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CYTOKININ—AGONISTIC AND ANTAGONISTIC ACTIVITIES OF 4-SUBSTITUTED-2-METHYLPYRROLO[2,3-*d*]PYRIMIDINES, 7-DEAZA ANALOGS OF CYTOKININ-ACTIVE ADENINE DERIVATIVES

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Key Word Index—Tobacco callus; lettuce seed germination; *Amaranthus* betacyanin synthesis; anticytokinins; cytokinins; pyrrolo[2,3-*d*]pyrimidines; structure–activity relationship.

Abstract—Sixteen 4-substituted-2-methylpyrrolo[2,3-*d*]pyrimidines were tested for their activities as cytokinin agonists and antagonists in three bioassay systems. With the systematic variation of the 4-substituent, the activity changed consecutively from agonistic to antagonistic in the tobacco callus test, and this agonist–antagonist relationship was analysed by a steric substituent parameter as a common quantitative measure. Two compounds were found to possess bud-forming activity in the absence of added cytokinins. Eight compounds promoted betacyanin synthesis in *Amaranthus* and 4 promoted germination in lettuce seeds. The 4-hydroxyethyl derivative, which was inactive in the tobacco test, suppressed betacyanin synthesis at 100 μM against 1 μM 6-(3-methyl-2-butenylamino)-purine. The 4-phenyl derivative showed antagonist activity in the tobacco test, little activity in the *Amaranthus* bioassay and significant agonist activity in the lettuce seed germination. The 4-cyclobutyl derivative exerted the strongest anticytokinin activity (I_{50} at 0.06 μM against 0.05 μM kinetin) in the tobacco callus assay, while little activity was observed in the other two assay systems.

INTRODUCTION

Development of antagonists as well as agonists is of potential value in studies of the mechanism of action of biologically active substances. Firstly, the synthesis of antagonists itself contributes to the recognition of structural differences associated with intrinsic activity and binding affinity, especially when both the agonists and antagonists have a common structural basis. Secondly, antagonists extend the studies of agonist action by blocking its utilization in biological systems. The latter is of particular importance in studies of cytokinins because most plant systems grow without added exogenous cytokinins. Cytokinins and other plant hormones generally have, however, multiple functions in intact plants and even in cultured cells. Most cytokinin agonists hitherto prepared have a N^6-substituted adenine structure and more or less possess a multiple nature. In contrast to this, the anticytokinins so far developed [1–5] have not been reported to be active as such in plant systems other than cultured callus cells. These facts prompt us to develop agonists having selective activity and the antagonists possessing specific applicability.

4-Substituted pyrrolo[2,3-*d*]pyrimidines, as the heterocyclic analogs of adenylate cytokinins, behave interestingly as both agonists and antagonists. 4-Substituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines exhibit anticytokinin activity against exogenously supplied cytokinins in tobacco callus bioassay [4], while the corresponding, deribosylated derivatives, 4-substituted pyrrolo[2,3-*d*]pyrimidines, act as cytokinins [6, 7]. Introduction of a methylthio group in the 2-position of the 4-substituted nucleus, however, restores strong anticytokinin activity [5]. Consequently, the introduction of a substituent into the 2 or 7 position appears to be important for the conversion of 4-substituted pyrrolo-[2,3-*d*]pyrimidines into anticytokinins‡. To test this further and to obtain more information on the agonist–antagonist relationship, we prepared a series of 4-substituted 2-methylpyrrolo[2,3-*d*]pyrimidines and tested them for activity in 3 different bioassay systems; the growth of tobacco callus [9, 10], the germination of lettuce seed [11] and the betacyanin synthesis of *Amaranthus* [12].

RESULTS AND DISCUSSION

Synthesis of 4-substituted 2-methylpyrrolo[2,3-d]*pyrimidines*

4-Benzylamino-, 4-*n*-propylamino-, 4-*n*-amylamino- and 4-anilino-2-methylpyrrolo[2,3-*d*]pyrimidines (**2**, **3**, **9**, **12**) have been previously prepared [13]. Other 4-substituted 2-methylpyrrolo[2,3-*d*]pyrimidines, **1**, **4**–**8**, **10**, **11**, **13**–**16**, were synthesized by treatment of the intermediate, 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine [13], with the appropriate amine by refluxing in *n*-

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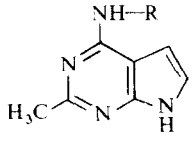
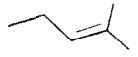
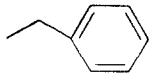
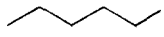
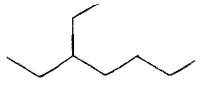
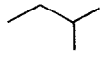
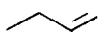
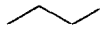
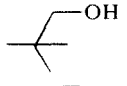
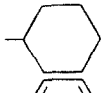
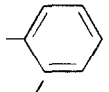
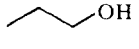
‡ We observed anticytokinin activity firstly in the tobacco callus bioassay of 4-substituted-7-methylpyrrolo[2,3-*d*]pyrimidines [8], where the ribofuranosyl moiety of the 4-substituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines had been replaced by methyl.

Table 1. Effects of 4-substituted-2-methylpyrrolo[2,3-*d*]pyrimidines on tobacco callus growth

Compound No.		Cytokinin activity Maximum response (μM)	Anticytokinin activity I_{50} (μM) against 0.05 μM kinetin	Maximum width (W_{max}) of N^4-substituent* (Å)
1		4.0		4.72
2		10.0		6.02
3		10.0		4.94
4		10.0	2.0	5.43
5			40.0	8.78
6			12.5	7.33
7			20.9	4.21
8			6.0	4.15
9			3.0	3.49
10			2.0	3.38
11			0.9	3.49
12			0.5	3.11
13			0.3	3.49
14			0.07	3.98
15			0.06	3.83
16			na†	3.38

* Calculation was based on the fully extended conformation or staggered conformation.
† Not active enough to estimate I_{50} value within the concentration range tested (~40 μM).

butanol. Table 1 summarizes the compounds tested in this study.

Effects on tobacco callus growth

Structure–activity relationship. Compounds **1**, **2**, **3** and **4** showed cytokinin activity (Fig. 1). Compounds **5**–**15** were inactive as cytokinins but inhibited the growth of tobacco callus cultured on a medium containing 0.05 μM kinetin (Fig. 2). Of these compounds, the cyclopentyl (**14**) and cyclobutyl (**15**) derivatives were most active, showing detectable inhibition at 0.01–0.04 μM. Characteristic for this series of compounds was the fact that the activity varied from agonistic to antagonistic with the systematic transformation of the side chains.

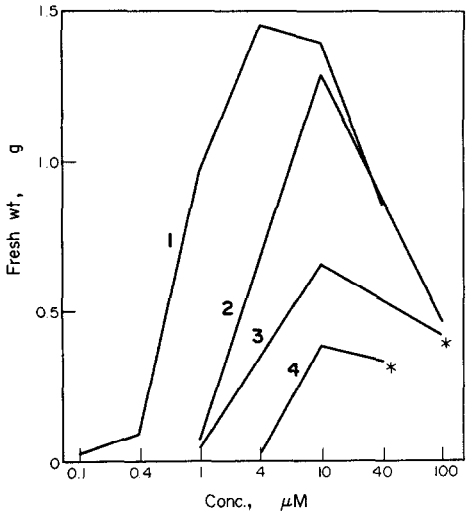


Fig. 1. Effects of compounds **1**, **2**, **3** and **4** on the fresh weight yield of tobacco callus. * Treatments in which buds formed.

Four cytokinins, **1**, **2**, **3** and **4** in Fig. 1, gave their optimum responses at 4–10 μM. The maximum yields of callus decreased, however, sharply in this order, indicating qualitatively the decrease of the intrinsic activity as cytokinins while the binding affinity itself is maintained *ca* constant. Because the molecular structure is altered only in the N^4-side chain, the variations in activity can be attributed to the structural modification of the substituents. Thus, the compounds where the side chains are further transformed would behave as cytokinin antagonists, the compounds which possess the binding affinity but not the intrinsic activity at the receptor site of cytokinins. Compounds **5**–**15** in Fig. 2 illustrate this. Compound **4** is considered to be a borderline case and to have fairly good binding affinity but weak intrinsic activity. Even though it exerts weak cytokinin activity by itself, it hinders the binding of the agonist at the receptor site when mixed with a stronger cytokinin like kinetin.

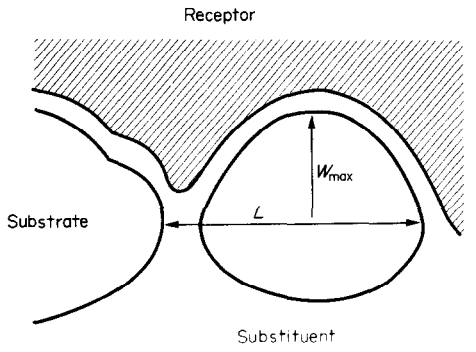


Fig. 3. Schematic representation of a substituent along the bond-axis, L, showing the W_{max} parameter.

The results are suggestive of the participation of a steric factor in the agonist–antagonist discrimination as well as the structure–activity relationship. The fact that the compounds which have branching at the α position to the exocyclic nitrogen atom (**11**–**15**) exhibited strong antagonistic activity indicates especially the importance of the molecular shape or a steric factor of N^4-substituents. Thus, we surveyed steric parameters currently used for the analysis of structure–activity relationship, and found that the maximum width, W_{max}, of substituents from the bond-axis between the exocyclic nitrogen atom and its α carbon atom illustrates the variation of the activity. The W_{max} is equivalent to the B_4 parameter, one of the STERIMOL parameters developed recently by Verloop, Hoogenstraaten and Tipker [14] based on the Corey–Pauling–Koltun models (Fig. 3).

Table 1 indicates that the W_{max} values of the cytokinin agonists **1**, **2**, **3** and **4** are within the range of 4.7–6. Those in which the W_{max} values are smaller than this range, e.g. **7**–**15**, and larger than 7, e.g. **5** and **6**, are cytokinin antagonists. The range of W_{max} values of the former 9 compounds is 3–4.5 and the value for the most active, cyclopentyl derivative (**15**) is 3.83. Thus, there seems to exist an activity maximum depending

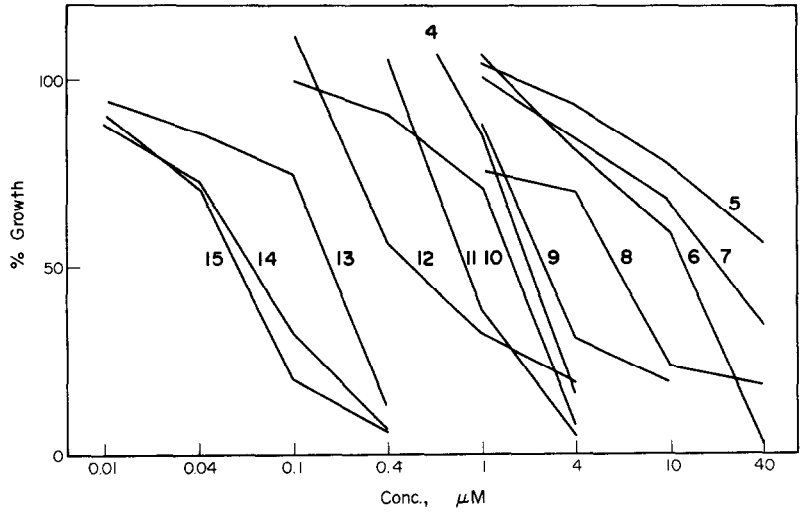


Fig. 2. Effects of compounds **4**–**15** on the fresh weight yield of tobacco callus cultured on medium containing 0.05 μM of kinetin.

upon the steric bulk of N^4-substituents. The *n*-decyl derivative (**5**) is weaker in antagonist activity than the *n*-octyl derivative (**6**), suggesting that their W_{max} values exceed the depth of the hypothetical receptor cavity in which the N^4-substituents are engulfed. Alternatively, this may be explained by their bond-axis lengths (*L* in Fig. 3); they are calculated to be 12.33 and 10.27, respectively, while those of the other compounds are less than the 8.22 of compound **4**. Skoog *et al.* have also examined the effects of N^6-alkyladenines on the growth of tobacco callus and found that the activity changed as a function of chain length [15]. Maximum width W_{max} of compound **4** is 5.43 which explains its cytokinin activity but not the anticytokinin activity. Thus a steric factor other than maximum width may be operative, although the exact characterization remains for future study. In general, however, it can be said that the compounds whose W_{max} values are smaller than 4.21 of **7** or larger than 7.33 of **6** do not possess intrinsic activity.

The introduction of quantitative measurements are common for the analyses of biologically active substances. However, auxins are the only other class of plant hormones hitherto analysed in this sense [16, 17]. The finding of the applicability of the steric parameter W_{max} would enable a quantitative approach also in the analysis of cytokinin activity.*

Bud-forming activity. Compounds **3** and **4** caused bud formation at 100 and 40 μM, respectively, in the presence of 11.4 μM of indole-3-acetic acid (IAA) and in the absence of added cytokinins (Fig. 1). Skoog *et al.* [5] have described the enhancement of cytokinin-promoted budding by anticytokinin derivatives, 4-cyclopentylamino- and 4-cyclohexylamino-2-methylthiopyrrolo[2,3-*d*]pyrimidines, at lower (1–10 μM) concentrations but at high levels of added cytokinins. Thus the mode of action appears to differ from that of **3** and **4** despite the similarity of the structure. The present results suggest the possiblity of differentiating between the two functions of cytokinins, callus growth promotion and bud formation, in terms of chemical structure.

Effects on Amaranthus *betacyanin synthesis*

Of the compounds tested, **1** and **2** showed the highest cytokinin activity and gave maximum response at 100 μM (Fig. 4A). Compounds **3**, **4**, **7**, **8**, **11** and **14** were at least 10 times less active than **1** and **2**. Of these, **7**, **8**, **11** and **14** were anticytokinins in the tobacco callus test. Compounds **12** and **13**, which were also anticytokinins in the tobacco assay, were inactive when tested on a medium containing 1 μM 6-(3-methyl-2-butenylamino)purine(2iP). These results thus indicate species difference of receptor molecules similar to that recently discussed by us for the optically active N^6-substituted adenylate cytokinins [18].

The hydroxyethyl derivative (**16**), which exerted little activity in the tobacco callus assay, suppressed betacyanin synthesis against 1 μM 2iP, the activity of which was detectable at 100 μM (Fig. 4B). Compound **10** also showed retarding activity although very weak at 1000 μM (data not shown). No conclusions can be drawn on their anticytokinin nature before we obtain

* In connection with this, of 7-substituted-3-methylpyrazolo-[4,3-*d*]pyrimidines prepared by Skoog *et al.* [1, 2] for the development of anticytokinins, two compounds, 4-(3-methyl-2-butenylamino)- and 4-isobutylamino-3 methylpyrazolo[4,3-*d*]-pyrimidines, were reported to be weakly active as cytokinins, but the authors stated only the role of the isopentenyl double bond as a factor to remove antagonist activity. If one considers the activity of isobutyl derivative as well, this series of compounds may be treated in a manner similar to the present procedure. It is also suggested that the antagonistic activity of 4-substituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [3, 4] and 4-substituted-2-methylthiopyrrolo[2,3-*d*]pyrimidine [5] anticytokinins could be analysed similarly. Quantitative details will be discussed separately.

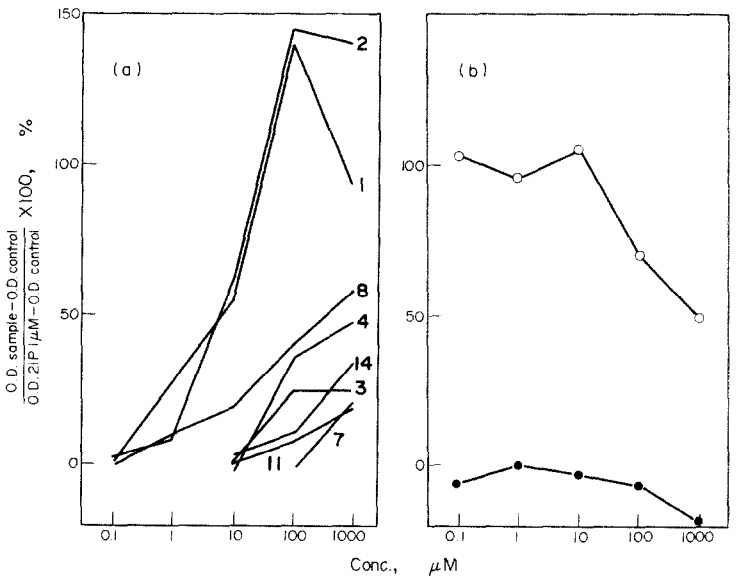


Fig. 4. A: Effects of compounds **1**–**4**, **7**, **8**, **11** and **14** on *Amaranthus* betacyanin synthesis. B: Effects of compound **16** on *Amaranthus* betacyanin synthesis in the presence (○) and in the absence (●) of 2iP. Vertical axis represents the yield of the pigment in percentage of that brought about by 1 μM of 2iP.

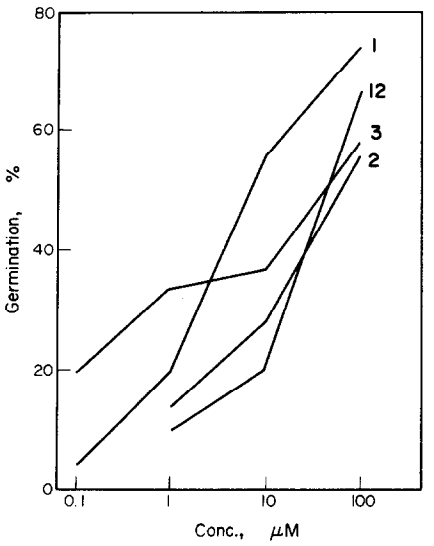


Fig. 5. Effects of compounds **1**, **2**, **3** and **12** on lettuce seed germination.

more analogs of similar activity, although anticytokinins hitherto developed have not been reported to act as such, in biological systems other than cultured tobacco cells [1–5]. The hydroxy group which structurally differentiate them from others appears, however, to imply the importance of lipohydrophilic factor if a common reactive site is assumed.

Effects on lettuce seed germination

Compounds **1**, **2**, **3** and **12** exerted significant agonist activity (Fig. 5) and other compounds were slightly active as both agonists and antagonists, indicating again the species difference. Striking were the results obtained with the phenyl derivative (**12**) in the 3 bioassay systems employed in this study, i.e. it exhibited anticytokinin activity in the tobacco callus bioassay, little activity in the *Amaranthus* betacyanin synthesis test and cytokinin activity in this test. This indicates the possibility of designing compounds having high selective activity. Compounds **3** and **4**, which promote bud-formation in particular, and compound **15**, for example, which is a strong antagonist in the tobacco callus bioassay and has little activity in the other two systems, fall in this category.

EXPERIMENTAL

All mps are corr.

4-(3-*Methyl*-2-*butenylamino*)-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**1**). A mixture of 540 mg (3.2 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine [13], 540 mg (4.4 mmol) of 3-methyl-2-butenylamine·HCl [19] and 5 ml of triethylamine in 20 ml of *n*-BuOH was refluxed for 19 hr. The reaction mixture was evapd *in vacuo* and the residue triturated with 28% NH_4OH and evapd *in vacuo*. The residue was washed with H_2O leaving 467 mg (67%) of white crystals. Recrystallization from EtOH–H_2O gave 328 mg (55%) of colorless needles, mp 179°: $\lambda_{max}^{H_2O}$ 280 nm (ε 14 600); $\lambda_{max}^{0.1N\,HCl}$ 280 (14 600); $\lambda_{max}^{0.1N\,NaOH}$ 279 (14 800). (Found: C, 66.56; H, 7.43; N, 26.15. $C_{12}H_{16}N_4$ requires: C, 66.64; H, 7.46; N, 25.91%).

4-(2-*Ethylhexylamino*)-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**4**). A mixture of 514 mg (3.1 mmol) of 4-chloro-2-methylpyrrolo-[2,3-*d*]pyridimidine [13] and 1 ml of 2-ethylhexylamine in 15 ml of *n*-BuOH was refluxed for 8 hr. The reaction mixture was evapd *in vacuo* and the residue was washed with H_2O to give 786 mg (99%) of white crystals. Recrystallization from EtOH–H_2O gave 575 mg (72%) of colorless needles, mp 127°; $\lambda_{max}^{H_2O}$ 279 nm (ε 13 900); $\lambda_{max}^{0.1N\,HCl}$ 280 (13 900); $\lambda_{max}^{0.1N\,NaOH}$ 278 (13 100). (Found: C, 69.23; H, 9.35; N, 21.45. $C_{15}H_{24}N_4$ requires: C, 69.19; H, 9.29; N, 21.52%).

4-n-*Decylamino*-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**5**). A mixture of 520 mg (3.1 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]-pyrimidine [13], 1 ml of *n*-decylamine and 5 ml of triethylamine in 30 ml of *n*-BuOH was refluxed for 8 hr. To the reaction mixture was added 28% NH_4OH and the soln evapd *in vacuo*. The residue was washed with H_2O and crystallized from EtOH–H_2O to give 505 mg (55%) of white crystals. Recrystallizations from EtOH–H_2O gave 283 mg (31%) of white crystals, mp 107°. *Picrate salt*. The reaction mixture obtained as above was evapd *in vacuo* and the residual oil dissolved in 5 ml of EtOH. To the EtOH soln was added dropwise a satd picric acid soln in EtOH and the mixture was cooled to deposit yellow crystals. Recrystallizations from EtOH gave yellow needles, mp 167°; $\lambda_{max}^{H_2O}$ 268 nm (ε 17 000), 358 (9900); $\lambda_{max}^{0.1N\,HCl}$ 268 (18 100), 357 (8900); $\lambda_{max}^{0.1N\,NaOH}$ 264 (16 700), 356 (10 700). (Found: C, 53.60; H, 6.09; N, 19.02. $C_{23}H_{31}O_7N_7$ requires: C, 53.37; H, 6.04; N, 18.95%).

4-n-*Octylamino*-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**6**). A mixture of 510 mg (3.0 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]-pyrimidine [13] and 0.8 ml of *n*-octylamine in 30 ml of *n*-BuOH was refluxed for 5 hr. The reaction mixture was evapd *in vacuo* and the residue was washed with H_2O to give 718 mg (91%) of crude crystals. Recrystallizations from EtOH–H_2O gave 546 mg (69%) of colorless needles, mp 118°; $\lambda_{max}^{H_2O}$ 276 nm (ε 15 500); $\lambda_{max}^{0.1N\,HCl}$ 276 (15 900); $\lambda_{max}^{0.1N\,NaOH}$ 275 (13 200). (Found: C, 69.49; H, 9.54; N, 21.50. $C_{15}H_{24}N_4$ requires: C, 69.19; H, 9.29; N, 21.52%).

4-(2-*Methylpropylamino*)-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**7**). A mixture of 300 mg (1.8 mmol) of 4-chloro-2-methylpyrrolo-[2,3-*d*]pyrimidine [13] and 1 ml of 2-methylpropylamine in 10 ml of *n*-BuOH was refluxed for 6 hr. The reaction mixture was evapd *in vacuo* and the residue washed with H_2O to give 323 mg (88%) of white crystals. Recrystallization from EtOH–H_2O gave 278 mg (76%) of colorless platelets, mp 192–193°; $\lambda_{max}^{H_2O}$ 278 nm (ε 12 900); $\lambda_{max}^{0.1N\,HCl}$ 278 (14 100); $\lambda_{max}^{0.1N\,NaOH}$ 274 (20 600). (Found: C, 64.81; H, 7.84; N, 27.65. $C_{11}H_{16}N_4$ requires: C, 64.67; H, 7.90; N, 27.43%).

4-*Allylamino*-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**8**). A mixture of 605 mg (3.6 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine [13] and 1 ml of allylamine in 25 ml of *n*-BuOH was refluxed for 5.5 hr. The reaction mixture was evapd *in vacuo* and the residue washed with H_2O to give 620 mg (91%) of crude crystals. Recrystallizations from EtOH–H_2O gave 390 mg (57%) of colorless needles, mp 204° (sublimed): $\lambda_{max}^{H_2O}$ 279 nm (ε 12 800); $\lambda_{max}^{0.1N\,HCl}$ 280 (12 800); $\lambda_{max}^{0.1N\,NaOH}$ 277 (14 600). (Found: C, 63.69; H, 6.27; N, 29.68. $C_{10}H_{12}N_4$ requires: C, 63.81; H, 6.43; N, 29.77%).

4-(1-*Hydroxymethyl*-1-*methylethylamino*)-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**10**). To 590 mg (3.5 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine [13] was added 10 ml of 2-amino-2-methyl-1-propanol. The soln was refluxed for 3 hr and evapd to dryness *in vacuo*. The residue was washed with H_2O to give 505 mg (65%) of colorless needles. Recrystallizations from EtOH–H_2O gave 364 mg (47%) of pure sample, mp 259° (dec): $\lambda_{max}^{H_2O}$ 278 nm (ε 15 300); $\lambda_{max}^{0.1N\,HCl}$ 278 (15 300); $\lambda_{max}^{0.1N\,NaOH}$ 277 (16 500). (Found: C, 66.04; H, 7.44; N, 25.39. $C_{11}H_{16}ON_4$ requires: C, 59.98; H, 7.32; N, 25.44%).

4-*Cyclohexylamino*-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**11**). A mixture of 665 mg (4.0 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine [13] and 2 ml of cyclohexylamine in 25 ml of *n*-BuOH was refluxed for 17 hr. The reaction mixture was evapd *in vacuo* and the residue was washed with H_2O to give 714 mg (78%) of crystalline solid. Recrystallization once from *iso*-PrOH–H_2O gave 517 mg (57%) of colorless platelets, mp 223–225°: $\lambda_{max}^{H_2O}$ 278 nm (ε 15 800); $\lambda_{max}^{0.1N\,HCl}$ 278 (15 800); $\lambda_{max}^{0.1N\,NaOH}$ 277 (17 800). (Found: C, 67.79; H, 7.89; N, 24.40. $C_{13}H_{18}N_4$ requires: C, 67.79; H, 7.88; N, 24.33%).

4-*sec*-*Butylamino*-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**13**). A mixture of 500 mg (3.0 mmol) of 4-chloro-2-methylpyrrolo-[2,3-*d*]pyrimidine [13] and 2 ml of *sec*-butylamine in 25 ml of *n*-BuOH was refluxed for 23 hr. The reaction mixture was evapd *in vacuo* and the residual oil extracted × 3 with hot *n*-hexane and the extracts evapd to dryness *in vacuo*. The residue was washed with H_2O to give 158 mg (26%) of colorless needles, mp 134°. *Picrate salt*. The reaction mixture obtained as above was evapd *in vacuo* and the residual oil was dissolved in 5 ml of EtOH. To the EtOH soln was added dropwise a satd picric acid soln in EtOH and the mixture was cooled to deposit yellow crystals. Recrystallizations from EtOH gave yellow needles, mp 260–263°: $\lambda_{max}^{H_2O}$ 274 nm (ε 16300), 355 (13700); $\lambda_{max}^{0.1N\,HCl}$ 274 (17100), 354 (12900); $\lambda_{max}^{0.1N\,NaOH}$ 275 (18000), 356 (14200). (Found: C, 47.27; H, 4.46; N, 22.85. $C_{17}H_{19}O_7N_7$ requires: C, 47.11; H, 4.42; N, 22.63%).

4-*Cyclopentylamino*-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**14**). A mixture of 635 mg (3.8 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine [13] and 1.5 ml of cyclopentylamine in 20 ml of *n*-BuOH was refluxed for 8 hr. The reaction mixture was evapd *in vacuo* and the residue was collected to give 685 mg (84%) of crystalline solid. Recrystallizations from EtOH–H_2O gave 502 mg (61%) of colorless platelets, mp 193°: $\lambda_{max}^{H_2O}$ 280 nm (ε 14200); $\lambda_{max}^{0.1N\,HCl}$ 281 (13600); $\lambda_{max}^{0.1N\,NaOH}$ 279 (16400). (Found: C, 66.77; H, 7.47; N, 25.98. $C_{12}H_{16}N_4$ requires: C, 66.64; H, 7.46; N, 25.91%).

4-*Cyclobutylamino*-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**15**). A mixture of 592 mg (3.53 mmol) of 4-chloro-2-methylpyrrolo-[2,3-*d*]pyrimidine [13], 1 g (9.3 mmol) of cyclobutylamine HCl and 5 ml of triethylamine in 25 ml of *n*-BuOH was refluxed for 25 hr. The reaction mixture was evapd *in vacuo* and the residue triturated with 28% NH_4OH and then evapd *in vacuo*. The residue was washed with H_2O to give 534 mg (75%) of crystalline solid. Recrystallizations from EtOH–H_2O gave 406 mg (57%) of colorless needles, mp 212° (dec): $\lambda_{max}^{H_2O}$ 281 nm (ε 14700); $\lambda_{max}^{0.1N\,HCl}$ 282 (14300); $\lambda_{max}^{0.1N\,NaOH}$ 279 (16000). (Found: C, 65.40; H, 7.00; N, 27.87. $C_{11}H_{14}N_4$ requires: C, 65.32; H, 6.98; N, 27.70%).

4-(2-*Hydroxyethylamino*)-2-*methylpyrrolo*[2,3-d]*pyrimidine* (**16**). A mixture of 527 mg (3.1 mmol) of 4-chloro-2-methylpyrrolo[2,3-*d*]pyrimidine [13] and 1 ml of ethanolamine in 20 ml of *n*-BuOH was refluxed for 5 hr. The reaction mixture was evapd *in vacuo* and the residue was washed with H_2O to give 486 mg (80%) of crude crystals. Recrystallizations from *iso*-PrOH–H_2O gave 212 mg (35%) of colorless needles, mp 241°: $\lambda_{max}^{H_2O}$ 278 nm (ε 13300); $\lambda_{max}^{0.1N\,HCl}$ 280 (13100); $\lambda_{max}^{0.1N\,NaOH}$ 277 (15300). (Found: C, 56.46; H, 6.25; N, 29.23. $C_9H_{12}ON_4$ requires: C, 56.23; H, 6.29; N, 29.15%).

Bioassay procedures. For the tobacco callus (*Nicotiana tabacum* cv Wisconsin No. 38) bioassay, the method described in ref. [9] as modified in ref. [10] was used. Compounds to be tested for cytokinin activity were dissolved in DMSO and added to the cooling, autoclaved medium after suitable dilution. Anticytokinin activity was tested similarly by adding 0.05 μM kinetin to the medium. Cytokinin-induced formation of betacyanin in *Amaranthus caudatus* was examined by the method of ref. [12]. Betacyanin synthesized in the *Amaranthus* explants consisting of the upper portion of the hypocotyl plus the cotyledons was determined by calculating the difference between the *A* at 542 and 620 nm. For the test of inhibitory activity against cytokinins, 1 μM of 2iP was added to the medium. Lettuce seed germination test was undertaken as described in ref. [11]. Briefly, seeds of *Lactuca sativa* cv New York 515 were incubated for 48 hr in darkness at 27° after sowing on filter paper wetted with an aq. soln of each sample in a Petri dish, and the percentage germination was measured. Antagonistic activity was examined by adding 10 μM of kinetin to the sample soln.

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