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CYTOKININ ACTIVITY OF *N*-PHENYL-*N'*-(4-PYRIDYL)UREA DERIVATIVES

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Key Word Index—Cytokinins; *N*-phenyl-*N'*-(4-pyridyl)urea derivatives; biological activity; structure–activity relationship.

Abstract—We have synthesized 35 *N*-phenyl-*N'*-(4-pyridyl)urea derivatives and tested their cytokinin activity in the tobacco callus bioassay. Among them, *N*-phenyl-*N'*-(2-chloro-4-pyridyl)urea is highly active, the optimum concentration of which is lower than 4×10^{-9} M (0.001 ppm), 3 compounds, i.e. *N*-(2-methylphenyl)-*N'*-(2-chloro-4-pyridyl)urea, *N*-(3-methylphenyl)-*N'*-(2-chloro-4-pyridyl)urea and *N*-(3-chlorophenyl)-*N'*-(2-chloro-4-pyridyl) urea are as active as N^6-benzyladenine (concentration for optimum yield: 4.4×10^{-8} M or 0.01 ppm), and *N*-phenyl-*N'*-(2-methyl-4-pyridyl)urea and *N*-(2-chlorophenyl)-*N'*-(2-chloro-4-pyridyl)urea are as active as *N*-phenyl-*N'*-(4-pyridyl)urea (concentration for optimum yield: 4.7×10^{-7} M or 0.1 ppm), while the activity of the other 29 compounds are not so remarkable and 11 of them are almost or completely inactive.

INTRODUCTION

We have been studying the structure–activity relationships of synthetic cytokinins using the tobacco callus bioassay. First, 4 active azaindene derivatives which are analogs of kinetin were synthesized [1]. Subsequently, considering the activity and chemical structure of these azaindene derivatives and *N,N'*-diphenylurea (DPU), various active amide and urea derivatives were synthesized [2]. Among them *N*-phenyl-*N'*-(4-pyridyl)urea (4PU, **1a**) showed rather high activity and gave a better callus yield at its optimum concentration of 0.1 ppm than that of the optimum concentration (0.01 ppm) of kinetin or N^6-benzyladenine (BA). 4PU had already been synthesized by Brude and Zwar [3] as one of 500 kinds of substituted ureas and thioureas and was reported to initiate cell division at 0.1 ppm in the bioassay devised by Bottomley *et al.* [4] using tobacco pith block in 1 ml of liquid nutrient medium containing IAA and the compound to be tested. However Dyson *et al.* [5] reported that urea derivatives are not active in tests of cytokinin activity in soybean callus cultures.

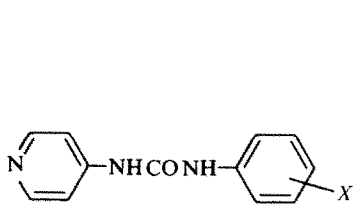
Now, we intended to synthesize 4PU derivatives having higher cytokinin activity and examined the structure–activity relationships of the substituted urea derivatives reported by Bruce and Zwar in order to get information for our purpose. Their bioassay method simply indicated the minimum concentration to initiate cell division on inoculated excised tobacco pith tissue blocks and seemed not to be strictly quantitative. DPU derivatives, most of which were reported by them to be highly active, were selected, and the activities (concentration range for cytokinin activity and fr. wt callus yield) of 24 of these compounds were determined in the tobacco callus bioassay and were compared to those reported by Bruce and Zwar.

As shown in Table 1, the results by Bruce and Zwar and by us were not identical, it was clear that half of the substituted DPUs showed higher activity than DPU, and that there was no DPU derivative having higher activity than 4PU in the tobacco callus bioassay. Therefore, referring to the results we synthesized 35 4PU derivatives and their cytokinin activity was assayed.

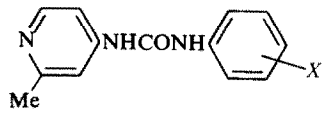
RESULTS AND DISCUSSION

Synthesis of the 4PU derivatives was carried out by the reaction of isonicotinoyl azide with appropriate aniline derivatives, or 4-aminopyridines with appropriate phenyl isocyanates.

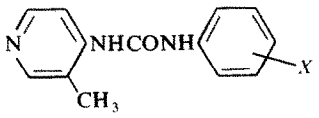
Cytokinin activity of the synthesized compounds was tested in the tobacco callus bioassay [1, 2]. The work was done in the following order. (a) Compounds with a mono substituted phenyl ring shown in Tables 2 and 6, were synthesized. As shown in Table 2 and Fig. 1, mono substitution of the phenyl ring with Me, OMe, OH, Cl or Br groups always resulted in reduction of activity. Especially in the compounds with *para* substituents (**1d**, **1g**, **1i**, **1k**, **1n**), activity was lost in most cases. But *meta* substitution (**1c**, **1f**, **1h**, **1j**, **1m**) gave a considerable range of activity, in which compounds with electronegative Cl or Br groups (**1f**, **1m**) show relatively higher activity than those with Me, OMe or OH (**1c**, **1h**, **1j**). However, *ortho*-methyl substitution (**1b**) leads to almost complete loss of activity. (b) Compounds with both mono methyl substituted pyridyl and unsubstituted or mono substituted phenyl rings, shown in Table 3 and 7, were synthesized. As shown in Table 3 and Fig. 2, the compound having an α-methyl substituted pyridyl and an unsubstituted phenyl ring (**2a**) gives similar activity as 4PU, but mono substitution on the phenyl ring with a Cl or Me group reduces activity in the following decreasing order: *meta* (**2c**, **2f**) > *ortho*



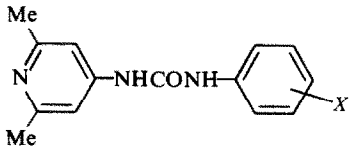
X
1a H
1b 2-Me
1c 3-Me
1d 4-Me
1f 3-Cl
1g 4-Cl
1h 3-OMe
1i 4-OMe
1j 3-OH
1k 4-OH
1l 4-COOEt
1m 3-Br
1n 4-Br



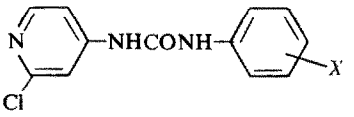
X
2a H
2b 2-Me
2c 3-Me
2d 4-Me
2e 2-Cl
2f 3-Cl
2g 4-Cl



X
3a H
3f 3-Cl



X
4a H
4b 2-Me
4c 3-Me
4d 4-Me
4e 2-Cl
4f 3-Cl
4g 4-Cl



X
5a H
5b 2-Me
5c 3-Me
5d 4-Me
5e 2-Cl
5f 3-Cl
5g 4-Cl

Table 1. Comparison of cytokinin activity of DPU derivatives between the assay of Bruce and Zwar and tobacco callus bioassay

Compound	CYTOKININ ACTIVITY Bruce and Zwar (ppm)	Tobacco callus bioassay† Optimum concn (ppm)	Minimum concn (ppm)	Callus yield	Satd. aq. soln (ppm)
DPU	0.5	10	<1	+ + + + +	10
4PU(**1a**)	0 3	0 1	<0.01	+ + + + + +	>100
N-(3-Methylphenyl)-*N'*-pu	0.6	10	0.1	+ + + + +	0.6
N-(4-Methylphenyl)-*N'*-pu	20‡	10	0.1	+ + + +	0.4
N-(3-Methoxyphenyl)-*N'*-pu	<1	10	1	+ + + +	10
N-(4-Methoxyphenyl)-*N'*-pu	3	5	0.1	+ + + +	0.7
N-(3-Hydroxyphenyl)-*N'*-pu	1	10	1	+ + + + +	10
N-(4-Hydroxyphenol)-*N'*-pu	10	10	1	+ + + + + +	10
N-(3-Chlorophenyl)-*N'*-pu	0.1	1	0.1	+ + + + +	0 5
N-(4-Chlorophenyl)-*N'*-pu	0.1	5	0.1	+ + +	0.5
N-(3-Bromophenyl)-*N'*-pu	0.1	1	<0.1	+ + + + +	0.5
N-(4-Bromophenyl)-*N'*-pu	33‡	5	<0.1	+ + + +	0.3
N-(4-Carboxyphenyl)-N'-pu	1	1	0.1	+ + + + +	1
N-(4-Ethoxycarbonylphenyl)-*N'*-pu	1	5	1	+ + + + +	0.8
N-(3-Chloro-4-methylphenyl)-*N'*-pu	<1	1	0.1	+ + +	0.6
N-(5-Quinolyl)-*N'*-pu	<1	10	1	+ + +	0.8
N,N-bis(4-Methylphenyl)urea	n.a.	5	1	+ +	0.3
N,N'-bis(4-Methoxyphenyl)urea*	—	5	1	+ +	0.7
N,N'-bis(3-Chlorophenyl)urea	20‡	n.a.	—	—	0 5
N-(3-Chlorophenyl)-*N'*-(4-chlorophenyl)urea	10‡	n.a.	—	—	0.5
N-(4-Chlorophenyl)-*N'*-(3,4-dichlorophenyl)urea	33‡	n.a.	—	—	insoluble
Phenylurea	75‡	n.a.	—	—	0.4
3-Chlorophenylurea	3	n.a.	—	—	10
4-Chlorophenylurea	<1	10	1	+ + + +	10
3-Bromophenylurea	0.6	10	1	+ +	10
(4-Pyridyl)urea	n.a.	n.a.	—	—	100

pu: phenylurea. n a.: not active. The result of Bruce and Zwar represents the minimum concentration to initiate cell division on inoculated tobacco pith block in the culture medium containing 2.5 ppm of IAA with 14 days of culture in the dark. Callus yield represents relative fr. wt and + corresponds *ca* 1g * These compounds are not reported by Bruce and Zwar. † Since all the compounds are sparingly soluble in water, DMSO is used to dissolve them in the tobacco callus bioassay. ‡ Bruce and Zwar did not use DMSO, so these values seem to be very difficult to attain.

Table 2. List of compounds with mono substituted phenyl ring and their optimum concentrations for cytokinin activity in the tobacco callus bioassay

	Compound	Optimum concn M (ppm)
1a	*N*-Phenyl-*N'*-4pu* (4PU)	4.7×10^{-7} (0.1)
1b	*N*-(2-Methylphenyl)-*N'*-4pu*	4.4×10^{-5} (10)
1c	*N*-(3-Methylphenyl)-*N'*-4pu*	4.4×10^{-5} (10)
1d	*N*-(4-Methylphenyl)-*N'*-4pu*	inactive
1f	*N*-(3-Chlorophenyl)-*N'*-4pu*	4.0×10^{-6} (1)
1g	*N*-(4-Chlorophenyl)-*N'*-4pu*	4.0×10^{-5} (10)
1h	*N*-(3-Methoxyphenyl)-*N'*-4pu	4.1×10^{-5} (10)
1i	*N*-(4-Methoxyphenyl)-*N'*-4pu	inactive
1j	*N*-(3-Hydroxyphenyl)-*N'*-4pu	4.4×10^{-5} (10)
1k	*N*-(4-Hydroxyphenyl)-*N'*-4pu	inactive
1l	*N*-(4-Ethoxycarbonylphenyl)-*N'*-4pu	inactive
1m	*N*-(3-Bromophenyl)-*N'*-4pu	3.4×10^{-6} (1)
1n	*N*-(4-Bromophenyl)-*N'*-4pu*	inactive
	BA	4.4×10^{-8} (0.01)

4pu: (4-pyridyl)urea. * Compounds synthesized and reported by Bruce and Zwar.

Table 3. List of compounds with both mono methyl substituted pyridyl and unsubstituted or mono substituted phenyl rings and their optimum concentrations for cytokinin activity in the tobacco callus bioassay

	Compound	Optimum concn M (ppm)
2a	*N*-Phenyl-*N'*-2-Me-4pu	4.0×10^{-7} (0.1)
2b	*N*-(2-Methylphenyl)-*N'*-2-Me-4pu	3.8×10^{-5} (10)
2c	*N*-(3-Methylphenyl)-*N'*-2-Me-4pu	3.8×10^{-6} (1)
2d	*N*-(4-Methylphenyl)-*N'*-2-Me-4pu	inactive
2e	*N*-(2-Chlorophenyl)-*N'*-2-Me-4pu	3.6×10^{-5} (10)
2f	*N*-(3-Chlorophenyl)-*N'*-2-Me-4pu	3.8×10^{-6} (1)
2g	*N*-(4-Chlorophenyl)-*N'*-2-Me-4pu	3.8×10^{-5} (10)
3a	*N*-Phenyl-*N'*-3-Me-4pu	4.2×10^{-5} (10)
3f	*N*-(3-Chlorophenyl)-*N'*-3-Me-4pu	3.8×10^{-5} (10)
	BA	4.4×10^{-8} (0.01)
	1a(4PU)	4.7×10^{-7} (0.1)

2- or 3-Me-4pu: (2- or 3-methyl-4-pyridyl)urea.

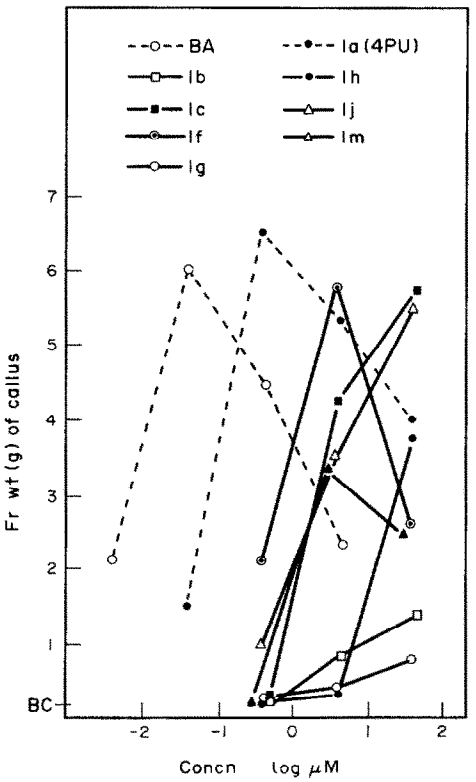


Fig. 1. Effect of compounds **1b**, **1c**, **1f**, **1g**, **1h**, **1j**, **1m**, BA and 4PU(**1a**) on fr. wt yield of tobacco callus.

(**2b**, **2e**) > *para* (**2d**, **2g**), while compounds with a β-methyl substituted pyridyl ring show lower activity than 4PU even if phenyl ring is unsubstituted as shown in Table 3 and Fig. 3. (c) Compounds with both dimethyl substituted pyridyl and unsubstituted or mono substituted phenyl rings shown in Tables 4 and 8 were synthesized. As shown in Table 4 and Fig. 3, 2,6-dimethyl substitution on the pyridyl ring greatly reduces activity and in most cases results in the loss of activity. Nevertheless, the compound with the unsubstituted phenyl ring (**4a**) retains considerable activity as compared to those with a substituted phenyl ring (**4b**–**4g**), which are inactive with the exception of *meta*-chloro substituent (**4f**). **4f** retains weak activity in the higher concentration. (d) Subsequently, compounds with both mono chloro substituted pyridyl and unsubstituted or mono substituted phenyl rings, shown in Tables 5 and 9, were synthesized. As shown in Table 5 and Figs 3 and 4, compounds with an α-chloro substituted pyridyl ring generally give higher cytokinin activity than 4PU (**1a**), especially the compound with the unsubstituted phenyl ring (**5a**) shows high activity. In this case, too, mono substitution on the phenyl ring with a Me or Cl group reduces activity and gives products with the following decreasing order of activity: *meta* (**5c**, **5f**) > *ortho* (**5b**, **5e**) > *para* (**5a**, **5g**).

Through all these studies, some interesting results on the structure–activity relationship mentioned below were obtained. As far as the 35 4PU derivatives are concerned, every substitution on the phenyl ring decreases

Table 4. List of compounds with both di-methyl substituted pyridyl and unsubstituted or mono substituted phenyl rings and their optimum concentrations for cytokinin activity in the tobacco callus bioassay

	Compound	Optimum concn M (ppm)
4a	*N*-Phenyl-*N'*-2,6-di-Me-4pu	4.0×10^{-6} (1)
4b	*N*-(2-Methylphenyl)-*N'*-2,6-de-Me-4pu	almost inactive
4c	*N*-(3-Methylphenyl)-*N'*-2,6-di-Me-4pu	almost inactive
4d	*N*-(4-Methylphenyl)-*N'*-2,6-di-Me-4pu	inactive
4e	*N*-(2-chlorophenyl)-*N'*-2,6-di-Me-4pu	inactive
4f	*N*-(3-Chlorophenyl)-*N'*-2,6-di-Me-4pu	3.4×10^{-5} (10)
4g	*N*-(4-Chlorophenyl)-*N'*-2,6-di-Me-4pu	inactive
	BA	4.4×10^{-8} (0.01)
	1a(4PU)	4.7×10^{-7} (0.1)

2,6-di-Me-4pu: (2,6-di-methyl-4-pyridyl)urea.

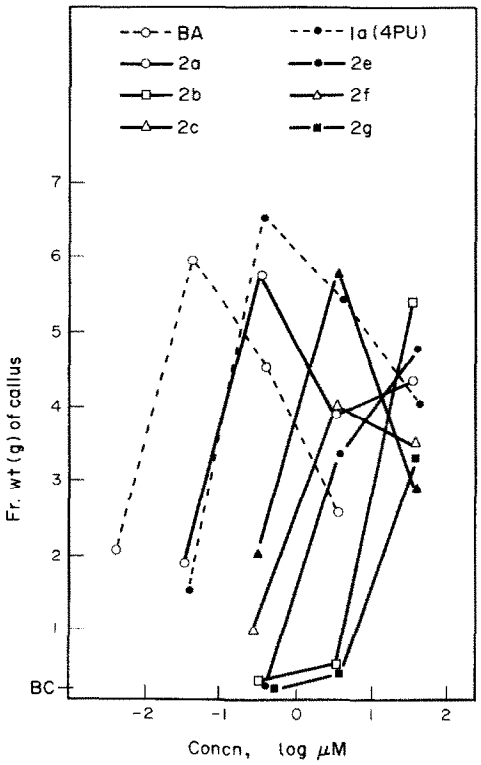


Fig. 2. Effect of compounds **2a**, **2b**, **2c**, **2e**, **2f**, **2g**, BA and 4PU(**1a**) on fr. wt yield of tobacco callus In the case of **2a**, marked shoot formation always occurred from the growing callus at the concentration of 4.0×10^{-5} M (10 ppm) and it was impossible to obtain the separate yield of the callus from the shoot weight. Therefore, the total sum of them was plotted at the point of an arrow It seems that this is the reason why the growth curve of **2a** bent at the concn of 4×10^{-6} M (1 ppm).

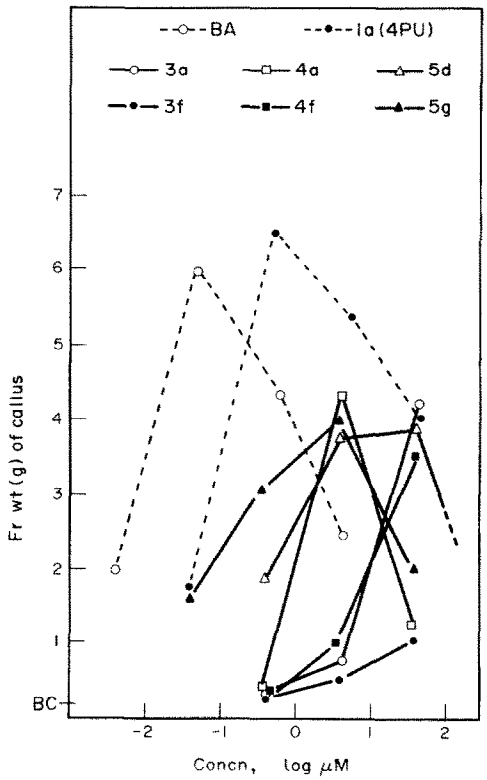


Fig. 3. Effect of the compounds **3a**, **3f**, **4a**, **4f**, **5d**, **5g**, BA and 4PU(**1a**) on fr. wt yield of tobacco callus.

biological activity and gives products with activities in the order: *meta* > *ortho* > *para*. In the DPU series, Bruce and Zwar [3] reported that substitution on one of the phenyl groups of DPU generally gave products with activities in the order: *meta* > *para* > *ortho*. Also, in the substituted 6-phenylureidopurine series, McDonald *et al.* [6] reported that substitution on phenyl ring showed

Table 5. List of compounds with both mono substituted pyridyl and unsubstituted or mono substituted phenyl rings and their optimum concentrations for cytokinin activity in the tobacco callus bioassay

Compound	Optimum concn M (ppm)
5a *N*-Phenyl-*N'*-2-Cl-4pu	$<4.0 \times 10^{-9}$ (<0.001)
5b *N*-(2-Methylphenyl)-*N'*-2-Cl-4pu	3.8×10^{-8} (0.01)
5c *N*-(3-Methylphenyl)-*N'*-2-Cl-4pu	3.7×10^{-8} (0.01)
5d *N*-(4-Methylphenyl)-*N'*-2-Cl-4pu	3.8×10^{-6} (1)
5e *N*-(2-Chlorophenyl)-*N'*-2-Cl-4pu	3.6×10^{-7} (0.1)
5f *N*-(3-Chlorophenyl)-*N'*-2-Cl-4pu	3.6×10^{-8} (0.01)
5g *N*-(4-Chlorophenyl)-*N'*-2-Cl-4pu	3.6×10^{-6} (1)
BA	4.4×10^{-8} (0.01)
1a(4PU)	4.7×10^{-7} (0.1)

2-Cl-4pu: Abbreviation for (2-chloro-4-pyridyl)urea.

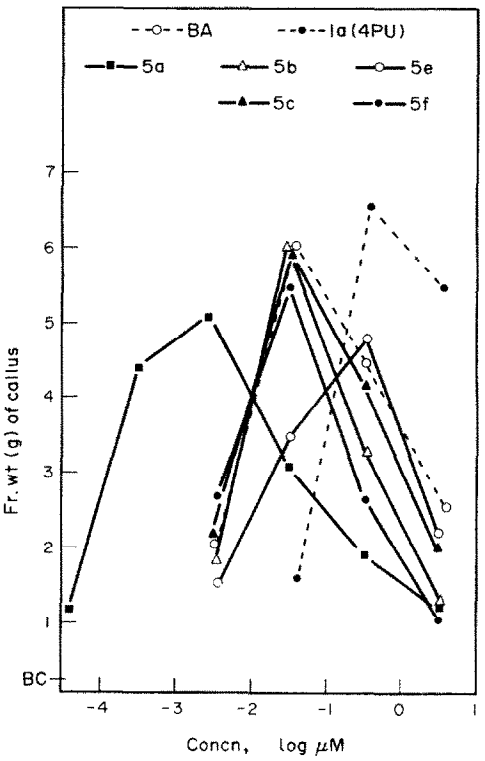


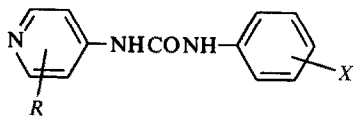
Fig. 4. Effect of compounds **5a**, **5b**, **5c**, **5e**, **5f**, BA and 4PU(**1a**) on fr. wt yield of tobacco callus

Table 6. *N*-(Substituted phenyl)-*N'*-(4-pyridyl)ureas.

	X	Formula	Yield, (%)	mp (°)	Carbon, % Calc.	Carbon, % Found	Hydrogen, % Calc.	Hydrogen, % Found	Nitrogen, % Calc.	Nitrogen, % Found
1b	2-Me	$C_{13}H_{13}N_3O$	85.3	187–189.5*	68.70	68.64	5.77	5.92	18.49	18.48
1c	3-Me	$C_{13}H_{13}N_3O$	88.5	199–200.5†	68.70	68.44	5.77	5.71	18.49	18.44
1d	4-Me	$C_{13}H_{13}N_3O$	86.7	187–188.5‡	68.70	68.81	5.77	5.81	18.49	18.39
1f	3-Cl	$C_{12}H_{10}ClN_3O$	92.9	211–214¶	58.19	58.04	4.07	4.07	16.96	17.18
1g	4-Cl	$C_{12}H_{10}ClN_3O$	93.1	241¶	58.19	58.08	4.07	4.09	16.96	16.94
1h	3-OMe	$C_{13}H_{13}N_3O_2$	91.4	180–182‡	64.19	64.19	5.39	5.38	17.27	17.37
1i	4-OMe	$C_{13}H_{13}N_3O_2$	91.1	171.5–172§	64.19	64.07	5.39	5.41	17.27	17.02
1j	3-OH	$C_{12}H_{11}N_3O_2.H_2O$	83.8	192–194*	58.29	58.12	5.30	5.24	17.00	16.65
1k	4-OH	$C_{12}H_{11}N_3O_2$	80.6	242–244‖	62.87	62.66	4.84	4.97	18.33	18.32
1l	4-COOEt	$C_{15}H_{15}N_3O_3$	96.0	186.5–188.5†	63.15	63.03	5.30	5.29	14.73	14.70
1m	3-Br	$C_{12}H_{10}BrN_3O$	87.5	227–230‖	49.34	49.54	3.45	3.53	14.38	14.36
1n	4-Br	$C_{12}H_{10}BrN_3O$	92.1	248–250‖	49.34	49.49	3.45	3.46	14.38	14.32

* From Me_2CO. † From 50% EtOH. ‡ From EtOAc–Me_2CO. § From EtOAc. ‖ From EtOH. ¶ From MeOH.

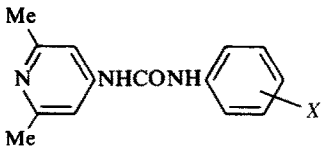
Table 7. *N*-(Substituted or unsubstituted phenyl)-*N'*-(2- or 3-methyl-4-pyridyl)ureas



	R	*X*	Formula	Yield, (%)	mp (°)	Carbon, % Calc.	Carbon, % Found	Hydrogen, % Calc.	Hydrogen, % Found	Nitrogen, % Calc.	Nitrogen, % Found
2a	2-Me	H	$C_{13}H_{13}N_3O.H_2O$	78.3	76–78*	63.66	63.61	6.16	6.17	17.13	17.28
2b	2-Me	2-Me	$C_{14}H_{15}N_3O.H_2O$	81.5	93–96†	64.84	64.30	6.61	6.41	16.21	15.94
2c	2-Me	3-Me	$C_{14}H_{15}N_3O.H_2O$	83.8	82–84‡	64.84	64.49	6.61	6.69	16.21	16.41
2d	2-Me	4-Me	$C_{14}H_{15}N_3O$	75.9	167–169§	69.69	69.66	6.27	6.27	17.42	17.43
2e	2-Me	2-Cl	$C_{13}H_{12}ClN_3O.H_2O$	76.3	96–99†	55.82	56.04	5.04	5.08	15.02	14.88
2f	2-Me	3-Cl	$C_{13}H_{12}ClN_3O$	82.2	170.5–171‡	59.66	59.52	4.62	4.55	16.06	15.94
2g	2-Me	4-Cl	$C_{13}H_{12}ClN_3O$	85.3	184–186‡	59.66	59.73	4.62	4.62	16.06	16.08
3a	3-Me	H	$C_{13}H_{13}N_3O.1/2H_2O$	82.7	129–130‖	66.08	65.74	5.97	5.86	17.78	17.79
3f	3-Me	3-Cl	$C_{13}H_{12}ClN_3O$	78.1	213.5–214¶	59.66	59.71	4.62	4.56	16.06	16.33

* From CH_2Cl_2. † From Me_2CO–Et_2O. ‡ From 50% EtOH. § From Me_2CO. ‖ From *n*-hexane–Me_2CO. ¶ From MeOH.

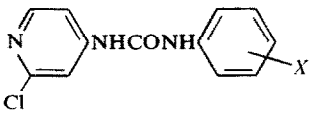
Table 8. *N*-(Substituted or unsubstituted phenyl)-*N'*-(2,6-dimethyl-4-pyridyl)ureas



	X	Formula	Yield, (%)	mp (°)	Carbon, % Calc.	Carbon, % Found	Hydrogen, % Calc.	Hydrogen, % Found	Nitrogen, % Calc.	Nitrogen, % Found
4a	H	$C_{14}H_{15}N_3O.1/2H_2O$	85.1	101–104*	67.18	67.19	6.44	6.47	16.79	16.94
4b	2-Me	$C_{15}H_{17}N_3O$	79.1	184–185†	70.56	70.30	6.71	6.68	16.46	16.33
4c	3-Me	$C_{15}H_{17}N_3O.H_2O$	82.4	159–160*	65.92	66.14	7.01	7.03	15.37	15.19
4d	4-Me	$C_{15}H_{17}N_3O$	80.8	201–202.5†	70.56	70.39	6.71	6.57	16.46	16.42
4e	2-Cl	$C_{14}H_{14}ClN_3O.H_2O$	76.4	110–113†	57.24	57.31	5.49	5.61	14.30	14.27
4f	3-Cl	$C_{14}H_{14}ClN_3O.H_2O$	77.1	107–110*	57.24	57.31	5.49	5.43	14.30	14.34
4g	4-Cl	$C_{14}H_{14}ClN_3O.H_2O$	75.7	106–108*	57.24	57.39	5.49	5.46	14.30	14.31

* From *n*-hexane–Me_2CO. † From Me_2CO.

Table 9. *N*-(Substituted or unsubstituted phenyl)-*N'*-(2-chloro-4-pyridyl)ureas



	X	Formula	Yield, (%)	mp (°)	Carbon, % Calc.	Carbon, % Found	Hydrogen, % Calc.	Hydrogen, % Found	Nitrogen, % Calc.	Nitrogen, % Found
5a	H	$C_{12}H_{10}ClN_3O$	73.5	173–174*	58.19	58.27	4.07	4.15	16.96	16.93
5b	2-Me	$C_{13}H_{12}ClN_3O$	73.0	184–185†	59.66	59.32	4.62	4.87	16.06	15.61
5c	3-Me	$C_{13}H_{12}ClN_3O.1/2H_2O$	70.6	93–95*	57.68	57.44	4.84	4.82	15.52	15.32
5d	4-Me	$C_{13}H_{12}ClN_3O$	76.7	188.5–190†	59.66	59.44	4.62	4.59	16 06	15 64
5e	2-Cl	$C_{12}H_9Cl_2N_3O$	79.8	183†	51.09	50.94	3.22	3.15	14.89	14.70
5f	3-Cl	$C_{12}H_9Cl_2N_3O$	77.0	198–199‡	51.09	51.29	3.22	3.23	14.89	14.80
5g	4-Cl	$C_{12}H_9Cl_2N_3O$	81.4	201–201.5*	51.09	51.02	3.22	3.11	14.89	14.73

* From Me_2CO–Et_2O. † From 70% MeOH. ‡ From *n*-hexane–Me_2CO.

the following decreasing order of activity: *ortho* > *meta* > *para*. Since the only difference among these 3 series is the replacement of a pyridyl group of 4PU by phenyl or a purin-6-yl group, these replacements have something to do with the effect of substitution in (other) phenyl ring on the activity. The exact reason for this remains for future study.

Introduction of an electronegative Cl group at the α-position of the pyridyl ring causes a striking increase in the cytokinin activity. For example, *N*-phenyl-*N'*-(2-chloro-4-pyridyl)urea (**5a**) shows outstanding activity, promotes vigorous growth of callus tissue and gives a fr. wt yield of callus similar to BA at a concentration less than 4×10^{-9} M (0.001 ppm), which means that **5a** has *ca* 100 times higher activity than 4PU, *ca* 10000 times higher than DPU, and is *ca* 10 times more active than BA in the present tobacco callus bioassays. Therefore, **5a** is one of the most active cytokinins so far known.

As for the concentration of various urea compounds required for optimum yield, that of DPU is 4.7×10^{-5} M, but the replacement of a phenyl group of DPU by 4-pyridyl or α-chloro-4-pyridyl groups enhances activity, so that the concentration of 4PU or **5a** was found to be 4.7×10^{-7} M or 4×10^{-9} M, respectively, in the tobacco callus bioassay. In the case of the ureidopurine derivatives, the most active compound reported by McDonald *et al.* [6], i.e. 6-phenylureidopurine produced optimum yields at a concentration of *ca* 1×10^{-7} M in the tobacco bioassay. Therefore, replacement of one of the phenyl groups of DPU gives products with the following increasing order of activity: phenyl < purin-6-yl, 4-pyridyl < α-chloro-4-pyridyl.

Since it is clear from the present study that four 4PU derivatives (**5a, 5b, 5c, 5f**), which have no purine ring in their structures, have activities similar to or higher than BA in the tobacco callus bioassay, we may say that there are, at present, two types of cytokinin active compounds, i.e. 'purine type' and 'urea type'. Whether they act on the same active site in the cells of tobacco callus tissue and promote the growth or not is an important problem which should be kept in mind by investigators who want to study the mechanism of cytokinin activity in the future.

The highly active Cl substituted compounds are quite highly active in shoot formation from tobacco callus. The activity of **5a**, for example, is *ca* 100 times higher than that of 4PU in the absence of auxin [12]. High activity in chlorophyll retention was also found. These results will be discussed in a separate paper.

EXPERIMENTAL

General synthetic procedure. All mps were recorded on a hot plate apparatus and are uncorr. IR and MS data were consistent with the assigned structure.

4PU (**1a**). A mixture of 2 mM of isonicotinoyl azide [7] and 2 mM of aniline dissolved in dry C_6H_6 was stirred and refluxed for 4 hr. After cooling, the mixture was evapd to dryness. The residue was purified by chromatography over Si gel and elution with Me_2CO–$CHCl_3$ (1:1). The appropriate fractions were combined and evapd to dryness and the solid residue was recrystallized from EtOAc (93.9% yield), mp 162–163°.

N-(*2-, 3- or 4-Substituted phenyl*)-N'-(*4-pyridyl*)*ureas* (**1b–1n**). These compounds were prepared from the corresponding aniline by the reaction with isonicotinoyl azide in the same way as described for 4PU(**1a**). Analytical data are reported in Table 6.

N-*Phenyl*-N'-(*2- or 3-methyl-4-pyridyl*)*urea* (**2a, 3a**). To 2 mM of 2- or 3-methyl-4-aminopyridine [8, 9] in dry Me_2CO was added 2 mM of phenylisocyanate and the soln was stirred at room temp for 8–12 hr. The reaction mixture was evapd to dryness. The residue was purified by chromatography over Si gel and elution with Me_2CO–$CHCl_3$ (1:1). The appropriate fractions were combined and evapd to dryness and the solid residue was recrystallized from CH_2Cl_2 or a mixture of *n*-hexane and Me_2CO. Analytical data are reported in Table 7.

N-(*2-,3- or 4-Substituted phenyl*)-N'-(*2- or 3-methyl-4-pyridyl*) *ureas* (**2b–2g, 3f**). These compounds were prepared from the corresponding phenylisocyanate by the reaction with 2- or 3-methyl-4-aminopyridine in the same way as described for *N*-phenyl-*N'*-(2- or 3-methyl-4-pyridyl)urea. Analytical data are reported in Table 7.

N-(*Substituted or unsubstituted phenyl*)-N'-(*2,6-dimethyl-4-pyridyl*)*ureas* (**4a–4g**). These compounds were prepared from the corresponding phenylisocyanate by the reaction with 2,6-dimethyl-4-aminopyridine [10] in the same way as described for *N*-phenyl-*N'*-(2- or 3-methyl-4-pyridyl)urea. Analytical data are reported in Table 8.

N-(*Substituted or unsubstituted phenyl*)-N'-(*2-chloro-4-pyridyl*)-*ureas* (**5a–5g**). These compounds were prepared from the corresponding phenylisocyanate by the reaction with 2-chloro-4-aminopyridine [11] in the same way as described for *N*-phenyl-*N'*-(2- or 3-methyl-4-pyridyl)urea. Analytical data are reported in Table 9.

Bioassay procedure. Cytokinin activity was determined in the tobacco callus bioassay which was described in [1, 2] and was based on the optimum conc range and fr. wt yield of callus tissue.

Since most of the synthetic compounds are sparingly soluble in H_2O, they were at first dissolved in a small quantity of DMSO and added to the nutrient medium. No effect of DMSO on the bioassay was observed.

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