The activity of the norbornyl derivative (**40**) deviated significantly from the predicted value; thus, it was excluded from the analysis. The steric parameters calculated from the model constructed by the normal sp^3 carbon may be deviated from the actual structure of the strained, bicyclic compound.

Discussion

The mode of interaction with the cytokinin receptor of both agonists and antagonists has a common feature within a congeneric series, as denoted by the equations developed in this study. The activity of phenyl derivatives is sterically governed by the maximum width of the N^4 substituents, W_{max}^{Ph}, and in the alkyl series of compounds, it is governed by W_{max}^{R}, W_u^R, and L^R. The optimum maximum width of the N^4-alkyl substituents, 4.7 Å, predicted from eq 3, coincides with the value, 5.2 Å, estimated previously for the congeneric N^6 substituents of adenylate cytokinins[20] and also with that of the N^4 substituents, 4.5 Å, preliminarily calculated for the anticytokinin activity of 2-(methylthio)pyrido[2,3-*d*]pyrimidine derivatives, another class of anticytokinins recently developed by us.[7] The fact that the optimum steric condition in terms of W_{max}^{R} is the same throughout these three series of compounds, irrespective of the intrinsic activity, agonistic or antagonistic, may provide us with an insight into the bulkiness of the receptor cavity, into which an active compound must fit. A similar optimum condition in terms of the W_{max}^{R} value has also been observed for another class of cytokinins with an apparently different structure, N,N′-disubstituted ureas.[20]

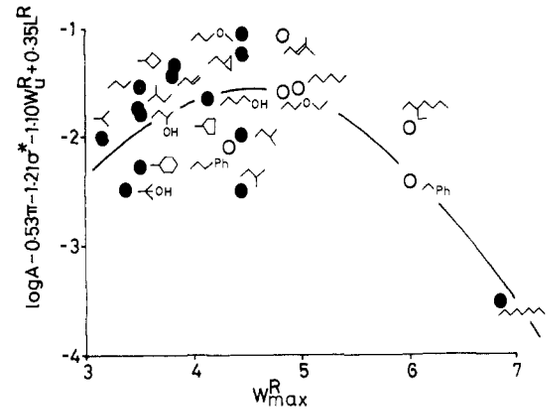

Figure 4. Relationship of the cytokinin agonistic and antagonistic activities of 4-alkylamino derivatives to W_{max} expressed by eq 3. The compounds denoted by open circles are those having agonistic activity.

Equations developed in this study explain the extent of activity irrespective of the quality of activity. We have previously and semiquantitatively shown the participation of the maximum width of N^4-alkyl side chains in agonist–antagonist discrimination.[6] Figure 4 shows in a more striking manner that the W_{max}^{R} values of agonists are in the range of 4.5–6.0 Å, and at the same time, they are on the parabolic curve of eq 3 in terms of W_{max}^{R}. Thus, the results coincide with and provide evidence for the hypothetical concept for hormonal action that agonist binding causes a conformational change of an otherwise inactive receptor to the active form and that antagonists are species that bind *similarly* to the receptor but do not cause the effective conformational change. In the present case with the N^4-alkyl derivatives, the interaction at the region in the W_{max}^{R} direction is responsible not only for the binding but also for the quality of activity, i.e., a conformational change leading to the active species. Also, in the phenyl series of compounds, the interaction at the W_{max}^{Ph} region has the same role. As Figure 5 shows, the agonists have W_{max}^{Ph} values larger than ca. 4.0 Å. The compounds that fall into this category are the meta-substituted derivatives that possess a projection in the W_{max} direction. *m*-Amino (**17**) and *o*-methyl (**18**) derivatives with weak anticytokinin activity are considered to be borderline cases.

The weak activity of meta derivatives, irrespective of the quality of activity, is explained in terms of binding affinity by the larger W_{max}^{Ph} values. Because of the large negative coefficient of the W_{max}^{Ph} term in eq 3, the larger the value, the lower the activity. Although the maximum width of

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(21) Henderson, T. R.; Frihart, C. R.; Leonard, N. J.; Schmitz, R. Y.; Skoog, F. *Phytochemistry* **1975**, *14*, 1687.

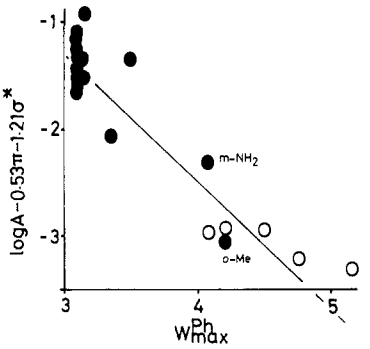

Figure 5. Relationship of the cytokinin agonistic and antagonistic activities of 4-anilino derivatives to W_{max} expressed by eq 3. The compounds denoted by open circles are those having agonistic activity.

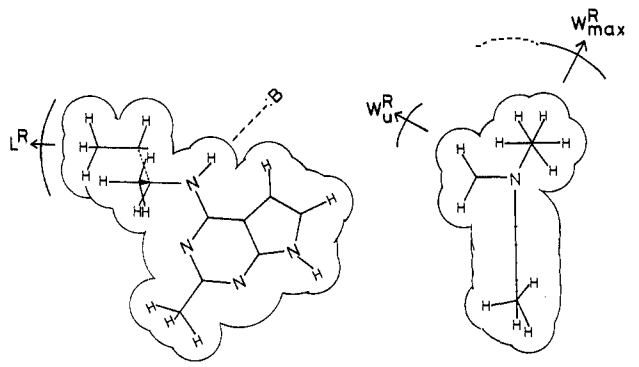

Figure 6. Schematic complex formation of the cytokinin receptor with 4-alkyl derivatives. The compound used as a model is 4-(*sec*-butylamino)-2-methylpyrrolo[2,3-*d*]pyrimidine. The solid lines show the interaction sites or spatial walls indicated by the $W_{max}{}^{R}$ and $W_{u}{}^{R}$ terms in eq 3, and the dotted line shows the interaction site indicated by the $W_{max}{}^{Ph}$ term (see Figure 7). :B represents the hydrogen acceptor site of the receptor.

the substituents is important for both sets of compounds in determining activity, the spatial arrangement on the receptor surface or in the receptor cavity appears to be different. This comes about because $W_{max}{}^{R}$ and $W_{max}{}^{Ph}$ were introduced into eq 3 separately as independent variables. In Figures 6 and 7, the schematic binding models of alkyl and phenyl derivatives to the receptor were drawn based on the information obtained from eq 3. The plane of the zigzag, main chain of the alkyl substituents was twisted 30° from the plane of the heterocyclic ring to avoid an eclipsed conformation and to minimize steric constraint, whereas the benzene ring of phenyl derivatives was laid on that plane, considering the coplanarity with the heteroaromatic ring due to the resonance effect. As a result, the spatial arrangements of the N^4-alkyl and N^4-phenyl substituents differ somewhat from each other as shown in Figures 6 and 7. The former faces the spatial wall in the $W_{max}{}^{R}$ direction and the latter in the $W_{max}{}^{Ph}$ direction, although the model drawings suggest that the interaction sites or surface may be continuous. On the other hand, the fact that the L^R term was incorporated into eq 3 with a negative coefficient whereas the L^{Ph} term was not significant in spite of the wide variation in its values seems suggestive that a spatial wall exists closer to the alkyl substituents in the L^R direction while the region where the para substituents of the phenyl derivatives are located is rather open. An attempt to examine this further by preparing a compound with a longer para substituent, *p*-*n*-butyl, was unsuccessful because of its sparing solubility in the bioassay medium. The spatial wall indicated by the $W_{u}{}^{R}$ term is considered to locate in the region to which the C-1 substituents of the alkyl side chain direct. The constancy, and thus insignificance, in the correlation of the width in the W_u direction of the phenyl derivatives is seen by the comparison of Figure 7 with Figure 6.

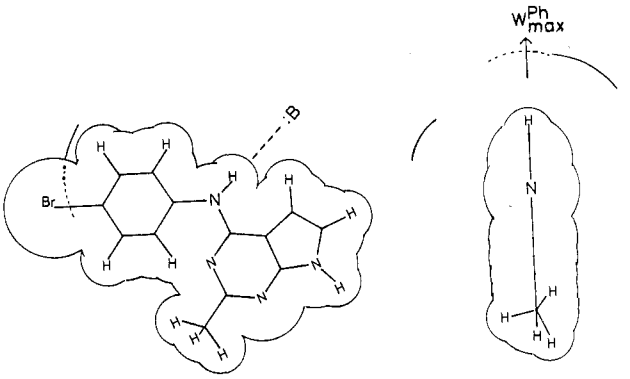

Figure 7. Schematic complex formation of the cytokinin receptor with 4-anilino derivatives (see Figure 6 and its legend). The compound used as a model is 4-(*p*-bromoanilino)-2-methylpyrrolo[2,3-*d*]pyrimidine. The double bonds of the heteroaromatic and benzene rings are abridged.

The common terms π and σ^* with positive coefficients in eq 3 for both series of compounds mean that hydrophobicity and an electron-withdrawing effect both enhance activity through whole set of compounds. The hydrophobicity of a molecule may be important not only in transport process(es) but also at the site of action. The electronic effect of the substituents is considered to operate on the NH group so as to form a hydrogen bond with a hydrogen acceptor on the receptor surface. The value of the coefficient, 0.89, is about a half of that, 2.03, estimated previously for N^6-adenylate cytokinins.[20] Since such hydrogen bonding is very sensitive to the geometry of binding, the difference in the coefficient values appears to reflect the somewhat differed geometry of interactions between these two classes of compounds; N^6-substituted adenines can form a substrate–receptor complex with more proper geometry of binding for hydrogen bonding. The different geometry of binding, although subtle, may explain the fact that all of the N_6-substituted adenines hitherto reported act as cytokinins, whereas some of the present compounds having substituents in common with adenylate cytokinins are anticytokinins.

We supposed the hydrogen bonding site at N^4 to be as shown in Figures 6 and 7. We prepared 4-(cyclopentyloxy)- and 4-(cyclohexyloxy)-2-methylpyrrolo[2,3-*d*]pyrimidines, in which the N^4 H of compounds **29** and **30** is replaced by an oxygen atom. The biological test showed that their activities are significantly lower than those of compounds, **29** and **30**, the I_{50} being ca. 10 μM (log $1/I_{50}$ = ca. −1.0). The hydrophobicity difference in terms of the log *P*, the logarithm of the partition coefficient between 1-octanol/water, is calculated by $\Delta \log P = \log P$ (C_6H_5-OEt)[11] − log *P* (C_6H_5-NHEt)[11] to be 0.3. The bond angles of –NH– and –O– are essentially the same, and thus the molecular shape is considered to be only slightly different between these two kinds of compounds. Therefore, the difference in activity is not explained by these factors but may be attributed to the lack of hydrogen-bonding ability at the O^4 position. The fact that they exerted any activity shows that the steric dimension of the molecule is of prime importance for activity; i.e., accomodation to the receptor cavity is the precedence factor, and the electronic effect, if any, intensifies the binding. These considerations coincide with the results of the quantitative analysis given above and may provide an explanation for the activity of the N^6-adenylate cytokinin analogues having different connecting links, such as –O–, –S–, $-CH_2-$ and –CH=,

Table VII. Properties of Previously Unreported 4-Substituted 2-Methylpyrrolo[2,3-*d*]pyrimidines[a]

no.	mp, °C	formula	yield, %
2	267	$C_{13}H_{11}N_4F$	73
3	267	$C_{13}H_{11}N_4Cl$	63
4	283	$C_{13}H_{11}N_4Br$	7
5[b]	274[c]	$C_{19}H_{14}O_7N_7I$	9
6	264	$C_{14}H_{14}N_4$	28
7	261	$C_{15}H_{16}N_4$	29
8	251	$C_{13}H_{13}N_5$	63
9[b]	264	$C_{19}H_{15}O_5N_7$	5
10	281	$C_{14}H_{14}ON_4$	49
11	272	$C_{15}H_{16}ON_4$	26
12[b]	262[c]	$C_{21}H_{17}O_8N_7 \cdot 0.1H_2O$	12
13	287-289	$C_{14}H_{11}N_4F_3$	81
14	265-266	$C_{16}H_{18}N_4$	27
15	>300	$C_{16}H_{18}N_4$	31
16	>300	$C_{14}H_{11}N_5$	43
17	260[c]	$C_{13}H_{13}N_5$	9
18	288	$C_{14}H_{14}N_4$	25
19	221-222	$C_{17}H_{20}N_4$	21
20	264	$C_{13}H_{11}N_4Br$	14
21	237	$C_{13}H_{11}N_4Cl$	34
22	232	$C_{13}H_{11}N_4I$	37
23	257	$C_{14}H_{14}N_4$	21
24	213	$C_{14}H_{14}ON_4$	35
26[b]	262-263[c]	$C_{16}H_{17}O_7N_7$	36
32	178-179	$C_{10}H_{14}ON_4$	46
33	207	$C_{10}H_{14}ON_4$	45
35[b]	214	$C_{16}H_{17}O_8N_7$	58
37	209-210	$C_{11}H_{14}N_4$	48
39	175-177	$C_{12}H_{18}N_4$	52
40	201-203	$C_{14}H_{18}N_4$	60
41	>300	$C_{10}H_{12}N_4$	57
47	205-207	$C_{15}H_{16}N_4 \cdot {}^1/_6H_2O$	28
48[b]	190	$C_{17}H_{19}O_8N_7$	38

[a] Analyses were carried out for C, H, and N and the results agreed with the calculated values within ± 0.3%. [b] Isolated as picrates. [c] Decomposed.

between the purine ring and side chain.[21]

The correlation eq 2 and 3 indicate that, within the congeneric phenyl derivatives, compounds having as large as possible π and σ^* values and as small as possible W_{max}^{Ph} values should be highly active as anticytokinins. The *p*-CF_3 (**13**) and *p*-isopropyl (**15**) derivatives are the compounds thus derived, the activities of which correspond to those of the highest active members in the alkyl series, the cyclobutyl (**28**) and cyclopentyl (**29**) derivatives, but the preparation of congeneric compounds with higher activity than these appears limited by the difficulty of synthesis and insolubility in the bioassay medium. The knowledge obtained from quantitative studies like the present and previous reports[6,7,20] may, however, be of help in obtaining insights into the mode of action of cytokinin agonists and antagonists and in thinking of new structures for active compounds.

Experimental Section

UV and ^{1}H NMR spectra were recorded on Shimadzu UV-200 and Hitachi R-22 spectrometers, respectively. All melting points were corrected.

Synthetic Procedure. 4-(Alkylamino)- and 4-anilino-2-methylpyrrolo[2,3-*d*]pyrimidines were prepared by refluxing a solution in which 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine[8] and appropriate alkylamines or anilines were dissolved in a 1:1.5 molar ratio. The end of the reaction was determined by TLC. The reaction mixture was evaporated in vacuo to dryness, and the residue was washed with water to give a solid, which was recrystallized from ethanol, ethanol–water, or water. Some compounds that could not be readily crystallized were isolated as picrate salts and recrystallized from ethanol. Refluxing of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine[8] in cyclopentanol and cyclohexanol, in which an appropriate amount of Na metal was dissolved, similarly gave 4-(cyclopentyloxy)-2-methylpyrrolo-[2,3-*d*]pyrimidine in 9% yield [mp 167 °C. Anal. ($C_{12}H_{15}ON_3$) C, H, N] and 4-(cyclohexyloxy)-2-methylpyrrolo[2,3-*d*]pyrimidine in 43% yield [mp 186 °C. Anal. ($C_{13}H_{17}ON_3$) C, H, N], respectively.

The physicochemical properties of previously unknown 4-(alkylamino)- and 4-anilino-2-methylpyrrolo[2,3-*d*]pyrimidines are summarized in Table VII. The analytical results for C, H, and N of these compounds were within ±0.3% of the theoretical values.

Bioassay Procedure. Compounds to be tested were added in different concentrations to the basal medium as specified previously.[9] The pH of the medium was adjusted to 5.6 with 1 N NaOH and autoclaved at 1.0 kg/cm^2 for 15 min. Three callus pieces of ca. 10 mg fresh weight derived from *Nicotiana tabacum* L. var Wisconsin No. 38 were implanted on the agar surface and maintained at 28 °C in darkness for 4 weeks, and then the average fresh weight was determined. The standard deviation of the activity measurements was within ±30%.

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Registry No. 1, 71149-48-9; **2**, 85250-72-2; **3**, 85250-73-3; **4**, 85250-74-4; **5** picrate, 85250-76-6; **6**, 85250-77-7; **7**, 85250-78-8; **8**, 85250-79-9; **9** picrate, 85250-81-3; **10**, 85250-82-4; 11, 85250-83-5; **12** picrate, 85250-85-7; **13**, 85250-86-8; **14**, 85250-87-9; **15**, 85250-88-0; **16**, 85250-89-1; **17**, 85250-90-4; **18**, 85250-91-5; **19**, 85267-30-7; **20**, 85250-92-6; **21**, 85250-93-7; **22**, 85250-94-8; **23**, 85250-95-9; **24**, 85250-96-0; **25**, 71149-45-6; **26** picrate, 85250-98-2; **27**, 71149-49-0; **28**, 71149-50-3; **29**, 71176-15-3; **30**, 71149-47-8; **31**, 71149-44-5; **32**, 85250-99-3; **33**, 85251-00-9; **34**, 85251-01-0; **35** picrate, 85251-03-2; **36**, 71149-42-3; **37**, 85251-04-3; **38**, 71149-43-4; **39**, 85251-05-4; **40**, 85251-06-5; **41**, 85251-07-6; **42**, 71149-51-4; **43**, 71149-39-8; **44**, 71149-38-7; **45**, 71149-40-1; **46**, 1866-43-9; **47**, 85251-08-7; **48** picrate, 85251-10-1; 4-chloro-2-methylpyrrolo-[2,3-*d*]pyrimidine, 71149-52-5; *p*-fluoroaniline, 371-40-4; *p*-chloroaniline, 106-47-8; *p*-bromoaniline, 106-40-1; *p*-iodoaniline, 540-37-4; *p*-methylaniline, 106-49-0; *p*-ethylaniline, 589-16-2; *p*-phenylenediamine, 106-50-3; *p*-hydroxyaniline, 123-30-8; *p*-methoxyaniline, 104-94-9; *p*-ethoxyaniline, 156-43-4; *p*-acetylaniline, 99-92-3; *p*-(trifluoromethyl)anline, 455-14-1; *p*-propylaniline, 2696-84-6; *p*-isopropylaniline, 99-88-7; *p*-cyanoaniline, 873-74-5; *m*-phenylenediamine, 108-45-2; *o*-methylaniline, 95-53-4; *p*-*n*-butylaniline, 104-13-2; *m*-bromoaniline, 591-19-5; *m*-chloroaniline, 108-42-9; *m*-iodoaniline, 626-01-7; *m*-methylaniline, 108-44-1; *m*-methoxyaniline, 536-90-3; isopropylamine, 75-31-0; 3-amino-1-propanol, 156-87-6; 1-amino-2-propanol, 78-96-6; 2-methoxyethylamine, 109-85-3; cyclopropylmethylamine, 2516-47-4; neopentylamine, 5813-64-9; 2-norbornanamine, 822-98-0; cyclopropylamine, 765-30-0; phenethylamine, 64-04-0; 2-ethoxyethylamine, 110-76-9; 4-(cyclopentyloxy)-2-methylpyrrolo[2,3-*d*]pyrimidine, 85251-11-2; 4-(cyclohexyloxy)-2-methylpyrrolo-[2,3-*d*]pyrimidine, 85251-12-3.