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Cytokinin Activities of *N*-(Purin-6-yl)amino Acids, *N*-(Purin-6-yl)peptides and Related Compounds

By

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Abstract

Four *N*-(purin-6-yl)amino acids, six *N*-(purin-6-yl)peptides, three ethyl esters of the latter, and some related compounds were tested for their cytokinin activities by the tobacco callus bioassay and lettuce seed germination. Among the *N*-(purin-6-yl)amino acids, *N*-(purin-6-yl)-L-phenylalanine exhibited a weak cytokinin activity at 100 μM in the tobacco callus bioassay, although around 10000 times less active than kinetin, but no any activity in lettuce seed germination. The *N*-(purin-6-yl)peptides tested were all inactive in both bioassays. However, esterification with the ethyl group in *N*-(purin-6-yl)glycylglycine and *N*-(purin-6-yl)-L-phenylalanylglycine enhanced the cytokinin activity; they were weakly active at 100 μM in the tobacco callus bioassay, but inactive in lettuce seed germination. (±)-2-Methyl-4-(purin-6-ylamino)butyric acid was active at 1-100 μM in the tobacco callus bioassay and its methyl ester showed a stronger cytokinin activity. Analogous amino acid or peptide derivatives with a naphthalene or pyrimidine ring instead of the purine ring were inactive.

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Introduction

N-(Purin-6-yl)amino acids and some amino acid conjugates of purine derivatives have been given attention to, because of their antitumor activity to certain mammalian cells.[1,2] On the other hand, it has been reported that a number of purine derivatives possess cytokinin activity, a cell-division-promoting activity in plant tissue cultures.[3-6] These facts have suggested some significant relationships via purine derivatives in the regulation of cell proliferation between mammalian cells and plant cultured tissues. Certain *N*-(purin-6-yl)amino acids and related compounds have been studied on their cytokinin activities.[7-12] These studies revealed that the *N*-(purin-6-yl)amino acids tested were generally inactive or weakly active and that hydroxymethylation or esterification of the carboxy group of them enhanced the cytokinin activity.[7-12] Nevertheless, no report on the cytokinin activity of *N*-(purin-6-yl)peptides has been found, so far as we know. This paper presents cytokinin activities of *N*-(purin-6-yl)peptides, their ethyl esters, and some related compounds, in addition to four *N*-(purin-6-yl)amino acids, tested by the tobacco callus bioassay and lettuce seed germination.

Materials and Methods

The syntheses and detailed information of *N*-(purin-6-yl)amino acids (**1**-**5**) were reported previously.[1] The syntheses of *N*-(purin-6-yl)peptides(**6**-**15**) were carried out as described in a previous paper.[13] Compounds **16** and **17** were synthesized by Dr. H. Iwamura (Agr. Chem., Kyoto Univ.) and **18** and **19** by Dr. K.

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Koshimizu (Food Sci. & Tec., Kyoto Univ.). Compounds **20**–**26** were synthesized by T. Fujii and T. Nishitani, two of the authors.[13,15] Kinetin was purchased from Nakarai Chem. LTD, Kyoto City.

For tobacco callus (*Nicotiana tabacum* L. cv Wisconsin No. 38) bioassay, the method reported previously[14,15] was used. The basal culture medium was the Linsmaier and Skoog medium[14] containing the mineral salts, 30 g/l sucrose, 10 g/l agar, 100 mg/l *myo*-inositol, 2 mg/l IAA and 0.4 mg/l thiamine-HCl. Aqueous solutions of all the compounds to be tested were filter-sterilized and added to the autoclaved basal media in 50-ml conical flasks, shortly before solidification. Each flask contained 20-ml medium and three pieces of tobacco callus (5-8 mg each, fr. wt) implanted on the agar surface. Each experimental treatment contained 4 replicates of flasks. The flasks were maintained at 28℃ in the dark for 30 days, and then the fr. wt of tissues was determined.

For auxin-assay, tobacco callus was implanted on the medium with 0.03 mg/l kinetin and compounds to be tested but without IAA. All other procedures were carried out as described above.

The cytokinin-assay by lettuce seed germination was carried out as described by Matusbara et al.[15,16] Lettuce (*Lactuca sativa* L. cv New York 515) seeds were sown on a sheet of filter paper wetted with 4 ml aqueous solution of the compound to be tested in a Petri dish (diam. 7 cm). After 48-h incubation in darkness at 27℃, the germination percentages were determined.

Results and Disccusion

As shown in Table 1, *N*-(purin-6-yl)glycine(**1**), -L-isoleucine(**2**) and -L-methionine(**3**) were inactive at the concentrations tested. *N*-(Purin-6-yl)-L-phenylalanine(**4**) and its racemic form(**5**) exhibited a weak cytokinin activity at 100 μM, although around 10000 times less active than kinetin(**27**). Higher activity of the L isomer (**4**) than the racemic form(**5**) suggests that the former possesses a higher cytokinin activity than the D isomer. Esterification of **3** and **4** to give **16** and **17**, respectively, or replacement of the carboxy group by the hydroxymethyl group, as in **18** and **19**, enhanced the cytokinin activity.

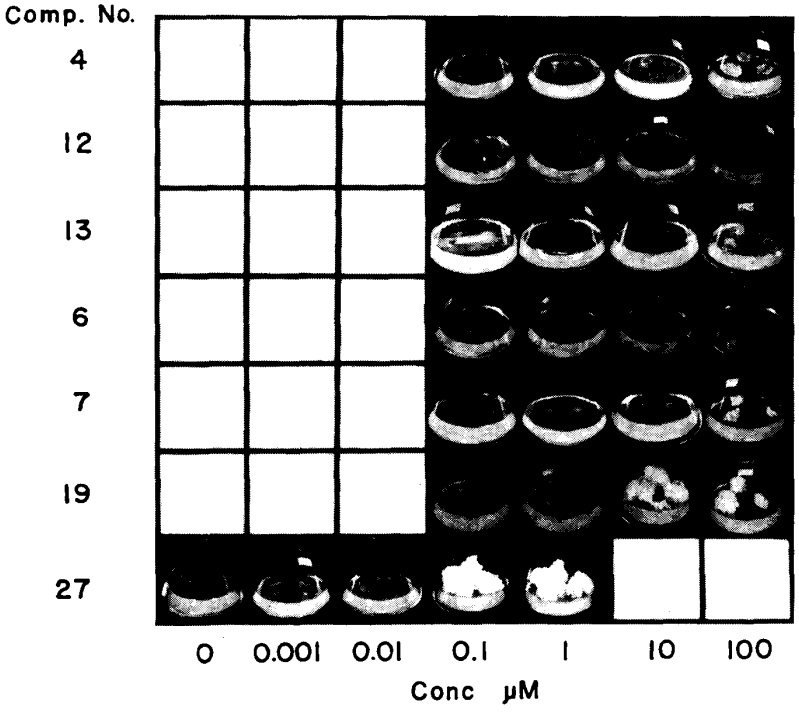

Fig. 1. Enhancement of cytokinin activity by esterification of the carboxy group and replacement of it by the hydroxymethyl group in an *N*-(purin-6-yl)amino acid or *N*-(purin-6-yl)peptides tested by the tobacco callus bioassay. Compound numbers are listed in Table 1.

Table 1. Cytokinin activity of *N*-(purin-6-yl)amino acids, *N*-(purin-6-yl)peptides and related compounds tested by the tobacco callus bioassay.

Compound	Average fresh weight of tobacco callus (mg)					
	0	0.01	0.1	1	10	100(μM)
N-(purin-6-yl)glycine (**1**)	12.6	-	25.8	18.8	30.4	19.3
N-(purin-6-yl)-L-isoleucine (**2**)	19.3	-	22.2	20.4	20.0	16.9
N-(purin-6-yl)-L-methionine (**3**)	21.3	-	16.3	19.2	25.3	13.3
N-(purin-6-yl)-L-phenylalanine (**4**)	13.0	-	12.7	13.0	67.1	217.3
N-(purin-6-yl)-DL-phenylalanine (**5**)	17.3	-	29.5	37.3	35.2	119.6
N-(purin-6-yl)glycylglycine (**6**)	12.6	-	21.7	11.8	16.4	20.9
N-(purin-6-yl)glycylglycine ethyl ester (**7**)	12.8	-	13.6	22.1	46.3	103.4
N-(purin-6-yl)glycylglycylglycine (**8**)	12.6	-	12.7	16.8	19.2	18.9
N-(purin-6-yl)glycylglycylglycine ethyl ester (**9**)	12.8	-	22.3	10.3	10.2	34.4
N-(purin-6-yl)glycylglycylglycylglycine (**10**)	12.6	-	13.6	10.6	12.8	25.0
N-(purin-6-yl)glycyl-DL-phenylalanine (**11**)	21.3	-	11.7	24.3	11.4	11.8
N-(purin-6-yl)-DL- phenylalanylglycine (**12**)	17.3	-	27.7	20.5	10.3	23.0
N-(purin-6-yl)-L-phenylalanylglycine ethyl ester (**13**)	19.3	-	24.6	20.3	19.8	169.4
N-(purin-6-yl)-DL-phenylalanylglycine ethyl ester (**14**)	12.8	-	19.3	15.9	13.8	8.8
N-(purin-6-yl)-DL-phenylalanylglycylglycine (**15**)	19.3	-	20.4	19.7	24.0	28.4
N-(purin-6-yl)-L-methionine methyl ester (**16**)	16.3	26.5	32.9	63.3	1681.5	73.5
N-(purin-6-yl)-L-phenylalanine methyl ester (**17**)*	16.3	39.3	27.7	283.4	1597.5	712.6
(S)-(−)-6-(α-hydroxymethyl-γ-methylmercaptopropylamino)purine (**18**)**	14.9	-	31.1	37.8	880.5	1525.6
(S)-(−)-6-(α-hydroxymethyl-β-phenylethylamino)-purine (**19**)**	14.9	-	25.5	20.3	1244.2	522.4
(±)-2-methy-4-(purin-6-ylamino)butyric acid (**20**)	18.1	-	20.3	105.7	446.4	339.8
(±)-2-methy-4-(purin-6-ylamino)butyric acid methyl ester (**21**)	18.1	-	141.7	1526.3	915.4	311.6

(Table 1 continued)

N-(α-naphthyl)phenylalanine (**22**)	18.1	-	20.3	19.8	17,3	24.7
N-(α-naphthyl)glycylglycine (**23**)	18.1	-	24.7	22.2	27.6	27.0
benzyl *N*-(α-naphthyl)carbamate (**24**)	7.7	-	24.4	11.7	6.8	6.3
N-(6-methyl-4-pyrimidyl)glycine (**25**)	21.3	-	27.2	22.5	28.3	34.5
N-(6-methyl-4-pyrimidyl)glycylglycine (**26**)	21.3	-	13.3	20.0	9.0	17.0
kinetin (**27**)	17.3	274.3	1080.4	-	-	-

* Data are cited from Ref. 10.
** Data are cited from Ref. 9.

N-(Purin-6-yl)-DL-phenylalanylglycine(**12**), which has a longer chain than the active **4** and **5** by a glycine molecule unit, did not show any cytokinin activity at the concentrations tested. *N*-(Purin-6-yl)peptides(**6, 8, 10–12, 15**) were all inactive. However, the ethyl esters (**7, 13**) of *N*-(purin-6-yl)-glycylglycine (**6**) and *N*-(purin-6-yl)-DL-phenylalanylglycine(**12**) recovered a slight cytokinin activity, when tested at 100 μM (Fig.1). This indicates that esterification of the carboxy group enhances the cytokinin activity. The ethyl ester(**9**) with a longer peptide chain was nearly inactive.

Among related compounds, **20** exhibited a considerable cytokinin activity at 1-100 μM, and esterification of **20** with the methyl group as in **21** likewise enhanced the activity. The amino acid and peptide derivatives (**22–26**) with a naphthalene or pyrimidine ring were all inactive.

N-(Purin-6-yl)amino acids(**1–4**), their methyl esters(**16, 17**), *N*-(purin-6-yl)peptides(**6, 8, 10–12, 15**), ethyl esters(**7, 9**) of **6** and 8, hydroxymethyl derivatives(**18, 19**) of **3** and **4**, and kinetin(**27**) were tested for their cytokinin activity by lettuce seed germination (Table 2). In this bioassay, these *N*-(purin-6-yl)amino acids, *N*-(purin-6-yl)peptides, and ethyl esters were all inactive. However, the methyl esters(**16,17**) of **3** and **4** and the hydroxymethyl derivatives(**18,19**) showed some cytokinin activity at 100 and 1000 μM. Kinetin(**27**) was active even at 0.1 μM.

α-Naphthaleneacetic acid is known to be a strong auxin. Therefore it is interesting to know whether or not the naphthalene derivatives such as **23** and **24** possess any auxin activity in tobacco callus bioassay. However, they were found to exhibit no auxin activity (Table 3).

Letham and Young[8] revealed that cytokinin activity was greatly reduced by the presence of an unesterified carboxy group in the N^6-substituent of a 6-(substituted amino)purine. Moreover, it has been known that unsuitably long N^6-substituents in 6-(substitued amino)purines caused the cytokinin activity to lower.[5,6] Therefore, it can be explained that these unfavorable structural characteristics of the *N*-(purin-6-yl)peptides removed the cytokinin activity and that esterification of the carboxy group in some of them recovered it.

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Table 2. Cytokinin activity of *N*-(purin-6-yl)amino acids, *N*-(purin-6-yl)peptides and related compounds tested by lettuce seed germination.

Compound	Lettuce seed germination (%)					
	0	0.1	1	10	100	1000 (μM)
N-(purin-6-yl)glycine (**1**)	11.9	-	-	9.5	14.8	12.0
N-(purin-6-yl)-L-isoleucine (**2**)	11.9	-	-	15.7	10.3	11.4
N-(purin-6-yl)-L-methionine (**3**)	11.9	-	-	15.4	20.0	12.7
N-(purin-6-yl)-L-phenylalanine (**4**)	11.9	-	-	12.7	15.2	7.8
N-(purin-6-yl)glycylglycine (**6**)	11.9	-	-	21.0	16.7	9.6
N-(purin-6-yl)glycylglycine ethyl ester (**7**)	11.9	-	-	9.5	10.0	4.3
N-(purin-6-yl)glycylglycylglycine (**8**)	11.9	-	-	17.8	15.9	16.4
N-(purin-6-yl)glycylglycylglycine ethyl ester (**9**)	11.9	-	-	9.3	13.3	6.8
N-(purin-6-yl)glycylglycylglycylglycine (**10**)	11.9	-	-	10.9	7.1	3.8
N-(purin-6-yl)glycyl-DL-phenylalanine (**11**)	11.9	-	-	11.9	21.6	12.5
N-(purin-6-yl)-DL-phenylalanylglycine (**12**)	11.9	-	-	10.0	10.4	8.6
N-(purin-6-yl)-DL-phenylalanylglycylglycine (**15**)	11.9	-	-	15.9	12.0	8.0
N-(purin-6-yl)-L-methionine methyl ester (**16**)	11.3	-	-	15.6	22.7	52.5
N-(purin-6-yl)-L-phenylalanine methyl ester (**17**)	11.9	-	15.4	14.1	18.3	44.4
(S)-(−)-6-(α-hydroxymethyl-γ-methyl-mercaptopropylamino)purine (**18**)	18.4	-	-	27.1	33.3	66.7
(S)-(−)-6-(α-hydroxymethyl-β-phenylethylamino)-purine (**19**)	18.4	-	31.7	30.4	28.6	62.3
kinetin (**27**)	11.9	35.8	60.8	82.6	-	-

Table 3. Auxin activity of naphthalene derivatives and IAA tested by the tobacco callus bioassay.

Compound	Average fresh weight of tobacco callus (mg)					
	0	0.01	0.1	1	10	100 (μM)
N-(α-naphthyl)glycylglycine (**23**)	7.7	-	13.3	17.1	16.3	29.1
benzyl *N*-(α-naphthyl)carbamate (**24**)	7.7	-	24.4	11.7	6.0	6.3
α-naphthaleneacetic acid	7.7	16.6	117.1	1040.1	617.2	61.0
IAA	7.7	-	129.6	591.1	1101.1	217.7

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