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Cytokinin Activities of 1'-Methyl Derivatives of Zeatin and N^6-(Δ^2-Isopentenyl)adenine and of their 9-β-D-Ribofuranosides in Lettuce Seed Germination

SATOSHI MATSUBARA,* TOZO FUJII** and MASASHI OHBA**

Abstract

Cytokinin activities of *trans*- and *cis*-zeatins (**1** and **2**), N^6-(Δ^2-isopentenyl)-adenine (**3**), their 1'-methyl derivatives [(1'*R*)-**1**, (1'*S*)-**1**, (1'*R*)-2OH-**1**, (1'*S*)-2OH-**1**, (1'*R*)-**2**, (1'*S*)-**2**, (1'*R*)-**3**, and (1'*S*)-**3**, listed in Table 1], and of their 9-β-D-ribofuranosides [**4**, (1"*R*)-**4**, (1"*S*)-**4**, **5**, (1"*R*)-**5**, (1"*S*)-**5**, **6**, (1"*R*)-**6**, and (1"*S*)-**6**, listed in Table 1] except for those of (1'*R*)-2OH-**1**, and (1'*S*)-2OH-**1**, assayed by lettuce seed germination, are summarized, and structure-activity relationships are reviewed.

(1) 1'- or 1"-Methylation in **1**, **2**, **4**, and **6** resulted in slightly less active (1'*R*)-**1**, (1'*R*)-**2**, (1"*R*)-**4**, and (1"*R*)-**6**, respectively, and did in much less active (1'*S*)-**1**, (1'*S*)-**2**, (1"*S*)-**4**, and (1"*S*)-**6**, respectively. However, 1'-methylation gave no effect on **3** : (1'*R*)-**3** and (1'*S*)-**3** were nearly as active as **3**. On the other hand, 1"-methylation in **5** gave slightly more active (1"*R*)-**5** and completely inactive (1"*S*)-**5**. The 2-hydroxy derivative (1'*R*)-2OH-**1** was more active than (1'*S*)-2OH-**1**. Thus, all the *R*-enantiomers were more active than the corresponding *S*-enantiomers, respectively.

(2) The *trans*-isomers [**1**, (1'*R*)-**1**, (1'*S*)-**1**, **4**, (1"*R*)-**4**, and (1"*S*)-**4**] were more active than the corresponding *cis*-isomers [**2**, (1'*R*)-**2**, (1'*S*)-**2**, **5**, (1"*R*)-**5**, and (1"*S*)-**5**], respectively.

(3) Hydroxylation at the 2-position of the purine ring in (1'*R*)-**1** and (1'*S*)-**1** gave much less active (1'*R*)-2OH-**1** and (1'*S*)-2OH-**1**, respectively.

(4) Ribosidations at the 9-position in **1**, (1'*R*)-**1**, (1'*S*)-**1**, **2**, (1'*R*)-**2**, (1'*S*)-**2**, **3**, (1'*R*)-**3**, and (1'*S*)-**3** all reduced the cytokinin activity. In the *cis* series, **5** still possessed a very weak cytokinin activity, but its 2'-deoxy derivative [9-(2'-deoxy-β-D-ribofuranosyl)-*cis*-zeatin (2'H-**5**)] did not exhibit any cytokinin activity even at the highest concentration tested.

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Introduction

Cytokinins are a group of phytohormones with various physiological activities to promote cell division in plant tissue cultures, seed germination, leaf and cotyledon enlargement, and lateral bud development, to inhibit chlorophyll degradation, and to induce buds on moss protonema.[1-4] More than 30 natural cytokinins have so far been isolated, and their chemical structures identified.[3,5-12]

*Laboratory of Applied Biology, Kyoto Prefectural University, Shimogamo, Kyoto 606, Japan
**Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan

Surico *et al.* (1985, 1986)[5,6] reported the isolation of two new cytokinins, 1'-methylzeatin and its 9-β-D-ribofuranoside, from the culture filtrate of the gall-forming phytopathogenic bacterium *Pseudomonas syringae* pv *savastanoi*. Fujii *et al.* (1989)[7] synthesized both enantiomers of 1'-methylzeatin and their 9-β-D-ribofuranosides, determined the absolute configurations of the new cytokinins as [*R*-(*E*)]-*N*-(4-hydroxy-1,3-dimethyl-2-butenyl)adenine [(1'*R*)-**1**] and its 9-β-D-ribofuranoside [(1"*R*)-**4**], and examined their cytokinin activity by tobacco tissue culture andl ettuce seed germination. These studies promoted further investigations to synthesize the *cis*-isomers [(1'*R*)-**2**, (1'*S*)-**2**, (1"*R*)-**5**, and (1"*S*)-**5**] of (1'*R*)-1'-methylzeatin, (1'*S*)-1'-methylzeatin, and their 9-β-D-ribofuranosides and to determine their cytokinin activity.[8,13] Farooqi *et al.* (1990)[9] isolated a novel cytokinin, the 2-hydroxy derivative of 1'-methylzeatin, from a marine green alga (code No. NIO-143). Fujii *et al.* (1992, 1993)[8,10] established the planar structure of this cytokinin and inferred its absolute configuration to be (1'*R*)-2-hydroxy-1'-methyl-*trans*-zeatin [(1'*R*)-2OH-**1**]. In relation to these new natural cytokinins, Fujii *et al.* (1994)[14] synthesized both enantiomers [(1'*R*)-**3** and (1'*S*)-**3**] of N^6-(1,3-dimethyl-2-butenyl)adenine and their 9-β-D-ribofuranosides [(1"*R*)-**6** and (1"*S*)-**6**] and determined their cytokinin activity by tobacco tissue culture.

These new cytokinins are unique in that their N^6-substituents consist of a branched allyl alcoholic C_6-unit with an asymmetric center adjacent to the N^6 atom. The present study summarizes the cytokinin activities, tested by lettuce seed germination, of *trans*- and *cis*-zeatins (**1** and **2**), N^6-(Δ^2-isopentenyl)adenine (**3**), their 1'-methyl derivatives [(1'*R*)-**1**, (1'*S*)-**1**, (1'*R*)-2OH-**1**, (1'*S*)-2OH-**1**, (1'*R*)-**2**, (1'*S*)-**2**, (1'*R*)-**3**, and (1'*S*)-**3**], and their 9-β-D-ribofuranosides [(1"*R*)-**4**, (1"*S*)-**4**, (1"*R*)-**5**, (1"*S*)-**5**, (1"*R*)-**6**, and (1"*S*)-**6**], and structure-activity relationships are discussed.

Materials and Methods

Compounds used in this study and their abbreviated names are listed in Table 1. The 2-hydroxy derivatives (1'*R*)-2OH-**1** and (1'*S*)-2OH-**1** were synthesized as described in refs. 8 and 10; (1'*S*)-**2**, (1"*S*)-**5**, and 2'H-**5** as described in refs. 8 and 15; and (1'*R*)-**3**, (1'*S*)-**3**, (1"*R*)-**6**, and (1"*S*)-**6** as described in ref. 14. Compounds **3**, **4**, **5**, and **6** were purchased from Sigma Chem. Co (St. Louis, MO, U. S. A.).

The cytokinin assay by lettuce seed germination was carried out as described previously.[16-18] Lettuce (*Lactuca sativa* L. cv New York 515) seeds were sown on a sheet of filter paper, wetted with 4 ml of an aqueous solution of test compound, in a Petri dish (7 cm in diameter). After a 48 h incubation in darkness at 27℃, the germination percentages were determined.

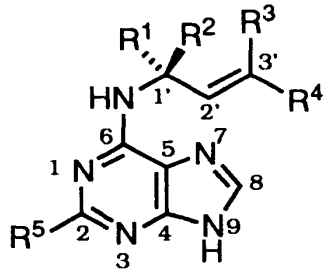
Results and Discussion

The chemical structures of the compounds used in this study and their abbreviated names are shown in Table 1. The cytokinin activities of these compounds tested by lettuce seed germination are summarized in Table 2. In this assay system, kinetin exhibited a detectable promotive activity at 0.04-0.4 μM and the maximum germination percentage at 10 μM. *trans*-Zeatin (**1**) and N^6-(Δ^2-isopentenyl)adenine (**3**) were nearly as active as kinetin, but *cis*-zeatin (**2**) was about 10 times less active than kinetin.

(1) 1'- *or* 1"-*Methylation in* N^6-*substituents of adenine derivatives*

1'- or 1"-Methylation in *trans*-zeatin (**1**), *cis*-zeatin (**2**), 9-β-D-ribofuranosyl-*trans*-zeatin (**4**), and N^6-(Δ^2-isopentenyl)adenosine (**6**) resulted in slightly less active (1'*R*)-**1**, (1'*R*)-**2**, (1"*R*)-**4**, and (1"*R*)-**6**, respectively, and did in much less active (1'*S*)-**1**, (1'*S*)-**2**, (1"*S*)-**4**, and (1"*S*)-**6**, respectively. However, such 1'-methylation in N^6-(Δ^2-isopentenyl)adenine (**3**) did not change the

Table 1. Compounds tested and their chemical structures.



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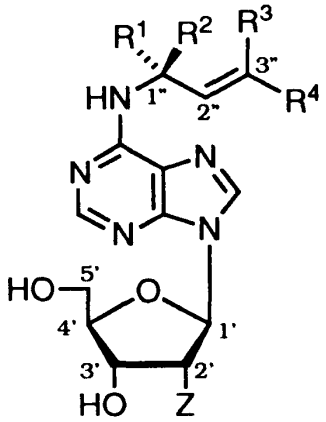

II

Structure I	R^1	R^2	R^3	R^4	R^5
trans-Zeatin (**1**)	H	H	CH_3	CH_2OH	H
(1'*R*)-1'-Methyl-*trans*-zeatin [(1'*R*)-**1**]	CH_3	H	CH_3	CH_2OH	H
(1'*S*)-1'-Methyl-*trans*-zeatin [(1'*S*)-**1**]	H	CH_3	CH_3	CH_2OH	H
(1'*R*)-2-Hydroxy-1'-methyl-*trans*-zeatin [(1'*R*)-2OH−**1**]	CH_3	H	CH_3	CH_2OH	OH
(1'*S*)-2-Hydroxy-1'-methyl-*trans*-zeatin [(1'S)-2OH−**1**]	H	CH_3	CH_3	CH_2OH	OH
cis-Zeatin (**2**)	H	H	CH_2OH	CH_3	H
(1'*R*)-1'-Methyl-*cis*-zeatin [(1'*R*)-**2**]	CH_3	H	CH_2OH	CH_3	H
(1'*S*)-1'-Methyl-*cis*-zeatin [(1'*R*)-**2**]	H	CH_3	CH_2OH	CH_3	H
N^6-(Δ^2-Isopentenyl)adenine (**3**)	H	H	CH_3	CH_3	H
(1'*R*)-1'-Methylisopentenyladenine [(1'*R*)-**3**]	CH_3	H	CH_3	CH_3	H
(1'*S*)-1'-Methylisopentenyladenine [(1'*S*)-**3**]	H	CH_3	CH_3	CH_3	H

Structure II	R^1	R^2	R^3	R^4	Z
9-β-D-Ribofuranosyl-*trans*-zeatin (**4**)	H	H	CH_3	CH_2OH	OH
(1"*R*)-1"-Methyl-9-β-D-ribofuranosyl-*trans*-zeatin [(1"*R*)-**4**]	CH_3	H	CH_3	CH_2OH	OH
(1"*S*)-1"-Methyl-9-β-D-ribofuranosyl-*trans*-zeatin [(1"*S*)-**4**]	H	CH_3	CH_3	CH_2OH	OH
9-β-D-Ribofuranosyl-*cis*-zeatin (**5**)	H	H	CH_2OH	CH_3	OH
9-(2'-Deoxy-β-D-ribofuranosyl)-*cis*-zeatin (2'H-**5**)	H	H	CH_2OH	CH_3	H
(1"*R*)-1"-Methyl-9-β-D-ribofuranosyl-*cis-zeatin* [(*1"R*)-**5**]	CH_3	H	CH_2OH	CH_3	OH
(1"*S*)-1"-Methyl-9-β-D-ribofuranosyl-*cis-zeatin* [(*1"S*)-**5**]	H	CH_3	CH_2OH	CH_3	OH
N^6-(Δ^2-Isopentenyl)adenosine (**6**)	H	H	CH_3	CH_3	OH
(1"*R*)-1"-Methylisopentenyladenosine [(1'*R*)-**6**]	CH_3	H	CH_3	CH_3	OH
(1"*S*)-1"-Methylisopentenyladenosine [(1"*S*)-**6**]	H	CH_3	CH_3	CH_3	OH

Table 2. Cytokinin activities of 1'-methyl derivatives of zeatin and N^6-(Δ^2-isopentenyl)-adenine and of their 9-β-D-ribofuranosides in the lettuce seed germination bioassay.

Compound	Lettuce seed germination (%)											
	Concentration of test compound (μM)											
	0	0.01	0.04	0.1	0.4	1	4	10	40	100	400	1000
Kinetin*	4.1	–	8.6	9.6	16.3	44.1	73.8	79.5	79.3	–	–	–
trans-Zeatin (**1**)*	3.7	8.8	13.5	32.1	43.7	40.2	70.9	74.0	–	88.4	–	–
(1'*R*)-**1***	3.8	9.6	15.0	19.4	21.4	32.4	57.4	65.7	–	88.2	–	–
(1'*S*)-**1***	3.8	–	4.4	5.8	4.3	8.1	11.7	16.0	27.3	30.3	–	–
(1'*R*)-2OH-**1**	5.0	–	–	–	–	–	–	4.0	4.0	11.2	14.4	28.6
(1'*S*)-2OH-**1**	5.0	–	–	–	–	–	–	5.0	4.5	4.1	5.6	6.7
cis-Zeatin (**2**)**	5.8	–	–	6.8	16.9	14.3	22.3	25.7	68.6	72.6	–	–
(1'*R*)-**2****	5.8	–	–	–	–	8.6	12.1	22.2	37.3	70.0	–	–
(1'*S*)-**2**	5.7	–	–	–	–	–	–	–	9.8	10.5	35.7	32.0
N^6-(Δ^2-Isopentenyl)-adenine (**3**)	5.8	5.8	12.0	11.7	21.8	45.6	69.4	79.8	–	–	–	–
(1'*R*)-**3**	4.3	–	–	5.4	26.4	40.1	61.2	70.7	80.2	–	–	–
(1'*S*)-**3**	4.3	–	–	–	21.8	35.6	65.2	73.2	74.8	78.7	–	–
9-β-D-Ribofuranosyl-*trans*-zeatin (**4**)	5.0	–	–	6.0	7.8	21.7	25.0	33.9	60.6	70.8	–	–
(1"*R*)-**4***	5.9	–	–	7.6	6.9	10.5	20.2	28.2	35.7	59.7	–	–
(1"*S*)-**4***	5.9	–	–	5.6	5.0	5.7	5.1	7.3	5.7	12.8	–	–
9-β-D-Ribofuranosyl-*cis*-zeatin (**5**)	4.2	–	–	–	–	5.2	4.7	4.6	6.2	4.8	8.0	15.6
2'H-**5**	4.2	–	–	–	–	4.7	4.3	4.1	4.7	5.5	5.9	8.2
(1"*R*)-**5****	5.8	–	–	–	–	7.4	7.3	9.3	12.8	21.8	–	–
(1"*S*)-**5**	5.7	–	–	–	–	–	–	–	–	4.7	–	5.5
N^6-(Δ^2-Isopentenyl)-adenosine (**6**)	5.8	–	–	9.9	8.1	23.5	36.1	61.5	73.0	83.1	–	–
(1"*R*)-**6**	4.3	–	–	–	4.3	5.1	11.3	32.0	55.4	70.4	–	–
(1"*S*)-**6**	4.3	–	–	4.3	4.2	3.4	3.8	4.0	8.3	17.4	–	31.0

* Taken from ref. 7.
** Taken from ref. 13.

cytokinin activity, giving nearly as active (1'*R*)-**3** and (1'*S*)-**3** as **3**. On the other hand, 1"-methylation in 9-β-D-ribofuranosyl-*cis*-zeatin (**5**) resulted in a little more active (1"*R*)-**5** and quite inactive (1"*S*)-**5**. The 2-hydroxy derivative (1'*R*)-2OH-**1** was more active than its enantiomer [(1'*S*)-2OH-**1**].

These results revealed that the *R*-enantiomers used in this study were more active than the corresponding *S*-enantiomers, respectively. Identical results have been obtained by us in the tobacco callus bioassay.[7,8,13,14] This suggests that these cytokinins act similarly in two different

physiological aspects, cell proliferation in tobacco tissue culture and lettuce seed germination.

(2) *Cytokinin activities of the* trans- *and* cis-*isomers of zeatin derivatives*

In various assay systems including tobacco tissue culture, *trans*-zeatin (**1**) always exhibits a stronger cytokinin activity than *cis*-zeatin (**2**).[2,4] In the present study using lettuce seed germination, **1**, (1'*R*)-**1**, (1'*S*)-**1**, **4**, (1"*R*)-**4**, and (1"*S*)-**4** were more than 10 times more active than **2**, (1'*R*)-**2**, (1'*S*)-**2**, **5**, (1"*R*)-**5**, and (1"*S*)-**5**, respectively. Similar results with these compounds have been reported in the case of tobacco tissue culture.[7,8,13]

(3) 2-*Hydroxylation in the purine ring*

In the tobacco tissue culture, substitution at the 2-position of N^6-substituted adenines mostly lowered the cytokinin activity to various degrees, depending on the substituents and the parent compounds: 2-substitutions with hydroxy, mercapto, methylsulfonyl, and benzylthio groups in *trans*-zeation (**1**) greatly lowered the cytokinin activity, while amino, methylthio, and methyl groups had a smaller effect on the activity, and 2-chloro substitution in *trans*-zeatin (**1**) gave a negligible effect or rather enhanced the activity.[19-21]

In the present study using lettuce seed germination, 2-hydroxylation in (1'*R*)-**1** and (1'*S*)-**1** drastically lowered the cytokinin activity and resulted in very weakly active (1'*R*)-2OH-**1** and completely inactive (1'*S*)-2OH-**1**, respectively.

(4) 9-*Ribosidation in the purine ring*

The 9-β-D-ribofuranosides of various cytokinins are generally less effective than their aglycons, respectively.[2,4] For example, the 9-β-D-ribofuranosides of kinetin, 6-benzylaminopurine, **1**, **2**, **3**, (*R*)-(+)-6-(4-hydroxy-3-methylbutylamino)purine, its (*S*)-(−)-isomer, and 6-isopentylaminopurine were all less active than their corresponding bases in the tobacco tissue culture.[17,22,23]

In the present study, ribosidation at the 9-position in **1**, (1'*R*)-**1**, (1'*S*)-**1**, **2**, (1'*R*)-**2**, (1'*S*)-**2**, **3**, (1'*R*)-**3** and (1'*S*)-**3** also reduced the cytokinin activity as observed in **4**, (1"*R*)-**4**, (1"*S*)-**4**, **5**, (1"*R*)-**5**, (1"*S*)-**5**, **6**, (1"*R*)-**6**, and (1"*S*)-**6**, respectively. These results, together with those obtained by tobacco tissue culture,[7,8,13,14] support a conclusion that cytokinins exogenously supplied can function without ribosidation at the 9-position.

Modification in the ribose moiety of N^6-substituted adenosines scarcely or slightly affects the cytokinin activity. It has been reported that the 9-(2'-deoxy-β-D-ribofuranosyl) derivative of **3** and its 5'-deoxy analog were nearly as active as **6** in tobacco tissue culture.[24] In our recent study,[14] a preliminary experiment using tobacco tissue culture revealed that 9-(2'-deoxy-β-D-ribofuranosyl)-*cis*-zeatin (2'H-**5**) was slightly less effective than **5**. In lettuce seed germination, **5** exhibited a very weak cytokinin activity at 1000 μM, the highest concentration applied, and 2'H-**5** did not show any cytokinin activity even at 1000 μM.

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