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CYTOKININ ACTIVITY OF O^6-SUBSTITUTED GUANINE AND HYPOXANTHINE DERIVATIVES*

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Abstract—O^6-Substituted guanine and hypoxanthine derivatives were prepared and tested for their cytokinin activity by the tobacco callus, radish cotyledons and lettuce seed bioassay systems. The results indicated that some derivatives of both types possess cytokinin activity.

INTRODUCTION

Since the isolation and identification of kinetin [1] and zeatin [2], various naturally-occurring cytokinins have been isolated and characterized [3]. All the highly active naturally occurring cytokinins that have been isolated so far are N^6-substituted adenines and their derivatives. On the other hand, a number of chemically related compounds have been synthesized and tested for their cytokinin activity [4]. Among the compounds which gave a positive response in the cytokinin tests, the most active compounds were N^6-substituted adenine derivatives. Although some exceptions [5] have recently been reported, it was not known whether the corresponding derivatives of the nucleic acid bases other than adenine would also show the cytokinin activity.

In order to examine the cytokinin activity of such compounds, we have synthesized eight compounds of O^6-substituted guanine and hypoxanthine types, and tested their activity in three bioassay systems: tobacco pith callus, radish cotyledons, and lettuce seed.

During the course of this study, Leonard and Skoog and their collaborators have reported the activity of 6-benzyloxypurine and 6-isopentenyloxypurine [6]. However, they did not deal with guanine type derivatives and the other derivatives of hypoxanthine type. In this paper, we describe the cytokinin activity of O^6-substituted guanine and hypoxanthine derivatives.

RESULTS AND DISCUSSION

All the compounds tested were synthesized in our laboratory (T.H.), and gave satisfactory elemental analysis (see Experimental). The results of tobacco bioassays, summarized in Fig. 1, indicate that the O^6-substituted derivatives of both the guanine and hypoxanthine types induce cell division, although such compounds have not yet been found to occur naturally. The results also indicate that the degree of cytokinin activity conferred by the O^6-side chain corresponds to the case of the N^6 side chain in adenylate cytokinins. Thus, O^6-benzylhypoxanthine was the most active of these compounds and reached an optimal growth response in a concentration range between 1×10^{-7} M and 1×10^{-6} M. The callus yield obtained at 1×10^{-7} M was nearly twice that produced by kinetin at the same concentration. The O^6-*n*-amyl derivative showed an optimal growth response between 1×10^{-6} M and 1×10^{-5} M. The other O^6-alkylhypoxanthines showed optimal responses at 1×10^{-5} M, and the callus yields decreased as a function of the side chain size in the order *iso*-amyl, butyl, hexyl, heptyl.

Table 1. List of compounds tested for cytokinin activity

1.	O^6-*n*-Butylhypoxanthine
2.	O^6-*n*-Amylhypoxanthine
3.	O^6-*iso*-Amylhypoxanthine
4.	O^6-*n*-Hexylhypoxanthine
5.	O^6-*n*-Heptylhypoxanthine
6.	O^6-Benzylhypoxanthine
7.	O^6-Benzylguanine
8.	O^6-*n*-Amylguanine

Guanine type compounds were less active than the corresponding hypoxanthine type compounds. Introduction of an amino group into the 2 position of the purine ring lowered the activity as can be seen from comparison of the activities of the guanine and hypoxanthine type compounds. Similar effects of the substituents in the 2-position have already been observed in N^6-substituted adenylate cytokinins except in the case of 2-chlorozeatin [7].

It is noteworthy that slight browning was observed in the callus tissue grown on the media containing the near to optimal concentration of 7.

The results obtained by the radish cotyledon test and the lettuce-seed germination test (Tables 2 and 3) were

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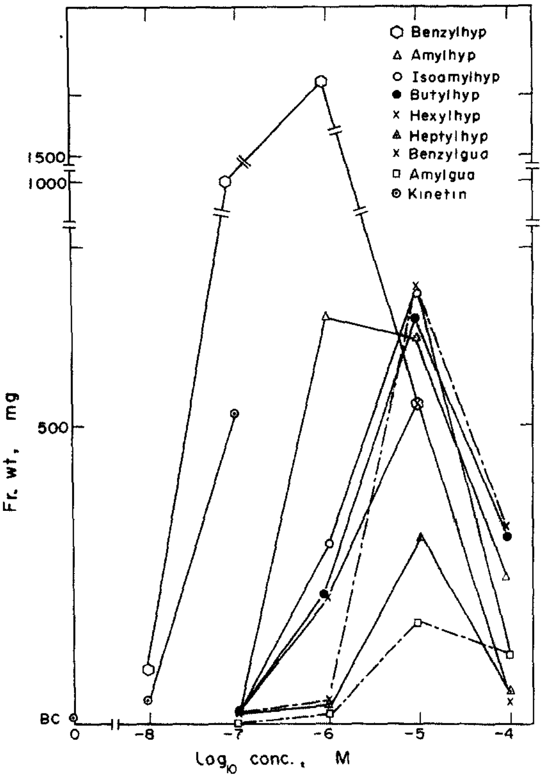

Fig. 1. Effect of compounds **1–8** and kinetin on fresh weight yield of tobacco callus.

Table 3. Results of lettuce-seed germination test

Compound	Conc. (M)	% Germination
Control	0	6.3
7	1×10^{-7}	16.1
	1×10^{-6}	12.7
	1×10^{-5}	35.2
	1×10^{-4}	71.8
8	1×10^{-7}	12.0
	1×10^{-6}	12.9
	1×10^{-5}	5.5
	1×10^{-4}	11.3
6	1×10^{-7}	29.5
	1×10^{-6}	71.0
	1×10^{-5}	86.0
	1×10^{-4}	92.0
2	1×10^{-7}	8.5
	1×10^{-6}	15.2
	1×10^{-5}	48.8
	1×10^{-4}	94.3
kinetin	1×10^{-7}	24.7
	1×10^{-6}	72.5
	1×10^{-5}	100.0
	1×10^{-4}	100.0

Lettuce (*Lactuca sativa* L. cv. New York) seeds were sown on a sheet of filter paper wetted with 3 ml of test soln in a Petri dish (diam. 7 cm) and maintained at 27° in darkness for 48 hr.

Table 2. Results of radish-cotyledon growth test

Compound	Conc. (M)	Observed wt of cotyledons (*A*, mg)	*A* − *B** (mg)	$\frac{A-B}{(A-B)_{\text{control}}}$ %
Control	0	15.5	10.5	100
7	5×10^{-7}	15.0	10.0	95.1
	5×10^{-6}	15.0	9.9	94.8
	5×10^{-5}	17.8	12.7	122
	5×10^{-4}	23.0	18.0	171
8	5×10^{-7}	13.9	8.9	84.8
	5×10^{-6}	11.8	6.8	64.6
	5×10^{-5}	12.9	7.8	74.9
	5×10^{-4}	17.7	12.7	121
6	5×10^{-7}	16.7	11.7	111
	5×10^{-6}	19.3	14.2	136
	5×10^{-5}	23.7	18.7	179
	5×10^{-4}	23.8	18.8	180
2	5×10^{-7}	14.5	9.4	90.1
	5×10^{-6}	15.1	10.1	96.6
	5×10^{-5}	15.7	10.7	102
	5×10^{-4}	16.1	11.0	105

* Average value of initial wt of 10 cotyledons. B = 5.02 mg. Radish (*Raphanus sativus* L. cv. Tokinashi, market strain) seed were germinated at 26° in darkness on well-wetted filter paper in large Petri dishes. After about 48 hr the larger cotyledon was excised from each seedling, the hypocotyl being removed completely. Cotyledons of uniform size (about 5 mg in fr wt) were selected and placed on filter paper in Petri dishes (diam. 7 cm). Each dish contained a sheet of filter paper, 4 ml of test soln (prepared in 2 mM KH_2PO_4) and 10 cotyledons. After 3 days at 27° under continuous fluorescent lighting (*ca* 3200 lx), the cotyledons were blotted dry and weighed.

essentially in agreement with those of the tobacco bioassay.

The significance of these results lies not simply in the extent of the cytokinin activity, but also in the qualification of the O^6-substituted guanine and hypoxanthine derivatives as cytokinins, though such cytokinins have not yet been found in plants.

EXPERIMENTAL

PMR spectra were recorded using TMS as an internal standard. UV spectra were measured in 95% EtOH.

Bioassay. Cytokinin activity of the compounds was assayed by the tobacco callus test [8], the radish cotyledon test [9], and the lettuce-seed germination test [10].

General procedures for synthesis of O^6-alkylhypoxanthine and O^6-alkylguanine. O^6-*n*-Butyl-, *n*-amyl-, *iso*-amyl-, *n*-hexyl-, and *n*-heptylhypoxanthines were prepared by Williamson synthesis involving 6-chloropurine and sodium alkoxide, as described in ref. [11]. O^6-Benzylhypoxanthine was prepared from 6-chloropurine by the procedure reported in ref. [12]. 6-Chloropurine was obtained by the reaction of hypoxanthine with phosphorus oxychloride in the presence of N,N-dimethylaniline [13]. Hypoxanthine was synthesized from thiourea and ethylcyanoacetate as described previously [14]. O^6-Benzyl- and *n*-amylguanines were prepared from 2-amino-6-chloropurine and sodium alkoxide in a manner like that described above [12]. 2-Amino-6-chloropurine was obtained from 2-amino-6-methylthiopurine derived from 2,6-diamino-4-hydroxy-5-formylaminopyrimidine [15]. The last compound was prepared from 2,6-diamino-4-hydroxypyrimidine hemisulfate [16]. Physicochemical characterizations of the test compounds synthesized are described below.

O^6-n-Butylhypoxanthine (**1**). mp 164°. Yield, 52.3%. (Found: C, 55.95; H, 6.01; N, 28.88. $C_9H_{12}N_4$ requires: C, 56.23; H, 6.29; N, 29.15%). UV λ_{max} nm (ϵ): 254 (10000), 259 *sh*, 267 sh, 95% EtOH (0.1N HCl) 255 (8700), 95% EtOH (0.1N NaOH) 264 (9700). PMR ($CDCl_3$) δ: 8.60 (1H, *s*, 2-H), 8.25 (1H, *s*, 8-H), 4.62 (2H, *t*, *J* 6.5Hz, $-OCH_2-$), 2.0–1.2 (4H, *m*, 2′, 3′-CH_2-), 0.95 (3H, *t*, *J* 6.5 Hz).

O^6-n-Amylhypoxanthine (**2**). mp 158°. Yield, 88.2%. (Found: C, 58.21; H, 6.87; N, 27.66. $C_{10}H_{14}N_4O$ requires: C, 58.23; H, 6.84; N, 27.17). UV λ_{max} nm(ϵ): 253 (10900), 259 *sh*, 267 *sh*, 95% EtOH (0.1N HCl) 255 (10700), 95% EtOH (0.1N NaOH) 264 (10200). PMR ($CDCl_3$) δ: 8.55 (1H, *s*, 2-H), 8.20 (1H, *s*, 8-H), 4.58 (2H, *t*, *J* 7Hz, $-OCH_2-$), 1.80 (2H, *m*, 2′-CH_2-), 1.40 (4H, *m*, 3′,4′-CH_2-), 0.89 (3H, *m*, -Me).

O^6-iso-Amylhypoxanthine (**3**). mp 162.5–163°. Yield 82.8%. (Found: C, 58.32; H, 7.01; N, 27.24. $C_{10}H_{14}N_4O$ requires: C, 58.23; H, 6.84; N, 27.17). UV λ_{max} nm(ϵ): 254 (9900), 259 *sh*, 267 *sh*, 95% EtOH (0.1N HCl) 255(11200), 95% EtOH (0.1N NaOH) 264 (9600). PMR ($CDCl_3$) δ: 8.60 (1H, *s*, 2-H), 8.25 (1H, *s*, 8-H), 4.65 (2H, *t*, *J* 6.5 Hz, $-OCH_2-$), 1.75 (3H, *m*, $-CH_2CH<$), 0.85 (6H, *d*, *J* 6 Hz, $-(Me)_2$).

O^6-n-Hexylhypoxanthine (**4**). mp 149–150°. Yield, 40.4%. (Found: C, 60.07; H, 7.29; N, 25.74. $C_{11}H_{16}N_4O$ requires: C, 59.98; H, 7.32; N, 25.44). UV λ_{max} nm (ϵ): 253.5 (8700), 259 *sh*, 267 *sh*, 95% EtOH (0.1N HCl) 255 (8800), 95% EtOH (0.1N NaOH) 264 (9500). PMR ($CDCl_3$): 8.54 (1H, *s*, 2-H), 8.22 (1H, *s*, 8-H), 4.59 (2H, *t*, *J* 7 Hz, $-OCH_2-$), 1.78 (2H, *m*, 2′-CH_2-), 1.34 (6H, *m*, 3′, 4′, 5′-CH_2-), 0.88 (3H, *m*, -Me).

O^6-Heptylhypoxanthine (**5**). mp 128°. Yield, 30.7%. (Found: C, 61.59; H, 7.78; N, 24.04. $C_{12}H_{18}N_4O$ requires: C, 61.51; H, 7.74; N, 23.91). UV λ_{max} nm(ϵ): 254 (9500), 259 *sh*, 267 *sh*, 95% EtOH (0.1N HCl) 255 (8400), 95% EtOH (0.1N NaOH) 264 (9600). PMR ($CDCl_3$): 8.55 (1H, *s*, 2-H), 8.25 (1H, *s*, 8-H), 4.60 (2H, *t*, *J* 6.5 Hz, $-OCH_2-$), 1.80 (2H, *m*, 2′-CH_2-), 1.30 (8H, *m*, 3′,4′,5′,6′-CH_2-), 0.85 (3H, *m*, -Me), 10-11(1H, *b*, NH).

O^6-Benzylhypoxanthine (**6**). mp 177–178°. Yield 60.0%. (Found: C, 63.63; H, 4.58; N, 25.04. $C_{12}H_{10}N_4O$ requires: C, 63.70; H, 4.46; N, 24.77%). UV λ_{max} nm (ϵ): 254 (10000), 259.5 *sh*, 268 *sh*, 95% EtOH (0.1N HCl) 255.5 (9100), 95% EtOH (0.1N NaOH) 264.5 (8500). PMR(DMSO-d_6): 8.49 (1H, *s*, 2-H), 8.33 (1H, *s*, 8-H), 7.46 (5H, *m*, phenyl-H), 5.73 (2H, *s*, $-CH_2-$).

O^6-Benzylguanine (**7**). mp 207–208° (reported [8] 202–204°). Yield, 24.7%. (Found: C, 59.55; H, 4.88; N, 28.81. $C_{12}H_{11}N_5O$ requires: C, 59.74; H, 4.60; N, 29.03%). PMR (DMSO-d_6): 7.82 (1H, *s*, 8-H), 7.42 (5H, *m*, phenyl-H), 6.17 (2H, *m*, 2-NH_2), 5.51 (2H, *s*, $-CH_2-$). IR(KBr) cm^{-1}: 3600, 3450 (NH_2), 2850 (CH), 1645, 1600 (purine ring), 1220 (-O-), 735, 693(monosubstituted benzene).

O^6-n-Amylguanine (**8**). mp 178°. Yield, 30%. (Found: C, 54.10; H, 6.76; N, 31.62. $C_{10}H_{15}N_5O$ requires: C, 54.28; H, 6.83; N, 31.66%). IR (KBr) cm^{-1}: 3600, 3480 (NH_2), 2960 (CH), 1625, 1595 (purine ring), 1460, 1380 (Me), 1220 (-O-).

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