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QUANTITATIVE ANALYSIS OF ANTICYTOKININ ACTIVITY OF 4-SUBSTITUTED-2-METHYLTHIOPYRIDO[2,3-*d*]PYRIMIDINES

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Key Word Index—Anticytokinins; tobacco callus; pyrido[2,3-*d*]pyrimidines; structure–activity relationship.

Abstract—Ten 4-substituted-2-methylthiopyrido[2,3-*d*]pyrimidines were synthesized and tested for their cytokinin-antagonistic activity by the tobacco callus bioassay. This series of compounds constitutes the first example of anti-cytokinins which possess a fused 6-6 membered ring system. The treatment of Lineweaver and Burk, the method of classical enzyme kinetics, revealed competitive inhibition of cytokinin-induced tobacco callus growth. The variation of activity with the systematic transformation of 4-substituents was analysed quantitatively with physicochemical substituent parameters and regression analysis. The results indicated the predominant importance of substituent width for binding of the antagonists at the receptor site of cytokinins.

INTRODUCTION

We have developed series of anticytokinins which possess pyrrolo[2,3-*d*]pyrimidine structure, i.e. 4-substituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines [1, 2] and 4-substituted-2-methylpyrrolo[2,3-*d*]pyrimidines [3]. Compounds of the latter class changed in inactivity from agonistic to antagonistic with the systematic variation of the 4-substituent in the tobacco callus growth test. This result suggested that the width of the substituent is important for the discrimination of agonist–antagonist relationship.

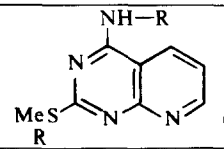
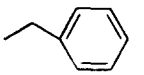
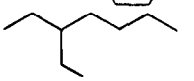
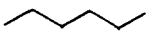
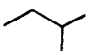
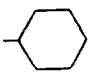
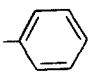
During the course of our study on the pyrrolo[2,3-*d*]-pyrimidine analogs of cytokinins, Skoog *et al.* reported anticytokinin activity of 4-substituted-2-methylthio-pyrrolo[2,3-*d*]pyrimidines [4]. Still earlier, Hecht *et al.* prepared 7-substituted-3-methylpyrazolo[4,3-*d*]pyrimi-dines for the development of anticytokinins [5, 6]. Structurally all of these anticytokinins hitherto reported have a fused 5-6 membered ring system. In this study, we prepared a series of substituted pyrido[2,3-*d*]pyrimidines for the first time as azanaphthalene analogs of N^6-substituted adenine cytokinins and of N^4-substituted pyrrolo[2,3-*d*]pyrimidine anticytokinins. The competi-tive anticytokinin nature of these compounds was shown by the method of Lineweaver and Burk [7]. The variation of activity with structures of the side chain was analysed quantitatively using physicochemical substituent param-eters and regression analysis. The results provide a basis for assessment of the roles of the side chain in the binding at the receptor site of cytokinins.

RESULTS AND DISCUSSION

Synthesis of test substances

First, 4-hydroxy-2-methylthiopyrido[2,3-*d*]pyrimidine was synthesized by methylating 4-hydroxy-2-mercapto-pyrido[2,3-*d*]pyrimidine [8] with methyl iodide in the

Table 1. Anticytokinin activity and constants employed in the derivation of Equations (1) and (2) for inhibition of tobacco callus growth by 4-substituted-2-methylthiopyrido[2,3-*d*]pyri-midines

Compound No.		I_{50} (μM)	log $1/I_{50}$	W^*_{max} (Å)	π
1		31.6	−1.50	6.02	2.18
2		15.9	−1.20	5.43	3.80
3		0.65	0.19	4.94	2.50
4		0.49	0.31	4.21	1.80
5		0.22	0.67	4.42	2.00
6		0.46	0.34	3.98	1.85
7		0.65	0.18	3.83	1.44
8		0.81	0.09	3.49	1.80
9		7.33	−0.87	3.49	2.26
10		30.83	−1.49	3.11	1.68

* Calculation was based on fully extended or staggered con-formation.

* To whom inquiries should be addressed.

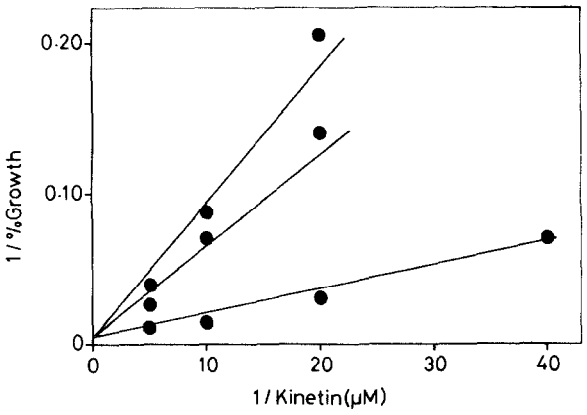


Fig. 1. The reciprocal of growth rate of tobacco callus plotted as a function of the reciprocal of the concentration of kinetin alone (bottom line) and in the presence of 0.4 μM (upper line) and 0.5 μM (middle line) compound **3**.

presence of catalytic sodium methoxide. 4-Hydroxy-2-methylthiopyrido[2,3-*d*]pyrimidine was then chlorinated by phosphorous oxychloride at refluxing temperature. Unstable 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine was immediately treated with the appropriate amine in *n*-butanol to give each of the 4-substituted-2-methylthiopyrido[2,3-*d*]pyrimidines (**1**–**10**). In cases where crystallization was difficult, glassy products were purified as picrate salts.

Characterization as specific anticytokinins

Except for the phenyl derivative (**10**), compounds did not show cytokinin activity in the tobacco bioassay within the concentration range tested. For the estimation of anticytokinin activity, the callus tissue was grown on media containing 0.05 μM kinetin and test substances. All compounds tested exerted growth inhibitory activity (Table 1). The phenyl derivative (**10**) showed very weak cytokinin activity at 10 μM; the maximum callus yield was half that caused by 0.05 μM kinetin. In the presence of 0.05 μM kinetin, however, it retarded the cytokinin-induced callus growth. This compound is thus considered to be a borderline case. A case similar to this, previously reported, is the activity of 4-(2-ethylhexylamino)-2-methylpyrrolo[2,3-*d*]pyrimidine [3].

The structural resemblance of the pyrido[2,3-*d*]-pyrimidine nuclei to purine appears to imply their specific anticytokinin nature. To confirm this, we applied the method of Lineweaver and Burk [7] which has been previously applied for the establishment of specific anti-auxin nature [9]. The result of the treatment on compound **3** is shown in Fig. 1, where the reciprocal of the growth response was plotted against the reciprocal of the concentration of added kinetin. Respective points on the upper two lines which express the effects of the anticytokinin on the kinetin-induced callus growth at 0.4 and 0.5 μM, respectively, were always determined in combination with the experiments with kinetin only. The growth response is presented as the percentage of that given by 0.2 μM kinetin, and the lowest line expresses the results obtained with kinetin only. The fact that the resultant family of straight lines possesses a common intercept fulfils the requisite for competitive inhibition and therefore suggests that compound **3** is a true anti-cytokinin. Although this treatment was not carried out on all compounds, the above results strongly suggest the specific antagonistic nature of the series of compounds.

Previously, 3 criteria have been proposed by Hecht *et al.* to distinguish compounds which are specific anticytokinins from those which inhibit growth in some other manner [10]. These criteria are briefly (i) structural resemblance to cytokinins, (ii) diminution of the utilization of cytokinins in some system and (iii) reversal of inhibition by more added cytokinin. These items themselves are considered to be qualitatively correct. Hecht *et al.* claimed, however, that 7-substituted-3-methylpyrazolo[4,3-*d*]pyrimidines and 4-substituted-2-methylthiopyrrolo[2,3-*d*]pyrimidines are the specific anticytokinins since they reversed, at relatively high concentrations, the supraoptimal inhibition of callus growth by cytokinins [4, 5]. The addition of the antagonists offsets the excess cytokinin so that a smaller 'effective' amount of cytokinin was being used. The term 'antagonism' used in this study does not include this function. In fact, the present series of anticytokinins did not reverse the supraoptimal inhibition caused by 1.5 μM kinetin, substantiating the difference between the mechanism of growth inhibition by anticytokinins and that of supraoptimal concentrations of cytokinins.

Quantitative structure–activity relationship

Recently we have analysed semiquantitatively the cytokinin and anticytokinin activities of 4-substituted-2-methylpyrrolo[2,3-*d*]pyrimidines by using a steric substituent parameter, W_{max} [3]. This parameter is equivalent to the B_4 value, one of the STERIMOL parameters developed recently by Verloop *et al.* [11] and represents the maximum width of a substituent from the bond-axis connecting the exocyclic nitrogen atom with its α-carbon atom. In this study, we applied the W_{max} parameter for quantitative analysis of structure–activity relationship in anticytokinin activity of the pyrido[2,3-*d*]pyrimidine anticytokinins.

Table 1 summarizes the biological data for inhibition of tobacco callus growth as well as W_{max} and lipohydrophilic π values [12] for the substituents. The electronic variable, σ* [13], which was found insignificant was not listed. The log $1/I_{50}$ values ranged from the least active −1.50 and −1.49 of benzyl (**1**) and phenyl (**10**) derivatives, respectively, to the most active 0.67 of *n*-butyl derivatives (**5**). Correspondingly the W_{max} values changed from 6.02 of benzyl derivative (**1**), via 4.42 of compound **5**, to 3.11 of phenyl derivative (**10**), indicating that there exists an activity maximum depending upon the width of the N^4-substituent. Multiple regression analysis of these log $1/I_{50}$ values thus yielded a quadratic equation (1) by use of W_{max} only.

$$\log 1/I_{50} = -0.93(\pm 0.43)W_{max}^2 + 8.26(\pm 3.96)W_{max} - 17.96(\pm 8.77) \quad (n=10,\ r=0.89,\ s=0.43) \qquad (1)$$

where the values in parentheses indicate the 95% confidence interval of the regression coefficients, n represents the number of data points employed, r is the correlation coefficient and s is the standard deviation.

Other physicochemical factors, i.e. electronic σ* and lipohydrophilic π, were examined and the use of π

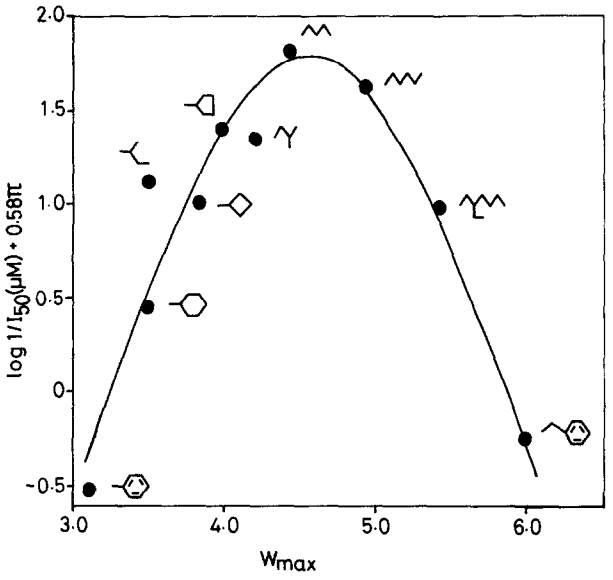


Fig. 2. Relation of anticytokinin activity to W_{max} expressed by Equation (2).

improved the correlation as shown in Equation (2).

$$\log 1/I_{50} = -0.96(\pm 0.29)W_{max}^2 + 8.75(\pm 2.67)W_{max} - 0.58(\pm 0.43)\pi - 18.32(\pm 5.86)$$
$$(n = 10, r = 0.96, s = 0.28) \quad (2)$$

Fig. 2 shows the correlation graphically. Although the number of data points per variable is not sufficiently great to place complete confidence in the correlation, according to the criterion issued by Topliss and Costello [14], the work in progress using compounds similar to those used here strongly supports the significance of both the steric and lipohydrophilic effects of substituents. Equation (2), together with Equation (1), provides insight into the role of the N^4-substituents in binding to the receptor molecule of cytokinins. The fact that σ^* was not significant in the correlation is considered to be due to its narrow range of variation within the series of compounds examined.

From Equations (1) and (2), it can be said that the most important variable is apparently W_{max}, indicating the predominant role of substituent width in the binding at the receptor site of cytokinins. π in Equation (2) functions as if it explains the activity of the 2-ethylhexyl derivative (**2**) which has the largest number of methylene units within the series of compounds. The negative coefficient of the π term suggests that lipophilicity has a certain detrimental effect at the site where the substituents bind. The quantitative structure–activity relationship presented in this study should be considered as complete but to constitute a useful starting point for future analyses of cytokinin agonists as well as antagonists, since both bind at the same receptor site.

EXPERIMENTAL

All mps are corr.

4-*Hydroxy-2-methylthiopyrido*[2,3-d]*pyrimidine*. To 50 ml dry MeOH were added 2.6 g 4-hydroxy-2-mercaptopyrido[2,3-*d*]-pyrimidine [8] and 5.3 ml 28% NaOMe in MeOH with stirring. To the mixture was then added 1.1 ml of MeI and the soln stood at room temp. for 18 hr. The reaction mixture was neutralized with HOAc and evapd *in vacuo* to dryness. The residue was washed with H_2O and collected giving 1.77 g (63%) of pale yellow powder, mp 230–235° (dec). Recrystallization several times from *iso*PrOH–H_2O gave an analytically pure sample, mp 248–249° (dec); $\lambda_{max}^{H_2O}$ 310 ($\varepsilon \times 10^{-4}$ 0.70); $\lambda_{max}^{0.2\,N\,HCl}$ 297 (0.51), 336 (1.41); $\lambda_{max}^{0.2\,N\,NaOH}$ 323 (0.82). (Found: C, 49.86; H, 3.67; N, 21.81. $C_8H_7N_3OS$ requires: C, 49.73; H, 3.65; N, 21.75%).

4-*Chloro-2-methylthiopyrido*[2,3-d]*pyrimidine*. To 0.5 g 4-hydroxy-2-methylthiopyrido[2,3-*d*]pyrimidine was added 20 ml phosphorous oxychloride and the mixture refluxed for 1.5 hr. The dark brown soln was evapd *in vacuo* to dryness and the residue treated with ice H_2O. The resulting soln was extracted with $CHCl_3$ and after washing with H_2O and drying (Na_2SO_4), the $CHCl_3$ layer was evapd *in vacuo* to dryness. The residue was extracted with hot *n*-hexane and the hexane extracts evapd *in vacuo* to dryness leaving 360 mg (66%) of yellow crystalline powder, mp 114–116°. Because of its instability, this compound was immediately treated with amines without further purification.

4-*Benzylamino-2-methylthiopyrido*[2,3-d]*pyrimidine* (*picrate*) (**1**). A mixture of 0.26 g 4-chloro-2-methylpyrido[2,3-*d*]pyrimidine and 0.4 ml benzylamine in 10 ml EtOH was left to stand at room temp. for 6 hr and then refluxed for 1 hr. The reaction mixture was evapd and the residue crystallized from H_2O to give 310 mg (94%) of glassy solid, mp 174–176°. A 150 mg portion was dissolved in EtOH and satd picric acid soln in EtOH was added dropwise to give yellow crystals which were recrystallized from EtOH to give analytically pure yellow prisms, mp 212–214°; $\lambda_{max}^{H_2O}$ 265 ($\varepsilon \times 10^{-4}$ 2.29), 340 (2.33); $\lambda_{max}^{0.2\,N\,HCl}$ 225 (2.89), 259 (2.11), 291 (1.61), 327 (2.27), 340 (2.46); $\lambda_{max}^{0.2\,N\,NaOH}$ 267 (2.48), 346 (2.24). (Found: C, 49.55: H, 3.37; N, 19.06. $C_{21}H_{17}N_7SO_7$ requires: C, 49.32; H, 3.35; N, 19.17%).

4-(2-*Ethylhexylamino*)-2-*methylthiopyrido*[2,3-d]*pyrimidine* (*picrate*) (**2**) was prepared from 4-chloro-2-methylthiopyrido-[2,3-*d*]pyrimidine and 2-ethylhexylamine in 80% yield using the method given for the prepn of **1** and then purified as picrate salts, mp 157–159°; $\lambda_{max}^{H_2O}$ 222 ($\varepsilon \times 10^{-4}$ 2.14), 341 (1.74); $\lambda_{max}^{0.2\,N\,HCl}$ 223 (2.30), 294 (1.07), 329 (1.76), 340 (1.82); $\lambda_{max}^{0.2\,N\,NaOH}$ 347 (1.77). (Found: C, 50.03; H, 5.08, N, 18.10. $C_{22}H_{27}O_7N_7S$ requires: C, 49.52; H, 5.10; N, 18.38%).

4-n-*Amyl-2-methylthiopyrido*[2,3-d]*pyrimidine* (**3**). A mixture of 0.34 g 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and 0.5 ml *n*-amylamine in *n*-BuOH was left to stand at room temp. for 1 hr and then evapd *in vacuo* to dryness. The residue was recrystallized from H_2O to give 0.38 g (90%) of pale yellow crystals, mp 144–147°. Recrystallization from H_2O–EtOH and EtOH gave analytically pure, colourless needles, mp 156–157°: $\lambda_{max}^{H_2O}$ 225 ($\varepsilon \times 10^{-4}$ 1.86), 268 (2.44), 338 (1.20); $\lambda_{max}^{0.2\,N\,HCl}$ 226 (1.98), 265 (1.80), 291 (1.61), 326 (1.88), 339 (1.79); $\lambda_{max}^{0.2\,N\,NaOH}$ 269 (2.40), 339 (1.14). (Found: C, 59.79; H, 7.01; N, 21.32. $C_{13}H_{18}N_4S$ requires: C, 59.51; H, 6.92; N, 21.35%).

4-n-*Butylamino-2-methylthiopyrido*[2,3-d]*pyrimidine* (**5**) was prepared from 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and *n*-butylamine in 66% yield using the method described for the prepn of **3**; mp 144–146°; $\lambda_{max}^{H_2O}$ 224 ($\varepsilon \times 10^{-4}$ 1.52), 268 (1.99), 337 (9.40); $\lambda_{max}^{0.2\,N\,HCl}$ 225 (1.77), 265 (9.40), 290 (1.30), 325 (1.48), 339 (1.41); $\lambda_{max}^{0.2\,N\,NaOH}$ 269 (1.69), 340 (8.09). (Found: C, 58.03; H, 6.32; N, 22.39. $C_{12}H_{16}N_4S$ requires C, 58.03; H, 6.49; N, 22.56%).

4-*Isobutylamino-2-methylthiopyrido*[2,3-d]*pyrimidine* (**4**) was prepared from 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and isobutylamine in 57% using the method described for the prepn of **3**; mp 175–177°; $\lambda_{max}^{H_2O}$ 225 ($\varepsilon \times 10^{-4}$ 1.55), 268 (2.00), 339 (1.39); $\lambda_{max}^{0.2\,N\,HCl}$ 226 (1.57), 265 (1.39), 292 (1.25), 326 (1.47), 339

(1.39); $\lambda_{max}^{0.2\,N\,NaOH}$ 269 (2.04), 338 (1.00). (Found: C, 58.03; H, 6.44; N, 22.56. $C_{12}H_{16}N_4S$ requires: C, 58.30; H, 6.54; N, 22.30%).

4-*Cyclopentylamino*-2-*methylthiopyrido*[2,3-d]*pyrimidine* (**6**) was prepared from 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and cyclopentylamine in 68% yield by the method given for prepn of **3**; mp 233–234°; $\lambda_{max}^{H_2O}$ 226 ($\varepsilon \times 10^{-4}$ 1.23), 269 (1.94), 339 (1.00); $\lambda_{max}^{0.2\,N\,HCl}$ 227 (1.56), 265 (1.31), 293 (1.26), 327 (1.56), 340 (1.42); $\lambda_{max}^{0.2\,N\,NaOH}$ 270 (1.97), 338 (1.00). (Found: C, 60.03; H, 6.22; N, 21.48. $C_{13}H_{16}N_4S$ requires: C, 59.97; H, 6.19; N, 21.52%).

4-*Cyclobutylamino*-2-*methylthiopyrido*[2,3-d]*pyrimidine* (**7**) was prepared from 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and cyclobutylamine in 62% using the method described for the prepn of **3**; mp 225–226°; $\lambda_{max}^{H_2O}$ 225 ($\varepsilon \times 10^{-4}$ 1.62), 270 (1.99), 344 (1.04); $\lambda_{max}^{0.2\,N\,HCl}$ 230 (1.47) 261 (1.21), 294 (1.21), 294 (1.15), 329 (1.38), 324 (1.34); $\lambda_{max}^{0.2\,N\,NaOH}$ 246 (3.86), 308 (1.69). (Found: C, 58.47; H, 5.93; N, 22.51. $C_{12}H_{14}N_4S$ requires: C, 58.51; H, 5.73; N, 22.75%).

4-sec-*Butylamino*-2-*methylthiopyrido*[2,3-d]*pyrimidine* (**8**) was prepared from 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and *sec*-butylamine in 70% using the method described for the prepn of **3**; mp 186–187°; $\lambda_{max}^{H_2O}$ 225 ($\varepsilon \times 10^{-4}$ 1.47), 268 (1.91), 337 (0.89); $\lambda_{max}^{0.2\,N\,HCl}$ 226 (1.36), 265 (1.25), 291 (1.12), 325 (1.32), 339 (1.26); $\lambda_{max}^{0.2\,N\,NaOH}$ 269 (1.42), 341 (0.68). (Found: C, 58.31; H, 6.51; N, 22.70. $C_{12}H_{16}N_4S$ requires: C, 58.04; H, 6.49; N, 22.56%).

4-*Cyclohexylamino*-2-*methylthiopyrido*[2,3-d]*pyrimidine* (**9**) was prepared from 4-chloro-2-methylthiopyrido[2,3-*d*]pyrimidine and cyclohexylamine in 59% yield from the method used in the prepn of **3**; mp 211–213°; $\lambda_{max}^{H_2O}$ 225 ($\varepsilon \times 10^{-4}$ 1.62), 269 (1.96), 340 (1.05); $\lambda_{max}^{0.2\,N\,HCl}$ 227 (1.69), 265 (1.39), 292 (1.33), 326 (1.59), 340 (1.50); $\lambda_{max}^{0.2\,N\,NaOH}$ 269 (1.99), 339 (1.03). (Found: C, 61.57; H, 6.72; N, 20.25. $C_{14}H_{18}N_4S$ requires C, 61.28; H, 6.61; N, 20.42%).

4-*Phenylamino*-2-*methylthiopyrido*[2,3-d]*pyrimidine* (*picrate*) (**10**) was prepared from 4-chloro-2-methylthiopyrido[2,3-*d*]-pyrimidine and aniline in 65% yield by an analogous method to that used for **1**; mp 225°; $\lambda_{max}^{H_2O}$ 239 ($\varepsilon \times 10^{-4}$ 3.20), 352 (2.72); $\lambda_{max}^{0.2\,N\,HCl}$ 241 (2.96), 347 (2.63); $\lambda_{max}^{0.2\,N\,NaOH}$ 246 (1.98), 364 (2.41). (Found: C, 48.53; H, 3.09; N, 19.78. $C_{20}H_{15}N_7S$ requires: C, 48.29; H, 3.04; N, 19.71%).

Tobacco callus bioassay. Anticytokinin activity was measured in terms of fr. wt yield of tobacco callus tissue 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. [15] to which kinetin and the test compound were added in different concentrations.

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REFERENCES

1. Iwamura, H., Ito, T., Kumazawa, Z. and Ogawa, Y. (1974) *Biochem. Biophys. Res. Commun.* **57**, 412.
2. Iwamura, H., Ito, T., Kumazawa, Z. and Ogawa, Y. (1975) *Phytochemistry* **14**, 2317.
3. Iwamura, H., Masuda, N., Koshimizu, K. and Matsubara, S. (1979) *Phytochemistry* **18**, 217.
4. Skoog, F., Schmitz, R. Y., Hecht, S. M. and Frye, R. B. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3508.
5. Hecht, S. M., Bock, R. M., Schmitz, R. Y., Skoog, F. and Leonard, N. J. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2608.
6. Skoog, F., Schmitz, R. Y., Bock, R. M. and Hecht, S. M. (1973) *Phytochemistry* **12**, 25.
7. Lineweaver, H. and Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658.
8. Robins, R. K. and Hitchings, G. H. (1955) *J. Am. Chem. Soc.* **77**, 2256.
9. McRae, D. H. and Bonner, J. (1953) *Physiol. Plant.* **6**, 485.
10. Hecht, S. M., Frye, R. B., Werner, D. and Hawrelak, S. D. (1975) *J. Biol. Chem.* **250**, 7343.
11. Verloop, A., Hoogenstraaten, W. and Tipker, J. (1976) *Drug Design* (Ariens, E. J., ed.) Vol. VII, p. 165. Academic Press, New York.
12. Iwasa, J., Fujita, T. and Hansch, C. (1965) *J. Med. Chem.* **8**, 150.
13. Taft, R. W., Jr. (1956) *Steric Effects in Organic Chemistry* (Newman, M. S., ed.) p. 567. John Wiley, New York.
14. Topliss, J. G. and Costello, R. J. (1972) *J. Med. Chem.* **15**, 1066.
15. Linsmaier, E. M. and Skoog, F. (1965) *Physiol. Plant.* **18**, 100.