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REVIEW

STRUCTURE–ACTIVITY RELATIONSHIPS OF CYTOKININS

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Key Word Index—Cytokinins; plant growth regulator; structure–activity relationships; tissue culture; chlorophyll retention; leaf growth; purine derivatives; urea derivatives; anticytokinins.

Abstract—Structure–activity relationships of cytokinins, which regulate many aspects of plant growth, have been reviewed.

INTRODUCTION

Shortly after Miller *et al.* [1, 2] isolated kinetin (6-furfurylaminopurine, **1**) as a cell division factor in the tobacco tissue culture from an acid hydrolysate of DNA, it was found to have other physiological activities including the promotion of seed germination [3–5], of leaf and cotyledon growth [3, 6–10] and of lateral bud development [11], the inhibition of chlorophyll degradation [12, 13] and the induction of buds on moss protonema [14, 15]. Many plant physiologists and plant biochemists were curious to know what part of the chemical structure of kinetin conferred such physiological activities. A number of analogs of kinetin were, therefore, synthesized and their biological activities were evaluated by various bioassay systems. Skoog *et al.* [16] named the compounds with kinetin-like activity 'cytokinins' and many papers on the relationship between the chemical structure of cytokinins and their biological activities and on anticytokinins have been published in recent years [17–21]. This paper reviews the available data on the relationship between the chemical structure and cytokinin activity.

The structure–activity relationship of cytokinins has been investigated in detail using tissue cultures of tobacco [22, 23], soybean [9] and carrot [24, 25] as bioassay materials, because of their high sensitivity, specificity and reliability. There are many other bioassay systems, but they are not as sensitive as those mentioned above. Though such systems require less time, little analysis has been conducted on data obtained by these systems. The activities of the cytokinins determined by different biossays are approximately the same [4, 7, 26–28], though there are some exceptions [29–31]. The bioassay systems for cytokinins have been reviewed [32, 33].

6-SUBSTITUTED PURINES

Ring-substituted aminopurines

Kinetin (6-furfurylaminopurine, **1**) produces little growth of translucent, watery tissue at the lowest concentration (*ca* 0.001 μM) in the tobacco callus culture which is one of the most standardized bioassays [34]. With increase in concentration, kinetin gives somewhat better growth with the maximum fr. wt yield at *ca* 0.1 μM. Higher concentrations give flat firm masses of white parencyma with less total volume.

The furfuryl group of kinetin (**1**) can be replaced by a phenyl, benzyl, 2-phenylethyl, 2-pyridylmethyl, 3-pyridylmethyl, 4-pyridylmethyl, 2-thenyl or α-naphthylmethyl group without much loss of cytokinin activity, when tested by various bioassay systems [4, 7, 15, 27]. Among these ring-substituted aminopurines, 6-benzylaminopurine (**5**) is the most active cytokinin and is somewhat more potent (requiring a lower concentration for the same callus growth) than kinetin in the tobacco callus bioassay [34]. Compounds with a saturated ring such as 6-cyclohexylaminopurine (**8**) and 6-tetrahydrofurfurylaminopurine (**3**) are less active than those with an unsaturated ring, **4** and **1**, respectively [7, 27, 34]. Other ring substituents lowered or eliminated the activity [7, 27, 35].

In the ring-substituted adenines, either removal of the methylene group between the ring and the amino group on the 6-position of the purine ring or lengthening the bridge results in a decrease of cytokinin activity. Thus, 6-phenylaminopurine (**4**), 6-(2-phenylethylamino)purine (**6**) and 6-[2-(2-furfuryl)ethylamino]purine (**2**) are as active as or less active than 6-benzylaminopurine (**5**) or kinetin (**1**) [4, 7, 34, 35]. 6-(3-Phenylpropylamino)purine (**7**) and 6-(α-naphthylethylamino)purine (**9**) still exhibit cytokinin activity, while compounds with longer bridges show no activity [4, 7].

Substitutions with CH_3, NH_2, NO_2, OH, CH_3O and Cl on the phenyl ring of 6-benzylaminopurine (**5**) were studied in the radish leaf discs [7]. Chlorine in the *ortho*-position increased cytokinin activity; in the *meta*-position it had little effect and in the *para*-position it reduced the activity. Other substituents all lowered the activity.

Alkyl-, alkoxy- and acylaminopurines and related compounds

The cytokinin activity of 6-alkylaminopurines varies according to the length of the alkyl chain, when tested by several bioassay systems [4, 7, 27, 34, 36, 37]. Adenine (**10**)

and 6-methylaminopurine (**11**) are nearly inactive or slightly active only at high concentrations [7, 34]. The activity increases with increase in chain length to an optimum length of five or six carbon atoms. Thus, 6-pentylaminopurine (**12**) compares closely with kinetin and the activity of 6-isopentylaminopurine (**14**) is similar to that of 6-*n*-pentylaminopurine [34]. Longer alkyl chains reduce the activity and 6-*n*-decylaminopurine (**13**) is very slightly active at concentrations greater than 25 μM [34].

Most of the 6-alkoxy- and 6-alkoxyalkylaminopurines tested are weakly active or nearly inactive in the chlorophyll retention test on wheat leaves and tobacco pith culture, except that 6-(β-ethoxyethylamino) purine (**15**), 6-(β-propoxyethylamino)purine (**16**), 6-(β-butoxyethylamino)purine (**17**) and 6-(β-ethoxy-β-methylethylamino)purine (**18**) exhibit strong cytokinin activity, though they are mostly less active than kinetin (**1**) [38]. It is clear from comparisons with the activity of 6-*n*-alkylaminopurines that substitution of O for CH_2 in the side chain depresses the activity. 6-Hydroxyalkylamino- and 6-hydroxyarylalkylaminopurines tested were found to be inactive [39]. Some 6-dimethylaminoalkylamino)purines exhibit weak cytokinin activity at high concentrations of 10 or 100 μM [40]. Several N^6-alkylthiomethyl- and N^6-arylthiomethyladenosines are at least 10 times weaker than their isosteric alkyl or arylalkyl derivatives in which sulfides are replaced by methylene [41].

The cytokinin activity of 6-acylaminopurines is also strongly influenced by the length of the substituents as observed in 6-alkylaminopurines. The activity is greatest with a 5-carbon chain such as is found in 6-valerylaminopurine (**19**); shorter side chains reduce the activity [42, 43]. 6-Benzoylaminopurine (**20**) and 6-furoylaminopurine (**21**) are nearly as active as or less active than 6-benzylaminopurine (**5**) or kinetin (**1**) [40, 43, 44].

Effect of the carboxyl group in the side chain

Early investigations suggested that a carboxyl group in the side chain would reduce or eliminate cytokinin activity [27]. Addition of a carboxyl group to the methylene in the side chain of 6-benzylaminopurine (**5**) results in the much less active *N*-(purin-6-yl)-α-phenylglycine (**22**) [39]. *N*-(Purin-6-yl)-L-valine (**23**) and *N*-(purin-6-yl)-L-leucine (**24**) are inactive [39]. However, replacement of the carboxyl group in these two *N*-(purin-6-yl)amino acids by a hydroxymethyl group or esterification of the carboxyl group with a methyl group enhances the cytokinin activity [45–47]. The negative charge of the carboxyl group is considered to prevent them from entering the cell.

Effect of double bond in the side chain

Letham was the first to isolate a natural cytokinin, zeatin [6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purine] (**25b**) from immature corn kernels [48, 49]. Zeatin (*trans*-zeatin) induced cell division in cultured carrot phloem tissue at a concentration of 5×10^{-5} μM [30] and was more active than any other known cytokinin [30, 34]. The olefinic double bond in the side chain attached to N^6-adenine seems to be one of the structural features which contribute to the high cytokinin activity. In fact, *trans*-zeatin and 6-(3-methyl-2-butenylamino)purine [6-(γ,γ-dimethylallylamino)purine, N^6-(Δ^2-isopentenyl)-adenine (**26**) are more active than their corresponding saturated analogs, 6-(4-hydroxy-3-methylbutylamino)purine (dihydrozeatin, **27**) and 6-isopentylaminopurine (**14**), respectively in the tobacco callus bioassay [28, 34, 50].

Hecht *et al.* consider that side-chain planarity is important in providing the most intense cytokinin activity, especially in the tobacco callus bioassay, and that if side-chain planarity is disturbed by adding substituents to the double bond, the activity is lowered [50]. Thus, 6-(2-bromo-3-methyl-2-butenylamino)purine (**28**) has only 1/100 activity of 6-(3-methyl-2-butenylamino)purine (**26**). The location of the double bond in the 2,3-position of the side chain in 6-(3-methyl-2-butenylamino)purine (**26**) might be important and shifting it to the 3,4-position as in 6-(3-methyl-3-butenylamino)purine (**29**) reduces the activity slightly in the tobacco callus bioassay [51].

The argument that the presence of a double bond in the side chain enhances cytokinin activity may not always apply to other plants. In *Phaseolus lunatus* L. cv Kingston, *trans*-zeatin (**25b**) has 3 times more activity than dihydrozeatin [6-(4-hydroxy-3-methylbutylamino)purine] (**27**) [31], while the activities of 6-(3-methyl-2-butenylamino)purine (**26**) and 6-isopentylaminopurine (**14**) are nearly the same. However, in *P. vulgaris* cv Great Northern, 30 times more *trans*-zeatin compared to dihydrozeatin is required to obtain the same effect and 6-isopentylaminopurine is 100-fold more active than 6-(3-methyl-2-butenylamino)purine, suggesting that genotypic differences in cytokinin function and metabolism exist between different *Phaseolus* cultivars. In the increase of cell number in carrot root tissue *in vitro*, 6-(3-methyl-2-butenylamino)purine is less active than its saturated derivative, 6-isopentylaminopurine [36].

Effect of methyl group in the side chain

The methyl group(s) in the side chain of N^6-substituted adenines and its position are important for cytokinin activity. In 6-allylaminopurine (**30**), introduction of a methyl group to the 1-, 2- or 3-position of the carbon atom in the side chain does not greatly change the activity [36]. However, disubstitution with two methyl groups at the 3-position of the side chain results in highly active 6-(γ,γ-dimethylallylamino)purine [6-(3-methyl-2-butenylamino)purine] (**26**) [34, 36]. On the other hand, 6-(α,α-dimethylallylamino)purine (**31**) having two methyl groups at the 1-position in the side chain is about 100 times less active than 6-(γ,γ-dimethylallylamino)purine [34].

The shift of the methyl group from the 3- to 2-position in the side chain of *cis*- and *trans*-zeatins (**25a** and **25b**) and dihydrozeatin [6-(4-hydroxy-3-methylbutylamino)-purine] (**27**) gives 10–100 times less active *cis*- and *trans*-isozeatins [6-(4-hydroxy-2-methyl-*cis*- and *trans*-2-butenylamino)purines] (**32a** and **32b**) and dihydroisozeatin [6-(4-hydroxy-2-methylbutylamino)purine] (**33**) respectively [52]. Removing the methyl group completely from *cis*-zeatin yields *cis*-norzeatin [6-(4-hydroxy-*cis*-2-butenylamino)purine] (**34**), which is less than one-fifth as active.

Effect of hydroxy group in the side chain

The position of the hydroxy group in the side chain of N^6-substituted adenines affects the cytokinin activity. Hydroxylation at the 4-position of the side chain of 6-isopentylaminopurine (**14**) somewhat enhances activity, but that at 2-, 3- or 2,3-positions lowers the activity ten times [53]. The higher activity of 6-(3,4-dihydroxy-3-methylbutylamino)purine (**35**) compared to that of 6-(2,3-

dihydroxy-3-methylbutylamino)purine (**36**) is also due to the position effect. Thus, the hydroxy group at the 4-position in the side chain seems to be partly responsible for the high cytokinin activity of *trans*-zeatin (**25b**). Removal of the 4-hydroxy group gives the slightly less active 6-(3-methyl-2-butenylamino)purine (**26**) [34] and replacement of the hydroxy by a methyl group also reduces the activity, as observed in 6-(3-methylpent-*trans*-2-enylamino)purine (**37**) [54].

When the hydroxy group of *trans*-zeatin (**25b**) is esterified by formic, acetic, propionic and indole-3-acetic acids, this produces esters (**38–41**) which are all slightly more active than *trans*-zeatin itself [53–55]. The slight but consistent increase in activity conferred on the zeatin molecule by esterification suggests that esterification serves to stabilize the molecule rather than to confer biological activity *per se* [55]. Gradual hydrolysis of the esters probably provides a continuous supply of active *trans*-zeatin, whereas an equivalent initial supply of unmodified *trans*-zeatin might be subjected to somewhat rapid loss during the culture period [53, 55]. These investigations indicate that the hydroxy group in the side chain of *trans*-zeatin need not be free. On the other hand, *O*-β-D-glucopyranosyl-zeatin (**42**) [56] and *O*-methyl-, *O*-ethyl-, *O*-propyl- and *O*-butylzeatins (**43–46**) [54] are all at least 10 times less active than *trans*-zeatin and in *O*-alkylzeatins the activity decreases with increasing size of the substituent [54].

Geometrical and optical isomers

Cytokinin activity is dependent on side chain configuration. The *trans*-forms of zeatin (**25b**), isozeatin (**32b**), 2-methylthio-zeatin (**74b**) and 6-(3-chloro-2-butenylamino)purine (**47b**) are more active than the corresponding *cis*-forms (**25a**, **32a**, **74a**, **47a**) [50, 52, 57, 58]. Hecht *et al.* [50] explained that 6-(3-chloro-*trans*-2-butenylamino)purine (**47b**) is more active than the *cis*-isomer because the chloro group in the former disturbs side-chain planarity less than that in the *cis*-form.

The naturally occurring (*S*)-(−)-dihydrozeatin [(*S*)-(−)-6-(4-hydroxy-3-methylbutylamino)purine] (**27b**) is 10 times less active than the synthetic (*R*)-(+)-isomer (**27a**) [59]. Such configurational differences in cytokinin activity are observed also in several pairs of synthetic N^6-(optically active alkyl and arylalkyl substituted) adenines [45, 46] and in those of methyl esters of *N*-(purin-6-yl)-amino acids [47]. The absolute configuration around the asymmetric carbon may contribute to the interaction between the cytokinin molecule and its receptor site.

6-*Ureidopurines*

N-(9-β-D-Ribofuranosylpurin-6-ylcarbamoyl)-L-threonine (**98**) has been shown to be present in some yeast and *Escherichia coli* *t*RNAs [60, 61]. This naturally occurring ureidopurine was, therefore, a logical candidate for study of its growth-promoting activity, but it was quite inactive in the tobacco callus bioassay and the soybean callus bioassay [62–64]. The negative charge of the carboxyl group in **98** may prevent it from penetrating the cell membrane. The lipophilic 6-isopentylureidopurine (**48**) and 6-allylureidopurine (**49**) exhibit slight cytokinin activity at high concentrations [63–65]. Some other ureidopurine derivatives tested are nearly inactive or are below 10% of the activity of 6-benzylaminopurine (**5**) [64, 65]. On the basis of length alone, the ureido group, contributing an additional NH–CO moiety, would be expected to reduce the activity. 6-Phenylureidopurine (**50**) actually averaged 8% of the activity of 6-benzylaminopurine [65].

Eighty-six analogs of 6-ureidopurines and their ribonucleosides were examined for cytokinin activity in soybean callus bioassay and also for the growth-inhibitory activity in cultured normal and leukemic human cells and revealed no direct relationship between the two types of activities [66]. 6-Allylureidopurine (**49**) and several other ureidopurines exhibited some cytokinin activity, but they showed only marginal or no activity for mammalian cell lines. 6-Isoamylureidopurine (**48**) showed both types of activity [66]. On the other hand, 6-benzylureidopurine (**51**) and some ureidopurines are inhibitory on mammalian tissue cultures and have no cytokinin activity.

Role of the amino group attached to the 6-position of the purine ring

The influence of an amino group attached to the 6-position of the purine ring on cytokinin activity has been studied by using various bioassay systems [4, 7, 15, 27, 34, 67]. Replacement of the amino group of kinetin (**1**) by a sulfur atom as in 6-furfurylthiopurine (**52**) results in a drastic reduction in activity [7, 27]. 6-Alkylthio-, 6-arylalkylthio-, 6-alkyloxy- and 6-arylalkyloxypurines, depending on the length of the side chain, are less active than the corresponding 6-alkylamino- or 6-arylalkylaminopurines [4, 68]. Studies on different connecting links (NH, S, O, CH_2) between the purine ring and isopentenyl or benzyl groups indicates that the biological activity decreases in the order: 6-(3-methyl-2-butenylamino)purine (**26**) > 6-(4-methyl-3-pentenyl)purine (**53**) > 6-(3-methyl-2-butenylthio)purine (**54**) ≫ 6-(3-methyl-2-butenyloxy)purine (**55**) and in the order: 6-benzylaminopurine (**5**) > 6-(2-phenylethyl) purine (**56**) = 6-benzyloxypurine (**57**) ≫ 6-benzylthiopurine (**58**) [67]. Thus, the amino group attached to the 6-position of the purine ring is important for high cytokinin activity in 6-substituted aminopurines.

POSITION EFFECT OF SUBSTITUENTS IN PURINE RING

Substitution at 1-position of the purine ring

1-Benzyladenine (**62**) and 1-(γ,γ-dimethylallyl)adenine (**63**), when filter-sterilized, have weak cytokinin activity in 0.1–12.5 μM range in the tobacco callus bioassay [29, 34]. However, activity is developed after autoclaving probably by conversion to their corresponding active 6-isomers. Mass spectrometric study revealed that the N atoms in the 1- and N^6-positions were interchanged by rotation of the C_5–C_6 axis in a process involving ring opening and reclosure between positions 1 and 2 [69]. 1-Benzyl- and 1-(γ,γ-dimethylallyl)purines (**64**, **65**) which have no amino group at the 6-position are, of course, inactive [34]. 1,7-Dibenzyladenine (**69**) and 1,9-dibenzyladenine (**70**) are active when autoclaved, but when they are filter-sterilized, only high concentrations have appreciable activity [34]. 6-Benzylamino-1-methylpurine (**66**) and 1-benzyl-6-methylaminopurine (**67**) are slightly active at 12.5 μM. 1-Benzyl-6-benzylaminopurine (**68**) shows a trace of activity at concentrations over 0.5 μM and this may be due to loss of the 1-benzyl group in the course of the bioassay [34]. These

Table 1. Purine derivatives and their cytokinin activity

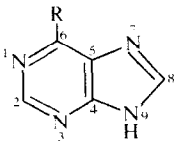
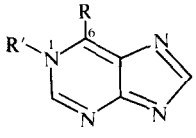
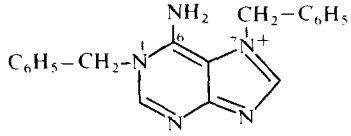
	Compounds	Bioassay	Min. concn (μM)*	Opt. concn (μM)†	Ref.
	R				
1	$NHCH_2$—FUR	T	0.001	0.1	[34]
		CAR	0.005	0.5	[32]
		S	0.02	50	[9]
		L	0.02	5	[7]
		COT	0.05	100	[10]
		W-CHL	0.1	10	[28]
		GER	1	100	[28]
2	$NHCH_2CH_2$—FUR	T	0.02	0.5	[34]
3	$NHCH_2$—TFUR	L	0.1	10	[7]
4	NH—C_6H_5	T	0.004	0.5	[34]
		L	0.03	5	[7]
5	$NHCH_2$—C_6H_5	T	0.0008	0.07	[34]
		L	0.02	5	[7]
6	$NHCH_2CH_2$—C_6H_5	L	0.02	10	[7]
7	$NH(CH_2)_3$—C_6H_5	L	0.2	50	[7]
8	NH—C_6H_{11}	T	0.5	12.5	[34]
9	$NHCH_2CH_2$—NAPH	L	0.02	—	[7]
10	NH_2	T	200	∝	[34]
		L	10	∝	[7]
11	NHMe	T	100	∝	[34]
		L	5	100	[7]
12	$NH(CH_2)_4Me$	T	0.0008	0.1	[34]
		L	0.03	10	[7]
13	$NH(CH_2)_9Me$	T	25	∝	[34]
14	$NHCH_2CH_2CH(Me)_2$	T	0.004	0.1	[34]
15	$NHCH_2CH_2OCH_2Me$	W-CHL	0.5	>100	[38]
		TP	0.1	1	[38]
16	$NHCH_2CH_2OCH_2CH_2Me$	W-CHL	0.5	10	[38]
		TP	0.1	1	[38]
17	$NHCH_2CH_2O(CH_2)_3Me$	W-CHL	5	>100	[38]
		TP	1	5	[38]
18	$NHCH_2CH(Me)OCH_2Me$	W-CHL	1	>100	[38]
		TP	1	1	[38]
19	$NHCO(CH_2)_3Me$	T	0.01	5	[43]
		S	—	10	[42]
20	NHCO—C_6H_5	S	0.005	0.5	[43]
21	NHCO—FUR	TP		10	[40]
22	NHCH(COOH)—C_6H_5	COT	5	—	[39]
23	$NHCH(COOH)CH(Me)_2$	COT	inactive		[39]
24	$NHCH(COOH)CH_2CH(Me)_2$	COT	inactive		[39]
25a	$NHCH_2CH^{E}C(Me)CH_2OH$	T	0.03	0.5	[57,52]
b	$NHCH_2CH^{Z}C(Me)CH_2OH$	T	0.0001 (0.00003–0.0005)	0.004–0.05	[34]
		CAR	0.00005	0.001	[30]
26	$NHCH_2CH{:}C(Me)_2$	T	0.0001 (0.00003–0.0008)	0.02	[34]
27a	$NHCH_2CH_2\overset{R}{C}H(Me)CH_2OH$	T	0.001	0.04	[59]
b	$NHCH_2CH_2\overset{S}{C}H(Me)CH_2OH$	T	0.01	0.4	[59]
28	$NHCH_2CBr{:}C(Me)_2$	T	0.03	0.3	[50]
29	$NHCH_2CH_2C(Me){:}CH_2$	T	0.0011	0.039	[51]
30	$NHCH_2CH{:}CH_2$	T	0.05	5	[34]
31	$NHC(Me)_2CH{:}CH_2$	T	0.004–0.2	0.5	[34]
32a	$NHCH_2C(Me)^{E}{:}CHCH_2OH$	T	0.05	1	[52]
b	$NHCH_2C(Me)^{Z}{:}CHCH_2OH$	T	0.03	0.5	[52]
33	$NHCH_2CH(Me)CH_2CH_2OH$	T	0.05	1	[52]
34	$NHCH_2CH^{E}{:}CHCH_2OH$	T	0.05	1	[52]
35	$NHCH_2CH_2C(OH)(Me)CH_2OH$	T	0.03	2	[53]

Table 1. (*Continued*)

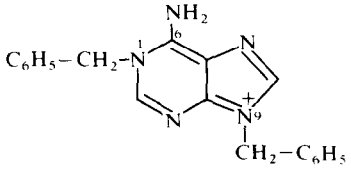
	Compounds	Bioassay	Min. concn (μM)*	Opt. concn (μM)†	Ref.
36	$NHCH_2CH(OH)C(OH)Me_2$	T	0.1	5	[53]
37	$NHCH_2CH{:}C(Me)CH_2Me$	COT	1	>10	[54]
38	$NHCH_2CH{:}C(Me)CH_2O-CHO$	T	0.0001	0.005	[55]
39	$NHCH_2CH{:}C(Me)CH_2O-COMe$	T	0.0001	0.02	[55]
40	$NHCH_2CH{:}C(Me)CH_2O-COCH_2Me$	T	0.0001	0.005	[55]
41	$NHCH_2CH{:}C(Me)CH_2O-COCH_2-IN$	T	0.0001	0.005	[55]
42	$NHCH_2CH{:}C(Me)CH_2O-GLU$	S	1	100	[56]
43	$NHCH_2CH{:}C(Me)CH_2O-Me$	COT	1	>10	[54]
44	$NHCH_2CH{:}C(Me)CH_2O-CH_2Me$	COT	1	>10	[54]
45	$NHCH_2CH{:}C(Me)CH_2O-CH_2CH_2Me$	COT	1	>10	[54]
46	$NHCH_2CH{:}C(Me)CH_2O-(CH_2)_3Me$	COT	10	—	[54]
47a	$NHCH_2CH{:}C(Cl)Me$	T	0.001	0.05	[50]
b	$NHCH_2CH{:}C(Cl)Me$	T	0.0003	0.05	[50]
48	$NHCONHCH_2CH_2CH(Me)_2$	S	2	10	[63]
49	$NHCONHCH_2CH{:}CH_2$	T	0.5	5	[65]
		S	2	50	[63]
50	$NHCONH-C_6H_5$	T	0.02	0.5	[65]
51	$NHCONHCH_2-C_6H_5$	S	inactive		[66]
52	SCH_2-FUR	L	10	∞	[7]
53	$CH_2CH_2CH{:}C(Me)_2$	T	0.01	0.5	[67]
54	$SCH_2CH{:}C(Me)_2$	T	0.5	5	[67]
55	$OCH_2CH{:}C(Me)_2$	T	5	∞	[67]
56	$CH_2CH_2-C_6H_5$	T	0.01	0.1	[67]
57	$OCH_2-C_6H_5$	T	0.01	0.1	[67]
58	$SCH_2-C_6H_5$	T	1	3	[67]
59	$N(CH_2CH_2Me)_2$	GER	—	—	[4]
60	$N(CH_2CH_2CH_2Me)_2$	GER	—	—	[4]
61	$N(CH_2CH_2CH_2CH_2Me)_2$	GER	—	—	[4]



	R	R′				
62	NH_2	$CH_2-C_6H_5$	T	0.1–1	2.5	[34]
63	NH_2	$CH_2CH{:}C(Me)_2$	T	0.5	2.5	[29]
64	H	$CH_2-C_6H_5$	T	inactive		[34]
65	H	$CH_2CH{:}C(Me)_2$	T	inactive		[34]
66	$NHCH_2-C_6H_5$	Me	T	12.5	∞	[34]
67	NHMe	$CH_2-C_6H_5$	T	12.5	∞	[34]
68	$NHCH_2-C_6H_5$	$CH_2-C_6H_5$	T	>0.5	12.5	[34]



69 T 0.5 ∞ [34]



70 T 2.5 ∞ [34]

Table 1. (*Continued*)

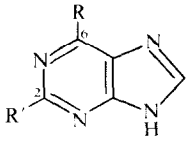
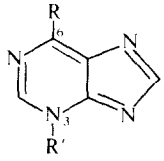
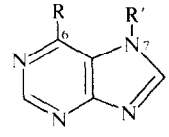
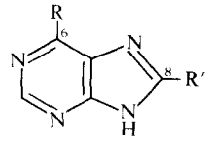
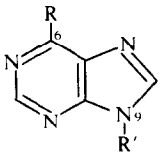
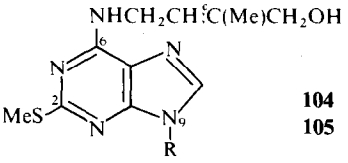
	Compounds	Bioassay	Min. concn (μM)*	Opt. concn (μM)†	Ref.
					
	R / R′				
71	NH_2 / $CH_2-C_6H_5$	T	inactive		[34]
72	H / $NHCH_2-FUR$	T	inactive		[34]
73	$NHCH_2CH{:}C(Me)_2$ / SMe	T	0.005	0.1	[71]
		W-CHL	0.1	10	[73]
74a	$NHCH_2CH{:}C(Me)CH_2OH$ / SMe	T	0.005	0.3	[58]
b	$NHCH_2CH{:}C(Me)CH_2OH$ / SMe	T	0.001	0.1	[70]
75	$NHCH_2CH{:}C(Me)CH_2OH$ / Cl	T	0.0001	0.01	[70, 71]
76	$NHCH_2CH_2CH(Me)CH_2OH$ / SMe	W-CHL	1	100	[73]
					
	R / R′				
77	NH_2 / $CH_2CH{:}C(Me)_2$	T	inactive		[76]
		T	0.5–1	2.5	[34]
78	NH_2 / $CH_2-C_6H_5$	T	0.1	∝	[34]
79	NH_2 / CH_2-FUR	T	inactive		[34]
80	$NHCH_2-C_6H_5$ / Me	T	0.1	∝	[79]
81	$NHCH_2-FUR$ / Me	T	0.1	>10	[79]
82	$NHCH_2CH{:}C(Me)_2$ / Me	T	0.01	1	[79]
83	$NHCH_2CH{:}C(Me)CH_2OH$ / Me	CAR	—	—	[80]
84	$NHCH_2-C_6H_5$ / $CH_2-C_6H_5$	T	12.5	25	[34]
85	$NHCH_2CH{:}C(Me)_2$ / $CH_2CH_2CH(NH_2)COOH$	T	0.05	10	[81]
					
	R / R′				
86	NH_2 / $CH_2CH{:}C(Me)_2$	T	5	∝	[34]
87	$NHCH_2-C_6H_5$ / $CH_2-C_6H_5$	T	0.5	12.5	[34]
88	$NHCH_2-C_6H_5$ / GLU	T	0.5	>10	[82]
89	$NHCH_2CH{:}C(Me)CH_2OH$ / Me	CAR	—	—	[80]
					
	R / R′				
90	NH_2 / $CH_2CH{:}C(Me)_2$	T	inactive		[74]
91	NH_2 / Br	T	5	∝	[83]
92	$NHCH_2-FUR$ / Me	B-CHL	—	—	[84]
93	$NHCH_2-C_6H_5$ / Me	B-CHL	—	—	[84]
94	$NHCH_2CH{:}C(Me)_2$ / Me	T	0.0005	0.01	[71]

Table 1 (*Continued*)

	Compounds	Bioassay	Min. concn (μM)*	Opt. concn (μM)†	Ref.
					
	R R′				
95	NH_2 $CH_2-C_6H_5$	—	—	—	[78]
96	NH_2 $CH_2CH{:}C(Me)_2$	T	2.5–12.5	∞	[34]
97	$NHCH_2-C_6H_5$ $(CH_2)_4Me$	T	0.01	0.1	[92]
98	NHCONHCH(COOH)-CH(OH)Me β-RIB	T	inactive		[62]
		S	inactive		[63]
99	$NHCH_2-C_6H_5$ β-RIB	T	0.02	1	[83]
		B	0.3	—	[94]
100a	$NHCH_2CH{:}C(Me)CH_2OH$ β-RIB	T	0.03	0.3	[52]
		B	inactive		[95]
b	$NHCH_2CH{:}C(Me)CH_2OH$ β-RIB	T	0.003	0.3	[52]
		B	inactive		[94, 95]
101	$NHCH_2CH{:}C(Me)_2$ β-RIB	T	0.01	0.5	[52, 34]
		B	0.3	—	[94]
102	$NHCH_2CH{:}C(Me)_2$ 2′-DRIB	T	0.02	2	[19]
103	$NHCH_2CH{:}C(Me)_2$ 5′-DRIB	T	0.02	1	[19]
					
	R				
104	α-RIB	T	5	∞	[58]
105	β-RIB	T	0.01	1	[58]

* Minimum concentration for detectable response.

† Optimum concentration.

Ref.: Reference, FUR: 2-furyl, TFUR: 2-tetrahydrofuryl, NAPH: α-naphthyl, IN: 3-indol, GLU: β-D-glucopyranosyl, RIB: D-ribofuranosyl, DRIB: deoxy-β-D-ribofuranosyl, T: tobacco callus growth, TP: tobacco pith growth, CAR: carrot tissue growth, S: soybean callus growth, L: radish leaf growth, COT: radish cotyledon growth, B-CHL: chlorophyll retention in barley leaf, W-CHL: chlorophyll retention in wheaf leaf, GER: lettuce seed germination, B: bud formation on moss protonema.

results suggest that the reactive 1-position must be free to make the substance active.

Substitution at 2-position of the purine ring

2-Benzyladenine (**71**) and 2-(2-furfurylamino)purine (**72**) are inactive in the tobacco tissue culture [34]. The effect of second substitution at the 2-position of N^6-substituted adenines varies depending on substituents. In the tobacco callus bioassay, 2-substitutions with hydroxy, mercapto, methylsulfonyl and benzylthio groups in *trans*-zeatin (**25b**) or 6-(3-methyl-2-butenylamino)purine (**26**) greatly lower the activity, while amino, methylthio and methyl groups have a smaller effect on activity [70, 71]. 6-(3-Methyl-2-butenylamino)-2-methylthiopurine (**73**), 2-methylthio-*cis*-zeatin (**74a**) and 2-methylthio-*trans*-zeatin (**74b**) are slightly less active than their corresponding unsubstituted analogs, **26**, **25a** and **25b** [58, 70–72]. A 2-chloro substituent has a negligible effect on *trans*-zeatin and 6-(3-methyl-2-butenylamino)purine, and 2-chloro-*trans*-zeatin (**75**) is possibly even more active than *trans*-zeatin itself [70, 71].

On the other hand, a chlorophyll retention test with wheat leaf segments revealed that 2-methylthio-6-(3-methyl-2-butenylamino)purine (**73**) and 6-(4-hydroxy-3-methylbutylamino)-2-methylthiopurine (**76**) are more active than unsubstituted analogs, **26** and **27**, respectively [73].

Substitution at 3-position of the purine ring

3-(γ,γ-Dimethylallyl)adenine (triacanthine) (**77**), a naturally occurring adenine derivative, has been reported to possess cytokinin activity in the tobacco pith culture [74]. Later, it was found that triacanthine has no growth-promoting activity, when sterilized by filtration

instead of autoclaving [75, 76]. Triacanthine is readily converted to an active form during autoclaving, possibly to the 6-isomer and promotes the growth of tobacco tissue [76]. 3-Benzyladenine (**78**), 3-furfuryladenine (**79**) and several other 3-substituted adenines are themselves inactive [34, 77]. Autoclaving 3-benzyladenine (**78**) produces N^6-benzyladenine (**5**) and 9-benzyladenine (**95**), although in very low yields [78].

A second substituent at the 3-position of N^6-substituted adenines gives inconsistent results. 6-Benzylamino-3-methylpurine (**80**) and 6-furfurylamino-3-methylpurine (**81**) are far less active than 6-benzylaminopurine (**5**) and kinetin (**1**) respectively, while 3-methyl-6-(3-methyl-2-butenylamino)purine (**82**) is a little less active than the unsubstituted analog, **26** [79]. 3-Methylzeatin (**83**) is slightly more active than zeatin (**25b**) itself [80]. 3,N^6-Dibenzyladenine (**84**) is far less active than N^6-benzyladenine [34].

Nomura *et al.* [81] isolated 3-(3-amino-3-carboxypropyl)-6-(3-methyl-2-butenylamino)purine (**85**) from the cellular slime mold, *Dictyostelium discoideum* and named it discadenine. Discadenine is about 10 times less active than kinetin (**1**) and 100 times less active than 6-(3-methyl-2-butenylamino)purine (**26**) in the tobacco callus bioassay.

N^6-*Disubstituted adenines*

N^6-Disubstituted adenines such as N^6-dipropyladenine (**59**), N^6-dibutyladenine (**60**) and N^6-dipentyladenine (**61**) are generally less active than their corresponding N^6-monosubstituted adenines, when tested by lettuce seed germination [4] and by bud formation on moss protonema [15]. Addition of a methyl group to the N^6-position of kinetin (**1**) and 6-benzylaminopurine (**5**) causes at least a 100-fold loss in activity in the tobacco callus bioassay [34].

Substitution at 7-position of the purine ring

7-Substituted adenines are nearly inactive [34, 74]. A highly purified sample of 7-(γ,γ-dimethylallyl)adenine (**86**) gives only a trace of activity at high concentration of 5 μM in the tobacco callus bioassay [34]. Substituents at the 7-position of N^6-substituted adenines often lower but do not eliminate the activity. N^6,7-Dibenzyladenine (**87**) is active even at 0.02 μM in the tobacco callus bioassay, when autoclaved with the nutrient medium, but is not so active when not exposed to heat [34]. The activation is presumably due to the removal of the substituent at 7-position. 6-Benzylamino-7-β-D-glucopyranosylpurine (**88**) has less than 1% of the activity of its unsubstituted counterpart, **5** [82]. It is rather exceptional that 7-methylzeatin (**89**) is nearly as active as *trans*-zeatin (**25b**) in the carrot callus bioassay [80].

Substitution at 8-position of the purine ring

8-Substituted adenines such as 8-(γ,γ-dimethylallyl)adenine (**90**) and 8-bromoadenine (**91**) are inactive or slightly active in the tobacco tissue culture [74, 83]. The effect of substitution at the 8-position of N^6-substituted adenines on the cytokinin activity depends on the type of substituents. 8-Methylkinetin (**92**) and 6-benzylamino-8-methylpurine (**93**) show enhanced activity over the unsubstituted compounds, kinetin (**1**) and 6-benzylaminopurine (**5**), respectively, in the chlorophyll retention bioassay with barley [84] and 8-methyl-6-(3-methyl-2-butenylamino)-purine (**94**) is also more active than its unsubstituted parent compound, **26**, in the tobacco callus bioassay [71]. A chloro substituent in the 8-position of 6-(3-methyl-2-butenylamino)purine (**26**) does not alter the activity. On the other hand, substitutions with methylthio, methylsulfonyl, benzylthio and mercapto groups in the 8-position of 6-(3-methyl-2-butenylamino)purine decrease the activity and generally the 2,8-disubstituted derivatives are definitely less active than the 2- or 8-substituted derivatives [71]. Substitution of the 8-position of *trans*-zeatin (**25b**) and 6-(3-methyl-2-butenylamino)purine (**26**) with a hydroxy group causes 1/10 activity and dihydroxylation of the 2 and 8 positions of **25b** and **26** causes 1/100–1/1000 activity [85].

Substitution at 9-position of the purine ring

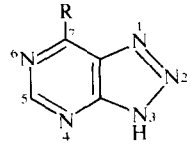
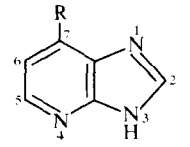
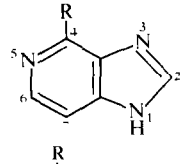
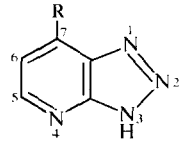
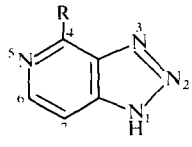
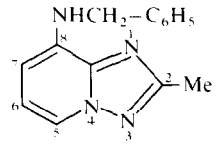
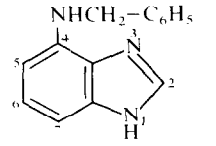
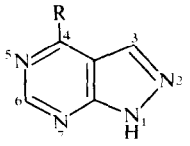
9-(γ,γ-Dimethylallyl)adenine (**96**) is activated by heating, possibly by conversion to N^6-substituted adenine [34]. A second substitution in the 9-position of N^6-substituted adenines generally lowers but does not eliminate the cytokinin activity. Methyl, methoxymethyl, propyl, butyl, benzyl, cyclohexyl, tetrahydropyran-2-yl and β-D-glucopyranosyl groups as substituents at the 9-position of 6-benzylaminopurine (**5**), *trans*-zeatin (**25b**) or 6-(3-methyl-2-butenylamino)purine (**26**) lower the activity [34, 80, 82, 86–91]. In 9-alkyl-6-benzylaminopurines the cytokinin activity depends on the length of the 9-substituents, the strongest activity being exhibited by 6-benzylamino-9-propylpurine (**97**) in the tobacco callus bioassay [92].

The 9-β-D-ribofuranosides of kinetin (**1**), 6-(3-methyl-2-butenylamino)purine (**26**), *cis*- and *trans*-zeatins (**25a** and **25b**), (*R*)-(+)-6-(4-hydroxy-3-methylbutylamino)purine (**27a**), its (*S*)-(−)-isomer (**27b**) and 6-isopentylaminopurine (**14**) are all less active than their corresponding bases [34, 51–53, 59, 72, 93]. In protonema of the moss *Funaria hygrometrica*, bud formation is induced by 6-benzylaminopurine (**5**), *cis*- and *trans*-zeatins (**25a** and **25b**) and 6-(3-methyl-2-butenylamino)purine (**26**); however, this moss protonema is almost insensitive or only slightly responsive to their ribosides (**99**–**101**) [94, 95]. These results suggest that cytokinins exogenously supplied can function without ribosidation at 9-position.

The 9-β-D-ribofuranosides of certain N^6-substituted adenines have been found to influence the cell division and viability of mammalian cells *in vitro*. The cell division of human myelogenous leukemia *in vitro* is inhibited by N^6-(Δ^2-isopentenyl)adenosine [6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine] (**101**), but its free base, N^6-(Δ^2-isopentenyl)adenine (**26**), has no effect on the cells [96]. Certain N^6-substituted adenosines show an inhibitory effect on cell cultures of Sarcoma 180 [97], leukemic myeloblast, Burkitt's lymphoma, leukemic lymphoblast [98] and mouse leukemia [99]. The inhibitory activity of these compounds on mammalian cells is thought to be caused by the presence of the sugar moiety [96, 98, 100].

Ribonucleotides and cyclic ribonucleotides of cytokinins are mostly less active than their corresponding bases and ribonucleosides. The cytokinin activities of several ribonucleotides and cyclic ribonucleotides possessing N^6,$O^{2'}$-dibutyryl, 8-bromo, N^6-Δ^2-isopentenyl and N^6-benzyl groups as substituents were compared with those of the corresponding nucleosides and bases in the tobacco callus bioassay [83]. In each of these series the cytokinin

Table 2. Modified purine derivatives and their cytokinin activity

	Compounds	BA	Min. concn (μM)*	Opt. concn (μM)†	Ref.
	R				
	106 $NHCH_2$—FUR	T	0.1	2	[34]
	107 $NHCH_2$—C_6H_5	T	0.02	0.5	[34]
	108 $NHCH_2CH{:}C(Me)_2$	T	—	—	[35]
					
	109 $NHCH_2$—FUR	T	0.05	0.2	[101]
	110 $NHCH_2CH{:}C(Me)_2$	T	0.001	0.02	[101]
	111 NHCO—C_6H_5	T	0.0001	0.01	[104]
	112 $NHCO(CH_2)_3Me$	T	0.004	0.04	[104]
					
	113 $NHCH_2$—FUR	T	5	∝	[101]
	114 $NHCH_2CH{:}C(Me)_2$	T	1	20	[101]
	115 NHCO—C_6H_5	T	0.02	0.1	[104]
	116 $NHCO(CH_2)_3Me$	T	1	10	[104]
					
	117 $NHCH_2$—FUR	T	0.05	1	[101]
	118 $NHCH_2CH{:}C(Me)_2$	T	0.003	0.02	[101]
					
	119 $NHCH_2$—FUR	T	1	5	[101]
	120 $NHCH_2CH{:}C(Me)_2$	T	0.05	1	[101]
121 	$NHCH_2$—C_6H_5	T	5	50	[106]
122 	$NHCH_2$—C_6H_5	T	5	50	[106]
123 	$NHCH_2$—C_6H_5	T	5	50	[106]
	R				
	124 $NHCH_2$—FUR	GER	inactive		[26]
		T	inactive		[27]
	125 $NHCH_2CH{:}C(Me)_2$	T	0.1	2	[19]

* Minimum concentration for detectable response.

† Optimum concentration.

BA: Bioassay, Ref.: Reference. FUR: 2-furyl, T: tobacco callus growth, GER: lettuce seed germination.

activity was in the decreasing order of bases ≫ nucleosides ≥ nucleotides > cyclic-nucleotides.

Modification of the ribose moiety in N^6-substituted adenosines affects the activity. 6-(4-Hydroxy-3-methyl-*cis*-2-butenylamino)-2-methylthio-9-α-D-ribofuranosylpurine (**104**) is less active than the corresponding 9-β-D-ribofuranosylpurine (**105**) [58]. The presence of the hydroxy group in the sugar moiety appears to be less crucial, for example, 9-(2'-deoxy-β-D-ribofuranosyl)-6-(3-methyl-2-butenylamino)purine (**102**) and its corresponding 5'-deoxy analog (**103**) have the same level of cytokinin activity as 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (**101**) [91]. This suggests that these compounds are converted to a common species, e.g. 6-(3-methyl-2-butenylamino)purine (**26**) by metabolic cleavage of the sugars.

Thus, investigations on the position effects presented above lead to the conclusion that only N^6-substituents confer cytokinin activity on adenine and that other derivatives (1-, 2-, 3-, 7-, 8- and 9-monosubstituted adenines) are nearly inactive or slightly active whereas 1-, 3- and 9-substituted adenines could be activated by conversion to the N^6-isomers [34]. On the other hand, the presence of a second substituent in N^6-substituted adenines mostly decreases the cytokinin activity except for certain 2-Cl and 8-methyl derivatives.

MODIFICATION OF THE PURINE RING

Aza and deaza purine derivatives

Early investigators reached the conclusion that highly active cytokinins are limited to N^6-monosubstituted adenines and that substitution of one atom for another in the purine ring, depending on the position, drastically lowers the cytokinin activity by 95% or more [7, 27, 34]. For example, the replacement of the 8-carbon atom by a nitrogen atom in the purine ring of kinetin (**1**), 6-benzylaminopurine (**5**) and 6-(3-methyl-2-butenylamino)purine (**26**) yields 8-aza-kinetin (**106**), 6-benzylamino-8-azapurine (**107**) and 6-(3-methyl-2-butenylamino)-8-azapurine (**108**), respectively, which have less than 10% the activity of the corresponding purine derivatives in the tobacco callus bioassay [34, 35].

1-Deaza-, 3-deaza-, 8-aza-1-deaza- and 8-aza-3-deaza-analogs of kinetin (**1**) and 6-(3-methyl-2-butenylamino)purine (**26**) were tested for their cytokinin activity in the tobacco callus bioassay [101, 102]. Replacement of nitrogen by carbon in the 1-position of the purine ring of kinetin and 6-(3-methyl-2-butenylamino)-purine gives the 15-fold less active 6-furfurylamino-1-deazapurine {7-furfurylaminoimidazo[4,5-*b*]pyridine} (**109**) and the 2-fold less active 6-(3-methyl-2-butenylamino)-1-deazapurine {7-(3-methyl-2-butenylaminoimidazo[4,5-*b*]pyridine} (**110**), respectively. 3-Deaza-analogs, 6-furfurylamino-3-deazapurine {4-furfurylaminoimidazo[4,5-*c*]pyridine} (**113**) and 6-(3-methyl-2-butenylamino)-3-deazapurine {4-(3-methyl-2-butenylamino)imidazo[4,5-*c*]pyridine (**114**), are 2000- and 1000-fold less active than the corresponding parent compounds, **1** and **26**, respectively. 8-Aza-1-deaza-analogs (**117**, **118**) are nearly as active as 1-deaza-analogs (**109**, **110**), but 8-aza-3-deaza-analogs (**119**, **120**) are more active than 3-deaza-analogs (**113**, **114**) respectively. In 6-acylaminodeazapurines too, 1-deazapurine derivatives exhibit high cytokinin activity [103, 104]. 6-Benzoylamino-1-deazapurine {7-benzoylaminoimidazo[4,5-*b*]-pyridine} (**111**) and 6-pentanoylamino-1-deazapurine {7-pentanoylaminoimidazo[4,5-*b*]pyridine} (**112**) are surprisingly more active than the corresponding purine analogs,6-benzoylaminopurine (**20**) and 6-pentanoylaminopurine (**19**), respectively, while their 3-deazapurine analogs (**115**, **116**) are less active than the corresponding purine analogs. 6-Benzoylamino-1-deazapurine (**111**) is as active as *trans*-zeatin (**25b**), one of the most active cytokinins [104]. This indicates that highly active compounds are not limited to adenylated cytokinins.

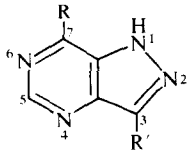
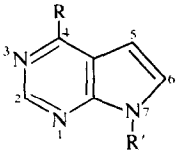
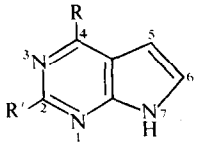
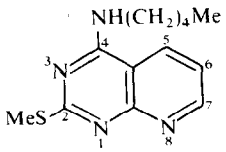
Compounds in which the purine ring is more drastically modified, such as azaindene, azanaphthalene, naphthalene and indole derivatives, are almost inactive except that 8-benzylamino-2-methyl-*s*-triazolo[1,5-*a*]pyrazine (**121**), 8-benzylamino-2-methyl-*s*-triazolo[1,5-*a*]pyridine (**122**) and 4(7)-benzylaminobenzimidazole (**123**) exhibit cytokinin activity at higher than 5 μM [105–107].

If the 8-carbon and the 7-nitrogen in kinetin (**1**) are interchanged, this completely eliminates cytokinin activity as observed in 4-furfurylaminopyrazolo[3,4-*d*]pyrimidine (**124**) [26, 27]. The same change in 6-(3-methyl-2-butenylamino)purine (**26**) yields 6-(3-methyl-2-butenylamino)-pyrazolo[3,4-*d*]pyrimidine (**125**) [19, 35], which is 300 times less active.

The exchange of the 8-carbon and the 9-nitrogen of 6-(3-methyl-2-butenylamino)purine (**26**) and 6-isopentylaminopurine (**14**) yields 7-(3-methyl-2-butenylamino)-pyrazolo[4,3-*d*]pyrimidine (**126**) and 7-isopentylamino-pyrazolo[4,3-*d*]pyrimidine (**127**), respectively, which are 300 times less active in the tobacco callus bioassay [108]. Hecht *et al.* [17] studied the development of anticytokinin activity in 7-substituted-pyrazolo[4,3-*d*]pyrimidines. Methylation at the 3-position of 7-(3-methyl-2-butenylamino)pyrazolo[4,3-*d*]pyrimidine (**126**) additionally lowers the cytokinin activity as observed in 3-methyl-7-(3-methyl-2-butenylamino)pyrazolo[4,3-*d*]-pyrimidine (**128**). Saturation of the double bond in the side chain of 3-methyl-7-(3-methyl-2-butenylamino)-pyrazolo[4,3-*d*]pyrimidine (**128**) yields 7-isopentyl-amino-3-methylpyrazolo[4,3-*d*]pyrimidine (**129**) which in fact is a potent inhibitor of cytokinin activity. Such structural modifications may thus remove weak cytokinin activity and produce inhibitory activity. 7-Isopentylamino-3-methylpyrazolo[4,3-*d*]pyrimidine (**129**) completely inhibits the growth of tobacco callus supplied with optimal concentrations of either 6-(3-methyl-2-butenylamino)purine (**26**) or 6-benzylaminopurine (**5**), when added to the tissue in an amount 100–200 times that of the cytokinins [17]. A higher concentration of the cytokinin has a restorative effect on growth, while a higher concentration of the inhibitor causes a further reduction in growth. Thus, the inhibitor is not merely toxic to the plant tissue but competes with the cytokinin as antagonist. Moreover, the growth-inhibiting effect of the antagonist **129** is surprisingly counteracted by *N*,*N'*-diphenylurea (**155**) or by non-adenylated cytokinin (see later), if used at high concentrations [18].

In 7-substituted-3-methylpyrazolo[4,3-*d*]pyrimidines, an N^7-substituent from four to seven carbon atoms in length is required for high anticytokinin activity, as in 7-hexylamino-3-methylpyrazolo[4,3-*d*]pyrimidine (**130**) and 3-methyl-7-pentylaminopyrazolo[4,3-*d*]pyrimidine (**131**) [18]. Linear substituents as in **131** and 7-butylamino-3-methylpyrazolo[4,3-*d*]pyrimidine (**132**) confer more antagonistic activity than do the

Table 3. Derivatives of pyrazolo[4,3-*d*]pyrimidine, pyrrolo[2,3-*d*]pyrimidine and pyrido[2,3-*d*]pyrimidine and their cytokinin activity and inhibitory activity

Compounds			BA	Cytokinin activity: Min. concn (μM)†	Cytokinin activity: Opt. concn (μM)‡	Inhibitory activity*: Min. concn (μM)†	Inhibitory activity*: Concn (μM) for CI	Ref.
								
	R	R′						
126	$NHCH_2CH{:}C(Me)_2$	H	T	0.1	2	—	—	[108]
127	$NHCH_2CH_2CH(Me)_2$	H	T	1	20	—	—	[108]
128	$NHCH_2CH{:}C(Me)_2$	Me	T	0.2	5	—	—	[108]
129	$NHCH_2CH_2CH(Me)_2$	Me	T	—	—	0.081	0.73	[17]
130	$NH(CH_2)_5Me$	Me	T	—	—	0.03	0.5	[18]
131	$NH(CH_2)_4Me$	Me	T	—	—	0.03	0.2	[18]
132	$NH(CH_2)_3Me$	Me	T	—	—	0.1	0.73	[18]
133	$NHCH_2CH(Me)_2$	Me	T	—	—	6.6	N.R.	[18]
134	$NH{-}C_5H_9$	Me	T	—	—	6.6	N.R.	[18]
135	$NH{-}C_6H_{11}$	Me	T	—	—	inactive		[18]
								
	R	R′						
136	$NHCH_2CH{:}C(Me)_2$	β-RIB	T	—	—	0.8	4	[111]
137	$NHCH_2{-}C_6H_5$	β-RIB	T	—	—	0.63	5	[111]
								
	R	R′						
138	$NHCH_2CH{:}C(Me)_2$	H	T	0.62	6.6	—	—	[20]
139	$NH(CH_2)_5Me$	H	T	5.8	>20	—	—	[20]
140	$NHCH_2CH{:}C(Me)_2$	SMe	T	—	—	0.24	2.2	[20]
141	$NH(CH_2)_5Me$	SMe	T	—	—	6.6	20	[20]
142	$NH{-}C_6H_{11}$	SMe	T	—	—	0.05	0.6	[20]
143	$NH{-}C_5H_9$	SMe	T	—	—	0.009	0.05	[20]
144	$NHCH_2CH{:}C(Me)_2$	Me	T	0.4	4	—	—	[21]
145	$NH{-}C_4H_7$	Me	T	—	—	0.04	0.4	[21]
146			T	—	—	0.4	4	[112]

* Against 0.003 μM **26** or 0.05 μM **1**.

† Minimum concentration for detectable response.

‡ Optimum concentration.

BA: Bioassay, CI: complete inhibition, Ref.: Reference, RIB: D-ribofuranosyl, T: tobacco callus growth, N.R.: not reached.

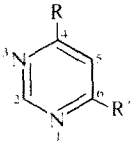
corresponding branched ones, as in 7-isopentylamino-3-methylpyrazolo[4,3-*d*]pyrimidine (**129**) and 7-isobutylamino-3-ethylpyrazolo[4,3-*d*]pyrimidine (**133**). Cyclization of the side chain as in 7-cyclopentylamino-3-methylpyrazolo[4,3-*d*]pyrimidine (**134**) and 7-cyclohexylamino-3-methylpyrazolo[4,3-*d*]pyrimidine (**135**) markedly reduce or eliminate antagonistic activity.

In 4-substituted-pyrrolo[2,3-*d*]pyrimidines in which the 7-nitrogen atom in the purine ring is replaced by a carbon atom, substituents such as 3-methyl-2-butenylamino, benzylamino, furfurylamino, propylamino, butylamino, pentylamino and hexylamino groups on the 4-position confer some cytokinin activity, though about 100–1000 times less than in the corresponding purine derivatives [19, 20, 109]. However, 7-ribosylation produces inhibitors [109, 110]. It is further observed that substituents which confer high cytokinin activity on 6-substituted aminopurines confer high anticytokinin activity on 4-substituted-pyrrolo[2,3-*d*]pyrimidines. Thus, 4-(3-methyl-2-butenylamino)- and 4-benzylamino-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidines (**136**, **137**) are strong inhibitors [111].

2-Methylthiolation of 4-substituted-pyrrolo[2,3-*d*]pyrimidines also results in loss of cytokinin activity and in development of anticytokinin activity [20]. 4-(3-Methyl-2-butenylamino)pyrrolo[2,3-*d*]pyrimidine (**138**) and 4-hexylaminopyrrolo[2,3-*d*]pyrimidine (**139**) are weak cytokinins in the tobacco callus bioassay, while their 2-methylthio derivatives (**140**, **141**) are inhibitory. In the case of 4-substituted-2-methylthiopyrrolo[2,3-*d*]pyrimidines, saturated cyclic substituents in the side chain produce rather strong antagonists when compared with 7-substituted-3-methylpyrazolo[4,3-*d*]pyrimidines; 4-cyclohexylamino-3-methylthiopyrrolo[2,3-*d*]pyrimidine (**142**) and 4-cyclopentylamino-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**143**) are very strong inhibitors. Only 0.009 μM of **143** is required to produce detectable inhibition of the growth in tobacco callus culture on a medium containing 0.003 μM 6-(3-methyl-2-butenylamino)purine (**26**) [20].

In 2-methyl derivatives of 4-substituted-pyrrolo[2,3-*d*]pyrimidine, the activity changes continuously from agonistic to antagonistic with systematic variation in the 4-substituent [21]. This agonist–antagonist relationship is illustrated as 'a steric substituent parameter' by the maximum width (W_{max}) of substituents from the bond-axis between the exocyclic nitrogen atom and its α-carbon atom. The W_{max} value of cytokinin-active compounds is 4.7 to 6.0 Å, while that of cytokinin antagonists is smaller or larger than this range. Cytokinin-active 4-(3-methyl-2-butenylamino)-2-methylpyrrolo[2,3-*d*]pyrimidine (**144**) has the W_{max} value of 4.72 Å and 4-cyclobutylamino-2-

Table 4. Pyrimidine, urea and amide derivatives and their cytokinin activity

	Compounds		BA	Min. concn (μM)*	Opt. concn (μM)†	Ref.
						
	R	R′				
147	$NHCH_2CH{:}C(Me)_2$	H	T	—	61	[115]
148	$NHCO-C_6H_5$	H	T	—	50	[115]
149	$NHCONH-C_6H_5$	H	T	—	4.7	[115]
150	$NH(CH_2)_3Me$	H	T	inactive		[115]
151	$NHCH_2-C_6H_5$	H	T	inactive		[115]
152	$NH(CH_2)_3Me$	Cl	T		54	[115]
153	$NHCH_2-C_6H_5$	Cl	T	—	23	[115]
	$R-CONH-R'$					
	R	R′				
154	$NH-C_6H_5$	H	TP	552	—	[118]
155	$NH-C_6H_5$	C_6H_5	T	1	50	[107]
156	$NH-C_6H_5$	PYR	T	0.047	0.47	[123]
157	$NH-C_6H_5$	Cl—PYR	T	0.0001	0.004	[123]
158	IM	C_6H_5	T	20	100	[107]
159	PYR	C_6H_5	T	5	50	[107]
160	PYR	Cl—PHE	T	5	50	[107]
161	$NHCH_2-C_6H_5$	C_6H_5	R-CHL	inhibitory		[124]

* Minimum concentration for detectable response.

† Optimum concentration.

BA: Bioassay, Ref.: Reference, PYR: 4-pyridyl, Cl-PYR: 4-(2-chloro)pyridyl, IM: 4-imidazole, Cl-PHE: 4-chlorophenyl, T: tobacco callus growth, TP: tobacco pith growth, R-CHL: chlorophyll retention in radish leaf.

methylpyrrolo[2,3-*d*]pyrimidine (**145**), the strongest antagonist in this series of compounds, has a W_{max} value of 3.83 Å.

Certain 4-substituted-2-methylthiopyrido[2,3-*d*]pyrimidines exhibit inhibitory activity on the growth of tobacco callus cultured on 0.05 μM kinetin (**1**) [112]. 4-*n*-Pentylamino-2-methylthiopyrido[2,3-*d*]pyrimidine (**146**) is shown to be a competitive inhibitor from Lineweaver and Burk's equation, a method of classical enzyme kinetics [113].

Pyrimidine derivatives

As described previously, modification of the purine ring in 6-substituted aminopurines mostly lowers or destroys cytokinin activity. Therefore, pyrimidine derivatives which are regarded as incomplete aminopurine derivatives lacking the imidazole ring have been presumed to be inactive. However, cytokinin activity has been observed in pyrimidine derivatives in the chlorophyll retention bioassay with barley and it is possible that the purine ring may not be an absolute requirement for cytokinin activity [114]. Takahashi *et al.* found that 4-isopentenylaminopyrimidine (**147**), 4-benzoylaminopyrimidine (**148**) and 4-(3-phenylureido)pyrimidine (**149**) possess cytokinin activity in the tobacco callus bioassay, although they are 100–1000 times less active than 6-benzylaminopurine (**5**) [115]. Moreover, addition of a chlorine atom at the 6-position of the pyrimidine ring in **147–149** enhances the activity. 4-Butylaminopyrimidine (**150**) and 4-benzylaminopyrimidine (**151**) are completely inactive, while their 6-chloro derivatives, **152** and **153**, are also significantly active. Thus, aminopyrimidine derivatives must be included in the group of compounds with significant cytokinin activity.

UREA AND AMIDE DERIVATIVES

N,*N*′-Diphenylurea (1,3-diphenylurea, **155**) was isolated from coconut milk as a growth stimulator for carrot root tissue *in vitro* [116]. *N*,*N*′-Diphenylurea and related urea derivatives were shown to possess cytokinin activity in various bioassay systems used for adenylated cytokinins, although much higher concentrations were required [117]. Bruce and Zwar [118] tested approximately 500 urea derivatives for their cytokinin activity in the tobacco pith bioassay. An intact —NH—CO—NH— bridge with a phenyl ring attached was required to exhibit activity. *N*-Phenylurea (**154**) was the simplest active compound and substitution on the ring with phenyl and electronegative groups increased the activity in the order: *meta* > *para* > *ortho*. Introduction of *N*′-phenyl ring to a *N*-phenylurea increased the activity. In compounds of the type RNHCONHR′ in which R and R′ are phenyl or substituted phenyl groups, the highest activity was usually found in compounds with one unsubstituted phenyl ring. Those with two substituted phenyl groups generally had lower activity. An NHCONH bridge conferred higher activity than an NHCSNH linkage.

In the chlorophyll retention bioassay with radish (*Raphanus sativus* L.) leaf, similar results as described above are obtained [119]. Thus, in urea derivatives the level of activity in chlorophyll retention closely corresponds to the level of activity in cell division. On the other hand, there is no general correlation in the activity of urea derivatives between lateral bud growth in pea (*Pisum sativum* L.) and cell division in tobacco pith tissue [120].

Among urea derivatives tested, *N*-phenyl-*N*′-(4-pyridyl)urea (**156**) exhibits strikingly high cytokinin activity comparable to 6-benzylaminopurine (**5**) in several different bioassay systems including tobacco callus bioassay [120–122]. Takahashi *et al.* [123] tested 35 derivatives of *N*-phenyl-*N*′-(4-pyridyl)urea in the tobacco callus bioassay and found that introduction of an electronegative Cl atom at the α-position of the pyridyl ring causes a striking increase in cytokinin activity. *N*-Phenyl-*N*′-(2-chloro-4-pyridyl)urea (**157**) exhibits high cytokinin activity which is about 10, 100 and 10000 times higher than that of 6-benzylaminopurine (**5**), *N*-phenyl-*N*′-(4-pyridyl)urea (**156**) and *N*,*N*′-diphenylurea (**155**), respectively. Several *N*-(substituted phenyl)-*N*′-(2-chloro-4-pyridyl)ureas are more active than or as active as *N*-phenyl-*N*′-(4-pyridyl)urea. In *N*-phenyl-*N*′-pyridylurea derivatives, monosubstitution on the phenyl ring with a CH_3, CH_3O, OH, Cl or Br group always results in reduction of activity [123]. Among some aromatic amides tested, imidazolecarboanilide (**158**), isonicotinic acid anilide (**159**) and its 4′-chloro analog (**160**) exhibit cytokinin activity at 5–100 μM in the tobacco callus bioassay [107]. They are less active than *N*,*N*′-diphenylurea (**155**).

Kefford *et al.* reported that the substitution of a benzyl group for the phenyl group of *N*,*N*′-diphenylurea (**155**) and some other *N*-phenyl-*N*′-(substituted phenyl)ureas converts them from active cytokinins to antagonists, for example *N*-benzyl-*N*′-phenyl urea (**161**), in the chlorophyll retention bioassay with radish [124]. The greater their cytokinin activities are, the more effective they are as inhibitors, when substituted with a benzyl group. In addition, inhibitors are antagonistic to adenylated cytokinins, kinetin (**1**) and 6-benzylaminopurine (**5**) as well as to phenylurea derivatives with cytokinin activity. This suggests that purine cytokinins and urea cytokinins function in the same or in related cellular processes. The resemblance in chemical structure between purine cytokinins and urea cytokinins has also been discussed [63, 65].

In conclusion, it is apparent that cytokinin active substances so far known can be classified [115] into four structural types: purine derivatives, modified purine (aza and deaza purine) derivatives, urea (and amide) derivatives and aminopyrimidine derivatives. Action mechanisms and relationships among these different types remain to be investigated.

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