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SYNTHESIS AND ANTICYTOKININ ACTIVITY OF 4-SUBSTITUTED-7-(β-D-RIBOFURANOSYL)-PYRROLO[2,3-*d*]PYRIMIDINES

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco callus bioassay; anticytokinins; pyrrolopyrimidines.

Abstract—A series of 4-substituted-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidines in which the 4-substituents were systematically varied has been synthesized and tested for anticytokinin activity in the tobacco callus bioassay. Qualitatively the activity of effective compounds were in order of cytokinin activity of the corresponding N^6-substituted adenine derivatives, suggesting that the site of action is related to that of cytokinins. *Single* modification at the 7-position of active cytokinins seemed to be required for the expression of anticytokinin activity.

INTRODUCTION

Recent development in the biochemistry of cytokinins has shown that a wide variety of plants convert cytokinins to their 7-glucosides [1, 2]. Because these metabolites are themselves active as cytokinins and are highly resistant to enzyme degradation, it has been suggested that the 7-glucosyl compounds may be the active form [1] or the storage form [2] of cytokinins. If this conversion process is indispensable to evoke the growth response, the 7-deaza analogs of cytokinins occur as possible cytokinin inhibitors because the structure is sufficiently similar to allow participation in the same type of enzyme–substrate complex with cytokinins but the lack of a N atom at the 7-position will prevent the successive glucosylation. In our preliminary communication we reported the results obtained with one member of that class, 4-furfurylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine, the 7-deaza analog of kinetin riboside [3]. Here we report on the synthesis and tests for anticytokinin activity of ten compounds in the pyrrolo[2,3-*d*]-pyrimidine series in which the 4-substituent was systematically varied.

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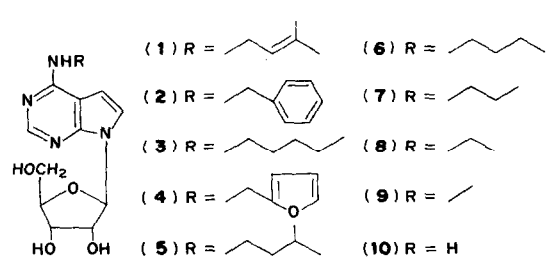
RESULTS

Synthesis of 4-substituted-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidines

Syntheses have been reported previously for 4-furfurylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*] pyrimidine(IV) [3], 4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine(IX) [4] and 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine(X) [5]. The following compounds were prepared by treatment of the intermediate, 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [5], with the appropriate amine at reflux temperature or at elevated temperature in a sealed tube: 4-(3-methyl-2-butenylamino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine(**1**), 4-benzylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyr-



imidine(**2**), 4-*n*-pentylamino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine(**3**), 4-(3-methylbutylamino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine(**5**), 4-*n*-butylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine(**6**), 4-*n*-propylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine(**7**) and 4-ethylamino-7- (β-D-ribofuranosyl) pyrrolo [2,3-*d*] pyrimidine(**8**). The compounds were isolated and purified as picrates.

Anticytokinin activity of the test substances

The tobacco tissues were grown on media containing various concentrations of kinetin and the test compounds. The results are shown in Figs.

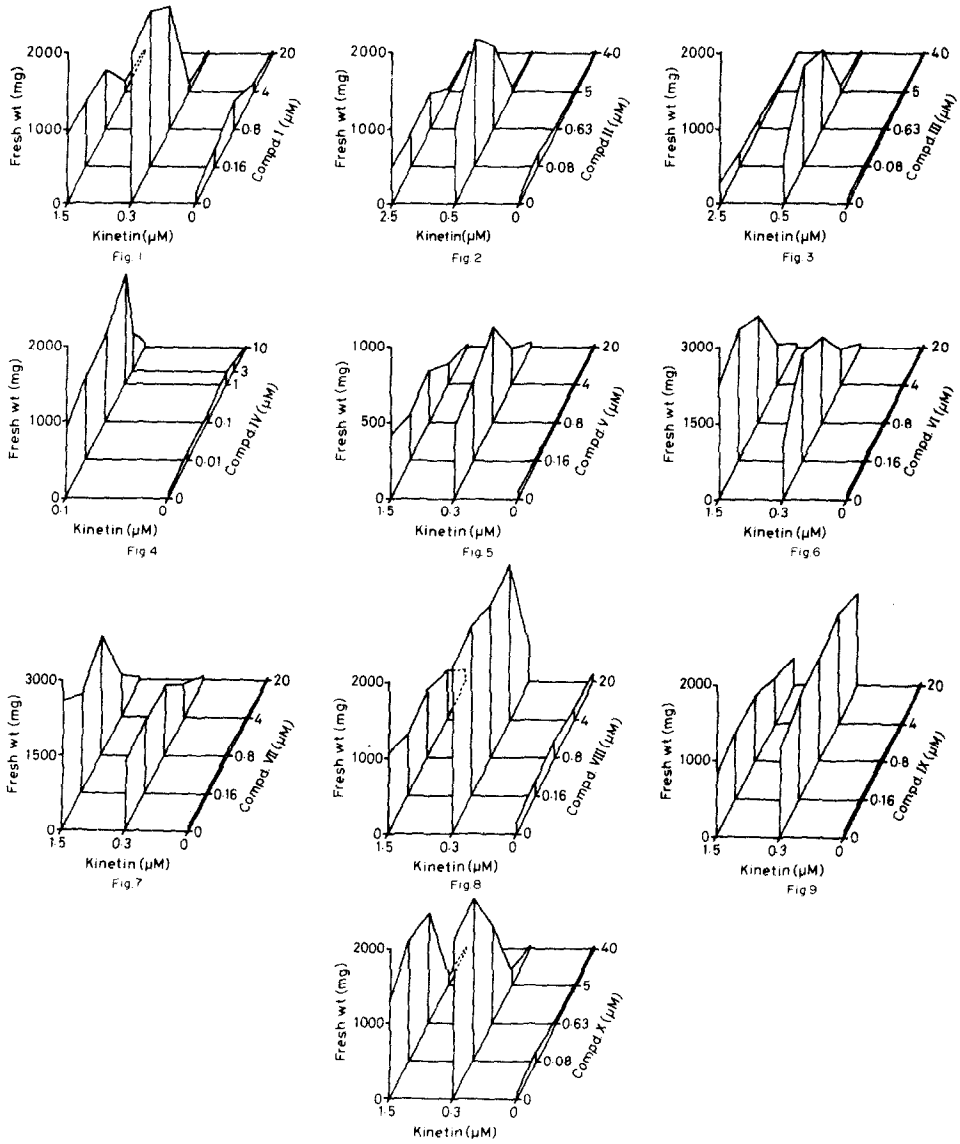


Fig. 1. Yield of tobacco tissue cultured on serial combination of kinetin and **1**; Fig. 2. Yield of tobacco tissue cultured on serial combination of kinetin and **2**; Fig. 3. Yield of tobacco tissue cultured on serial combination of kinetin and **3**; Fig. 4. Yield of tobacco tissue cultured on serial combination of kinetin and **4** (filter-sterilized); Fig. 5. Yield of tobacco tissue cultured on serial combination of kinetin and **5**; Fig. 6. Yield of tobacco tissue cultured on serial combination of kinetin and **6**; Fig. 7. Yield of tobacco tissue cultured on serial combination of kinetin and **7**; Fig. 8. Yield of tobacco tissue cultured on serial combination of kinetin and **8**; Fig. 9. Yield of tobacco tissue cultured on serial combination of kinetin and **9**; Fig. 10. Yield of tobacco tissue cultured on serial combination of kinetin and **10**.

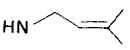
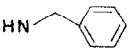
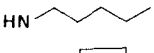
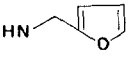
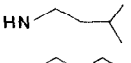
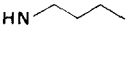
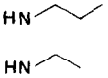
1–10. At zero concentration of kinetin, none of the test compounds showed significant growth-promoting activity in the concentration range tested. Compounds **1–3** showed a strong growth inhibitory effect at a concentration of 4 or 5 μM under conditions where the growth of the callus tissue is promoted by kinetin. Because it has previously been shown that the N^6-substituted adenylate cytokinins which have the isopentenyl, benzyl and pentyl side chains are highly active cytokinins [6], it seemed reasonable to expect the above results. In line with this, furfuryl (**4**), isopentyl (**5**), butyl (**6**), propyl (**7**) and ethyl (**8**) derivatives are expected to be less active. This effect can be observed, qualitatively, from the yields of tobacco callus tissue grown on media containing the antagonists at 3–4 μM in Figs. 4–8 in comparison with those of the compounds **1–3** in Figs. 1–3. Markedly reduced inhibitory activity was seen in the case of compound **8**. This serial decrease of the anticytokinin activity could not be quantitatively estimated as was done for the cytokinin activity of N^6-substituted adenine derivatives [6], but the striking results in connection with this were obtained in the tests of methylamino (**9**) and amino (**10**) derivatives. As seen from Fig. 9, compound **9** did not show either anticytokinin activity or cytokinin activity. On the other hand, the amino derivative (**10**) exhibited strong inhibitory effects (Fig. 10), the extent of which almost corresponded to that of the most active ones (**1–3**). Compound **10** is known as an antibiotic, tubercidin, and has been isolated from culture filtrates of *Streptomyces tubercidus* [7]. It exhibits antimicrobial and at the same time strong cytotoxic activity [8]. The cytotoxicity of tubercidin is greatly reduced when substituted at N^4 by an alkyl group [5]. The lack of anticytokinin activity of methylamino derivative (**9**) is then explained by the fact that it has lost tubercidin-like cytotoxicity by N^4-alkylation and at the same time has no more anticytokinin activity in the sense that its Me side chain is not that of active adenylate cytokinins. This means that the growth inhibitory activity of the N^4-alkylated pyrrolopyrimidines is not due to the tubercidin-like cytotoxicity, and suggests that the site of their action is related to the processes through which N^6-adenylate cytokinins express their activity. In addition, the behaviour of this derivative is important in the sense that it makes the possibility unlikely that the series of active 7-deaza compounds may exert their growth-inhibiting activity after the degradation *in vivo* of alkyl side chains, i.e. the really active species may be tubercidin. If the side chains were removed after incorporation into the tobacco tissues, compound **9** should exert as strong a growth inhibitory effect as tubercidin. Alternatively the side chains may be eliminated during autoclaving, but this possibility is also unlikely because compound (**4**) exhibited the activity after filter-sterilization (Fig. 4)* as well as after autoclaving.

DISCUSSION

Ten compounds in the 4-substituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine series were synthesized and tested for anticytokinin activity. These analogs represent the systematic variation of the 4-substituent from isopentenyl to methyl. If the site of anticytokinin action of these compounds were related to the mechanism through which cytokinins themselves exert their effect, the structure-activity relationship for the adenylate cytokinins would be valid for the pyrrolo[2,3-*d*]pyrimidine series of anticytokinins as well. The results depicted in Figs. 1–10 are summarized in Table 1 with the reported data [6] of corresponding adenylate cytokinins. The N^4-alkylated pyrrolopyrimidine derivatives (Table 1) can be arranged in order of cytokinin activity of the corresponding adenine derivatives without significant inconsistency with the order of their anticytokinin activity. The lack of anticytokinin activity of the methylamino derivative (**9**) strongly supports this because the corresponding methyladenine is not active as a cytokinin in the tested range of concentrations. Formal removal of the side chains gives compound (**10**) (tubercidin) which is known as antimicrobial and cytotoxic [8]. Thus the apparent anticytokinin activity of this compound (**10**) is considered to belong to that of non-specific growth inhibitors like, for example, 2,6-diaminopurine [9]. If the activity of the anticytokinins described here is real and if it is due to tubercidin generated by degradation of the side

* This test was carried out at Kyoto Prefectural University by Dr. S. Matsubara. The authors express sincere gratitude for his help.

Table 1. Relation of the structure of 4-substituents to anticytokinin activity of 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines

No.	Compound 4-Substituent	Anticytokinin activity	Cytokinin activity of the corresponding adenine derivative [6]	
			Concentrations needed for:— Maximum yield (μM)	Detectable response (μM)
(1)		+ + +	0·02	(0·0001)
(2)		+ + +	0·07	(0·0008)
(3)		+ + +	0·1	(0·0008)
(4)		+ +	0·1	(0·001)
(5)		+ +	0·1	(0·004)
(6)		+ +	0·1–0·5	(0·004)
(7)		+ +	5	(0·02)
(8)		+	12·5	(0·5)
(9)		–	∞	(0·5)
(10)	H_2N	+ + +	∞	(200)

chains, methylamino compound (**9**) should be active.

The pyrrolopyrimidine anticytokinins were designed at first as blocking agents for the 7-glucosylation of cytokinins which may be an indispensable process for the expression of cytokinin activity. Because the glucosidic bond of a pyrrolo[2,3-*d*]pyrimidine nucleoside is resistant to acidic hydrolysis, it is also possible that the anticytokinin activity may be due to the resistance to enzymic cleavage of that bond prior to metabolic conversion. Incidentally a recent report [10] has shown that, in the case of *Zea mays*, a major metabolite of exogenously supplied zeatin is 9-glucosylzeatin, although its physiological significance is unknown. The speculations described here do not, however, necessarily mean that any pyrrolo[2,3-*d*]pyrimidine species is active as anticytokinin. Some pyrazolo[4,3-*d*]pyrimidines are active as cytokinin antagonists but others are active as cytokinins [10]. We feel from the evidence presented here that the close structural resemblance of our compounds to cytokinins, in other words, the *single* modification at the 7-position in adenine moiety of active cytokinins, rendered them active as anticytokinins.

EXPERIMENTAL

4-(3-*Methyl*-2-*butenylamino*)-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]*pyrimidine(picrate)* (**1**). A mixture of 512 mg of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [5], 0·6 g of 3-methyl-2-butenylamine hydrochloride [12] and 5 ml of triethylamine in 20 ml of *n*-BuOH was refluxed for 5 hr. The reaction mixture was evaporated *in vacuo* leaving an oil which was extracted with hot Me_2CO. The extract was evaporated *in vacuo* and residue was dissolved in 3 ml of EtOH. To the EtOH soln was added dropwise a satd picric acid soln in EtOH and the mixture was stood at 4° for 18 hr depositing 905 mg (90%) of yellow crystals. Recrystallization from EtOH gave 793 mg (78%) of yellow platelets, mp 200–203°; $\lambda_{max}^{H_2O}$ 269 nm (ε 21600), 353 (15200); $\lambda_{max}^{0\cdot1\,N\,HCl}$ 270 (22100), 352 (13800); $\lambda_{max}^{0\cdot1\,N\,NaOH}$ 269 (21600), 353 (15300). (Found: C, 46–84; H, 4·35; N, 17·43. $C_{22}H_{25}O_{11}N_7$ requires C, 46·90; H, 4·47; N, 17·40%).

4-*Benzylamino*-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]*pyrimidine*-(*picrate*) (**2**). A mixture of 330 mg of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [5] and 3 ml of benzylamine in 20 ml of *n*-BuOH was refluxed for 5 hr. The reaction mixture was evaporated *in vacuo* leaving an oil which was dissolved in 3 ml of EtOH. To the soln was added dropwise a satd picric acid soln in EtOH. The mixture was stood at 4° for 18 hr depositing 666 mg (99%) of yellow crystals. Recrystallization once from MeOH–EtOH gave 369 mg (55%) of yellow leaflets, mp 185–188°; $\lambda_{max}^{H_2O}$ 270 nm (ε 21100), 353 (14800); $\lambda_{max}^{0\cdot1\,N\,HCl}$ 272 (21100), 352 (13500); $\lambda_{max}^{0\cdot1\,N\,NaOH}$ 270 (21600), 353 (14800). (Found: C, 49·04; H, 3·80; N, 16·68. $C_{24}H_{23}O_{11}N_7$ requires C, 49·23; H, 3·96; N, 16·74%).

4-n-*Pentylamino*-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]*pyrimidine(picrate)* (**3**). A mixture of 301 mg of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [5] and 3 ml of *n*-pentylamine in 20 ml EtOH was refluxed for 5 hr. The reaction mixture was evaporated *in vacuo* leaving an oil which was extracted with EtOAc–Me_2CO (3:1) with heating. Removal of solvents left a glass which was dissolved in 3 ml of EtOH. To the soln was added dropwise a satd picric acid soln in EtOH and the mixture was stood at 4° for 18 hr depositing 562 mg (95%) of yellow crystals, mp 201–204°. Recrystallization from EtOH–MeOH mixture gave 410 mg (69%) of yellow platelets, mp 204–206°; $\lambda_{max}^{H_2O}$ 270 nm (ε 19600), 355 (14600); $\lambda_{max}^{0\cdot1\,N\,HCl}$ 272 (19900), 354 (13200); $\lambda_{max}^{0\cdot1\,N\,NaOH}$ 271 (19400), 355 (14600). (Found: C, 47·01; H, 4·68; N, 17·44. $C_{27}H_{27}O_{11}N_7$ requires C, 46·73; H, 4·81; N, 17·34%).

4-(3-Methylbutylamino)-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]-*pyrimidine(picrate)* (**5**). A mixture of 312 mg of 4-chloro-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-*d*]pyrimidine [5] and 3 ml of 3-methylbutylamine in 20 ml of EtOH was refluxed for 5 hr. The reaction mixture was evaporated *in vacuo* leaving an oil which was dissolved in 3 ml of EtOH. To the soln was added dropwise a satd picric acid soln in EtOH and the mixture was stood at 4° for 18 hr depositing 563 mg (91%) of yellow crystals. Recrystallization from EtOH gave 394 mg (64%) of needles, mp 202–205°; $\lambda_{max}^{H_2O}$ 269 nm (ε 20800), 353 (15200); $\lambda_{max}^{0\cdot1\,N\,HCl}$ 269 (21000), 352 (13900); $\lambda_{max}^{0\cdot1\,N\,NaOH}$ 269 (20700), 352 (15400). (Found: C, 46·98; H, 4·65; N, 17·61. $C_{22}H_{27}O_{11}N_7$ requires C, 46·73; H, 4·81; N, 17·43%).

4-n-*Butylamino*-7-(β-D-*ribofuranosyl*)*pyrrolo*[2,3-d]*pyrimidine(picrate)* (**6**). A mixture of 301 mg of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [5] and 3 ml of *n*-BuOH

in 20 ml EtOH was refluxed for 5 hr. The reaction mixture was evaporated *in vacuo* leaving an oil which was dissolved in 3 ml of EtOH. To the soln was added dropwise a satd picric acid soln in EtOH and the mixture was stood at 4° for 18 hr depositing 536 mg (92%) of yellow crystals. Recrystallization from EtOH gave 443 mg (76%) of yellow needles, mp 210–212°; $\lambda_{max}^{H_2O}$ 271 nm (ε 19300), 356 (14500); $\lambda_{max}^{0.1\,N\,HCl}$ 270 (19100), 355 (14100), $\lambda_{max}^{0.1\,N\,NaOH}$ 271 (19100), 356 (14400). (Found: C, 45·71; H, 4·38; N, 17·73. $C_{21}H_{25}O_{11}N_7$ requires C, 45·74; H, 4·57; N, 17·38%).

4-n-*Propylamino-7-(β-*D*-ribofuranosyl)pyrrolo*[2,3-d]*pyrimidine(picrate)* (**7**). A mixture of 311 mg of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [5] and 3 ml of *n*-propylamine in 20 ml EtOH was refluxed for 5 hr. The reaction mixture was evaporated *in vacuo* leaving an oil which was dissolved in 3 ml EtOH. To the soln was added dropwise a satd picric acid soln in EtOH and the mixture was stood at 4° for 18 hr depositing 535 mg (91%) of yellow crystals. Recrystallization from EtOH gave 455 mg (78%) of yellow needles, mp 210–213°; $\lambda_{max}^{H_2O}$ 270 nm (ε 18900), 355 (14300); $\lambda_{max}^{0.1\,N\,HCl}$ 271 (19100), 354 (12100); $\lambda_{max}^{0.1\,N\,NaOH}$ 270 (18700), 355 (14300). (Found: C, 44·64; H, 4·09; N, 17·99. $C_{20}H_{23}O_{11}N_7$ requires C, 44·70; H, 4·31; N, 18·24%).

4-*Ethylamino-7-(β-*D*-ribofuranosyl)pyrrolo*[2,3-d]*pyrimidine-(picrate)* (**8**). A mixture of 373 mg of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine [5]. 110 mg of ethylamine hydrochloride and 0·6 ml of triethylamine in 15 ml EtOH was heated at 120° for 4 hr in a sealed tube. The reaction mixture was made alkaline using 25% NH_4OH soln and evaporated *in vacuo* leaving an oil which was extracted with EtOH. Removal of the volatiles left an oil which was dissolved in 3 ml EtOH. To the soln was added dropwise a satd picric acid soln in EtOH and the mixture was stood at 4° for 18 hr depositing 436 mg (64%) of yellow crystals. Recrystallization from EtOH gave 294 mg (43%) of yellow needles, mp 202–205°: $\lambda_{max}^{H_2O}$ 271 nm (ε 18700), 355 (14400); $\lambda_{max}^{0.1\,N\,HCl}$ 271 (19200), 354 (13200); $\lambda_{max}^{0.1\,N\,NaOH}$ 271 (18600), 355 (14400). (Found: C, 43·75; H, 3·75; N, 18·93. $C_{19}H_{21}O_{11}N_7$ requires C, 43·60; H, 4·05; N, 18·73%).

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 [13] to which kinetin and the test compound were added in different concentrations. Testing was done between December 1973 and December 1974.

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