ANTICYTOKININ ACTIVITY OF 4-FURFURYLAMINO-7-(β-D-RIBOFURANOSYL)PYRROLO[2,3-d]PYRIMIDINE

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SUMMARY: 4-Furfurylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]-pyrimidine, the 7-deaza analog of kinetin riboside, has been synthesized and found to be a potent anticytokinin in the tabacco callus bioassay.

Deleuze et al.(1) have recently reported that when 6-benzylaminopurine was supplied to soybean callus tissue it was converted into a stable long-lived compound which was tentatively identified as 6-benzylamino-7-glucofuranosylpurine. Since the soybean tissue continued to grow long after there was any detectable 6-benzylaminopurine, they suggested that the stable metabolite might be a protected or active form of the cytokinin. The similar 7-glucosyl derivative of zeatin has been isolated by Parker et al.(2) from the extracts of radish seedlings supplied with zeatin. This compound exhibited activity in the radish-cotyledon cytokinin bioassay, also suggesting that the cytokinin might be activated by conversion into the 7-glucosyl derivative.

The above two recent works prompted us to synthesize the 7-deaza analogs of cytokinin ribosides and test their anti-cytokinin activity because the single modofication at the 7-position in adenine moiety of cytokinins may render them

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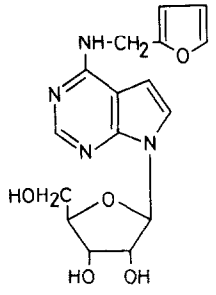
effective as anticytokinins. In this communication are reported the preliminary results obtained on one member of the class; 4-furfurylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (I), the 7-deaza analog of kinetin riboside.

MATERIALS AND METHODS

4-Furfurylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]-pyrimidine(picrate)(I).———A mixture of 500 mg of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine(3), 1 ml of



4-Furfurylamino-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine

furfurylamine and 25 ml of n-butanol was refluxed for 5 hr. The reaction mixture was evaporated in vacuo to dryness leaving a brownish glass which was extracted with ethyl acetate-acetone (3:1) mixture with heating. Removal of the solvents in vacuo gave a glass which was dissolved in 3 ml of ethanol. To the ethanol solution was added dropwise the saturated picric acid solution in ethanol. The reaction mixture was stood in a refrigerator overnight depositing 644 mg(56%) of yellow crystalls, mp. 185-192°. Recrystallizations from ethanol gave analytically pure sample, mp. 190-195°; $\lambda_{max}^{H_2O}$ 268 nm(ε 24,000),354(15,000); $\lambda_{max}^{0.1\underline{N}HCl}$ 230(38,600), 271(20,400),353(13,500); $\lambda_{max}^{0.1\underline{N}NaOH}$ 269 (20,300), 354(14,900). Anal. Calcd. for $C_{22}H_{21}O_{12}N_7$: C, 45.92;

H, 3.68; N, 17.04. Found: C, 45.96; H, 3.68; N, 16.91.

Tabacco callus bioassay.———Anticytokinin activity was measured in terms of fresh weight yields of tabacco callus tissue derived from Nicotiana tabacum var. Wisconsin No.38.

The tabacco callus was grown at 28°C for 4 weeks on the standard medium specified by Linsmair and Skoog(4) to which kinetin and the test compound were added in different concentrations.

RESULTS AND DISCUSSION

The findings reported by Deleuze et al.(1) and Parker et al. (2) has led to the suggestion that the conversion of exogenously supplied cytokinin into the 7-glucosyl derivative may be an important step for the expression of its activity. The anti-cytokinin(I) described here was designed with the idea that the structure is sufficiently similar to kinetin to allow participation in the same type of enzyme-substrate complex with the cytokinin, but the lack of nitrogen atom at the 7-position will prevent the successive glucosylation.

The tabacco tissues were grown on the media containing various concentrations of kinetin and compound I. The results were shown in Table 1 and Fig. 1. At zero concentration of kinetin, compound I did not show any growth promoting activity, showing that it is ineffective as a cytokinin. It exhibited strong growth inhibitory effect, however, against kinetin when applied at concentrations of 3 μM and 12 μM. The inhibition was complete at the concentration of 12 μM regardless of the cytokinin concentration within the range tested in this experiment. At the concentrations below 3 μM of compound I, the inhibitory effect was counteracted by kinetin. This suggests the potential

Table 1. Yields(mg) of Tabacco Tissues Caltured on Serial Combination of Compound I and Kinetin.

Kinetin (μM)	Compound I (μM)						
	0	0.01	0.05	0.19	0.75	3.0	12.0
0	52	31	103	112	15	26	27
0.01	472	283	311	283	398	113	7
0.05	955	987	605	1010	809	148	17
0.19	507	1373	965	916	331	16	16
0.75	535	746	624	654	608	230	14
3.0	301	599	308	116	358	149	14
12.0	68	53	172	43	34	32	8

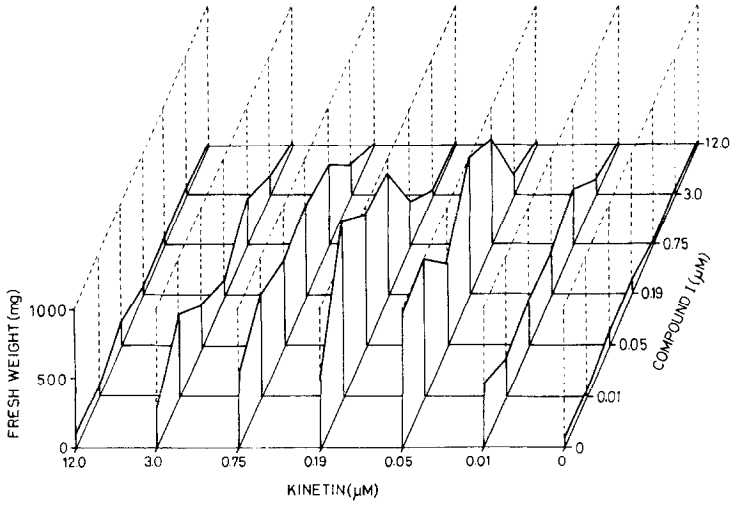


Fig. 1.———Effect of various concentrations of kinetin and compound I on the fresh-weight yield of tabacco callus.

utility of the compound in studies of the mechanism of cytokinin action because with other growth inhibitors structurally related to the adenylate cytokinins like 6-methylaminopurine(5) the counteraction by cytokinins is slight. As depicted in Fig. 1, the tissues grown on media containing lower concentrations of compound I ($\leq$0.75 μM) grew as well as the controls.

The biological effects of this compound seem to be similar to those of the 7-substituted 3-methylpyrazolo[4,3-d]pyrimidines which are the cytokinin antagonists developed by Hecht et al.(6) and Skoog et al.(7). This series of compounds was designed as the structural analogy of 6-(3-methyl-2-butenylamino)purine, one of the known, intact N^6-substituted adenylate cytokinins, and the alkyl side chains at the 7-position are different from that of the cytokinin as well as the heterocyclic moiety. Thus the mode of action of the pyrazolo[4,3-d]pyrimidine anticytokinins may differ from that of compound I. The results of this study appear to strengthen the suggestion that the adenylate cytokinins might be activated via the 7-glucosyl derivatives. Further synthetic and biological studies are extensively in progress.

REFERENCES

1. Deleuze, G. G., McChesney, J. D., and Fox, J. E. (1972) Biochem. Biophys. Res. Commun. 48, 1426-1432.
2. Parker, C. W., Letham, D. S., Cowley, D. E., and McLeod, J. K. (1972) Biochem. Biophys. Res. Commun. 49, 460-466.
3. Gerster, J. F., Carpenter, B., Robins, R. K., and Townsend, L. B. (1967) J. Med. Chem. 10, 326-331.
4. Linsmaier, E. M., and Skoog, F. (1965) Physiol. Plant. 18, 100-127.
5. Klämbt, D., Thies, G., and Skoog, F. (1966) Proc. Natl. Acad. Sci. USA. 56, 52-59.
6. Hecht, S. M., Bock, R. M., Schmitz, R. Y., Skoog, F., and Leonard, N. J. (1971) Proc. Natl. Acad. Sci. USA. 68, 2608-2610.
7. Skoog, F., Schmitz, R. Y., Bock, R. M, and Hecht, S. M. (1973) Phytochem. 12, 25-37.