Effect of 4-Substituted Pyrrolo[2,3-d]pyrimidines on the Tobacco Callus Growth

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Recent advances in the synthesis of cytokinin analogs have revealed the peculiar nature of pyrrolo[2,3-d]pyrimidine derivatives some of which behave as anticytokinins.¹⁻³⁾ The development of the synthetic studies of anticytokinins immediately suggests their practical usage as plant growth regulators⁴⁾ as well as the utilization for the studies on the mechanism of cytokinin action. Here we wish to report an experiment in which the growth promoting activity of some 4-substituted pyrrolo[2,3-d]pyrimidines (Ia-If) was compared with the growth retarding activity of the corresponding, previously reported anticytokinins, 4-substituted 7-(β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidines (IIa-IIf).^{1,2)}

MATERIALS AND METHODS

Test compounds Ib-If were synthesized according to the previously reported methods.^{5,6)} Compound Ia was synthesized accordingly: A mixture of 4-chloro[2,3-d]pyrimidine⁵⁾ (1.0 g), 3-methyl-2-butenylamine-HCl⁷⁾ (1.2 g), triethylamine (10 ml) in *n*-butanol was refluxed for 10 hr, and filtered and evaporated. Recrystallization of the residue from ethanol gave 1.05 g (78%) of colorless crystals. Further crystallization gave an analytically pure sample; mp 199~200°C. *Anal.* Calcd. for C₁₁H₁₄N₃; C, 65.23; H, 6.98; N, 27.70. Found: C, 65.51; H, 6.68; N, 27.97%.

The bioassay procedure has been described previously.²⁾

RESULTS AND DISCUSSION

Compounds Ia~If were tested for their cytokinin

activity which was measured in the absence of an additional cytokinin (kinetin) and for anticytokinin activity which was examined in the presence of 0.3 and 1.5 μ M of kinetin. Because the test substances are structurally very similar to the previously reported anticytokinins, 4-substituted 7-(β -D-ribofuranosyl)pyrrolo[2,3-d]pyrimidines,^{1,2)} the reproducibility of the assays was ascertained by two independent tests. Two representatives of the results are shown in Fig. 1 for the compound Ia and Ib. These assays indicated that none of the series of the compounds exhibited anticytokinin activity within the concentration range used. The decrease of the callus growth at 100 μ M of compound Ia in Fig. 1 was considered to be due to its supra-optimal inhibition which is common to the adenylate cytokinins.

FIG. 1. Yields of Tobacco Tissue Cultured on Serial Combination of (A) Kinetin and Ia and (B) Kinetin and Ib.

A summary for the relative cytokinin activity of the compound Ia~If is shown in Fig. 2. It clearly indicates that the structure-activity relationship observed for the corresponding N⁶-substituted adenine derivatives is valid for the present series of compounds as well although the activity of 100 to 1000 times less. Significance of the present results exists, however, not on this quantitative difference of the activity but on their qualification as cytokinins. As previously reported,^{1,2)} the formal ribofuranosylation at the position 7 of these compounds renders them anticytokinin

FIG. 2. Relative Effects on Tobacco Callus Yield of Compounds Ia (○), Ib (△), Ic (□), Id (×), Ie (●) and If (▲). Control: 0.3 μ M kinetin.

activity (II). This indicates that the present series of compounds exerted their cytokinin activity without ribosylation *in vivo* at position 7 and thus a suggestion has been made that there exists at least a path in the tobacco tissue through which exogenously added cytokinins exert their activity without ribosylation.

As to the question of the necessity and/or role of the ribosyl moiety of the 9 position of exogenously added cytokinins, Hecht *et al.*^{8,9} recently prepared N⁸-isopentenyl-8-aza-9-deaza adenine (7-isopentenyl-pyrazolo[4,3-d]pyrimidine) and its 9-methyl and 9- β -D-ribofuranosyl derivatives and identified their cytokinin activity by the tobacco callus assay. Because these derivatives possess a carbon rather than a nitrogen atom at 9 position and thus ribosylation or deribosylation is unlikely, they suggested that the cytokinins function without ribosylation. Their conclusion, however, does not exclude the possible existence of a path(s) where exogenously added cytokinins are ribosylated at 9 position and then function,¹⁰ because

their 9-ribosyl compound is active at 1 μ M level as a cytokinin despite of the unlikely scission of the C-C glycosyl bond. Incidentally, recent experiments on the cytokinin metabolites identified 7-glucosyl¹¹ and 9-glycosyl¹² derivatives as stable metabolites of exogenously added cytokinins. Although their physiological significance is presently unclear, these results are suggestive of the existence of metabolic loops.

The results of the present investigation do not exclude the existence of plural routes through which cytokinins function, but show straightforwardly that the present series of compounds exert the cytokinin activity without ribosylation at 7 position which is analogous to 9 position in adenine. If glycosylation occurred at 7 nitrogen atom, the resulting compounds should behave as anticytokinins. In connection with this it is noteworthy that the single modification at the 7 position of adenine ring prevented the ribosylation because the cytokinin system is quite capable of attaching or removing the sugar moiety.¹³ Thus this fact may imply an important role of 7 nitrogen atom or the imidazole moiety in the system.

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