

CHEMICAL STRUCTURE AND PLANT GROWTH REGULATING ACTIVITY OF SOME NOVEL 1,1_i-POLYMETHYLENEBIS(3-SUBSTITUTED)UREAS

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Summary. The plant growth regulating activity of some novel 1,1'-polymethylenebis(3-substituted)ureas is described. Most of the compounds possessed plant growth regulating activity – they stimulated betacyanin synthesis in *Amaranthus caudatus* cotyledons, growth of excised radish cotyledons and of tobacco callus tissues, and inhibited root growth in intact wheat seedlings. Some chemical structure – plant growth regulating activity relationships have been discussed.

Key words: bioassays, bis-ureas, diamines, plant growth regulating activity, structure–activity relationships

Abbreviations: DPU – N,N'-diphenylurea; DAE – 1,2-diaminoethane; PUT – 1,4-diaminobutane (putrescine); DAH – 1,6-diaminohexane; BA – benzyladenine; 2,4-D – 2,4-dichlorophenoxyacetic acid

Introduction

Much current interest exists in discovering natural products and their derivatives which would impact plant growth. Such compounds have a potential for development as pesticides and plant growth regulators. They may also serve as “starting points” for laboratory synthesis to optimize biological activity.

The active phenylurea cytokinins, derivatives of natural N,N'-diphenylurea and di- and polyamines possess common physiological properties. Senescence can be prevented by exogenous cytokinins or polyamines (Tetley and Thimann, 1974; Altman,

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1982). This has prompted us to design more compounds with certain structural modifications in the framework of disubstituted urea and of diaminoalkanes. In connection with our continuing studies of biologically active aryl-substituted ureas (Yonova and Vassilev, 1992; Yonova et al., 1992), we targeted the synthesis of 1,1'-polymethylenebis(3-substituted)ureas having both diamine and urea moieties. Further, it was interesting to probe how this combination could contribute to the plant growth regulating activity.

1,1'-Polymethylenebis(3-substituted-1-nitroso)ureas have been pointed out as antitumour agents against AH-13 and L-1210 (Miyahara, 1978).

The aim of the present study was to investigate the plant growth regulating activity of 1,1'-polymethylenebis(3-substituted)ureas and to compare it with that of the parent compounds (diphenylurea and diaminoalkanes). The effects of a 3-substituent (phenyl, pyridyl and thiazolyl rings, and the substituents on them) and the length of the polymethylene chain on plant growth regulating activity were also studied.

Materials and Methods

Chemicals

1,1'-Polymethylenebis(3-substituted)ureas, subject of the present study, were synthesized earlier by us for further information about the role of the fused polymethylene chain and substituted urea in plant growth regulating activity. The synthesis and the full physicochemical characteristics of the compounds were described in another paper (Yonova and Ionov, 1997). Compounds with the following common formula were investigated

$$\text{Ar-NHCONH(CH}_2\text{)}_n\text{NHCONH-Ar}$$

No	Ar	n	No	Ar	n
1.	phenyl	2	11.	phenyl	5
2.	4-fluorophenyl	2	12.	4-fluorophenyl	5
3.	3-chlorophenyl	2	13.	4-chlorophenyl	5
4.	4-chlorophenyl	2	14.	phenyl	6
5.	phenyl	3	15.	4-fluorophenyl	6
6.	4-fluorophenyl	3	16.	4-chlorophenyl	6
7.	4-chlorophenyl	3	17.	2-thiazolyl	6
8.	phenyl	4	18.	4-pyridyl	6
9.	4-fluorophenyl	4	19.	(3,5-dichloro-4-pyridyl)	6
10.	4-chlorophenyl	4			

Only five of the 19 compounds - **1**, **6**, **9**, **12** and **15** have been described in the available references (Miyahara, 1978). However, there no data about their plant growth regulating activity were found.

Biological Evaluations. Bioassay Procedures

Synthesis of betacyanins in *Amaranthus caudatus* L. Ten *Am. caudatus* explants in each replicate, consisting of the upper portion of the hypocotyl plus the cotyledons, were placed in Petri dishes on filter paper moistened with 2 ml of test solutions. After incubation at 25°C for 20 h in the dark, betacyanin was extracted with 2 ml of distilled water by means of two cycles of freezing and thawing. The quantity of betacyanin was determined by the procedure of Biddington and Thomas (1973). Optical density was measured at 542 and 620 nm.

Radish cotyledon expansion. Cytokinin-stimulated growth of excised radish cotyledons was measured after the method of Green and Muir (1978). Seeds of radish (*Raphanus sativus* L., cv. Red) were germinated on moistened filter paper in darkness at 25°C for 3 days. Ten cotyledons per replicate were floated with the adaxial face down in 6 cm Petri dishes, containing 3 ml of 6.7 mM Na-K phosphate buffer pH 6.7 or the test solutions. The cotyledons were blotted dry and weighed after 20 h of incubation in darkness at 25°C.

Root growth inhibition. Influence of the compounds on the root growth of young wheat seedlings in the dark was determined according to Stenlid (1982). Wheat (*Triticum aestivum* L. cv. Sadovo-1) grains were soaked in distilled water for 18 h, sown on filter paper moistened with distilled water and incubated in the dark for 48 h at 22°C. Plants with a central root 10–15 mm long were selected and, in groups of ten, were placed in 6 cm Petri dishes, containing 4 ml of 6.7 mM Na-K phosphate buffer pH 5.9 or the test solutions. After 20 h at 22°C in the dark, the length of the central root was measured.

Growth of tobacco callus tissues. The cytokinin effect of some from the investigated compounds was tested on *Nicotiana tabacum* L., cv. CMS/81 callus tissues, cultivated on a nutrient medium of Murashige and Skoog (1962) under thermostat conditions with 70% air humidity and $24 \pm 2^\circ\text{C}$. The cytokinin (0.05 mg.l^{-1} kinetin) in the nutrient medium was substituted with the new growth regulators at different concentrations and in the presence of 0.2 mg.l^{-1} 2,4-D as auxin. Their effects on biomass accumulation (fresh and dry weights) were registered after six week growth of calluses.

The compounds were tested in a concentration range of $1 \mu\text{M}$ –10 mM. We selected this wide concentration range since the obtained compounds are derivatives of diamines which have optimal concentration about 7.5 mM (Feraý et al., 1992) and of phenylurea cytokinins with optimal concentration about 0.01–0.001 mM (Yonova and Vassilev, 1987). The data presented are means from two experiments, each in five or seven replications. Student–Fisher's procedures were used for statistical calculation.

Table 1. Effect of some bis-urea derivatives of diaminoalkanes on the betacyanin accumulation in *Amaranthus* cotyledons. Results are expressed as % induction of the control \pm SD.

Compsds No	% Betacyanin at concentrations of mM										
	0.001	0.005	0.01	0.05	0.1	0.5	1	3	5	8	10
1	121 \pm 2.1	124 \pm 1.8	124 \pm 2.5	117 \pm 1.1	123 \pm 1.5	140 \pm 2.4	137 \pm 2.2	122 \pm 1.6	117 \pm 1.2	122 \pm 1.9	117 \pm 1.3
2	90 \pm 3.0	-	95 \pm 2.2	-	71 \pm 1.1	92 \pm 2.4	85 \pm 1.2	78 \pm 1.5	-	-	-
3	138 \pm 3.1	147 \pm 2.8	140 \pm 2.5	166 \pm 4.1	167 \pm 3.8	182 \pm 5.0	198 \pm 4.3	174 \pm 6.1	172 \pm 4.4	172 \pm 3.8	159 \pm 4.5
4	83 \pm 1.5	-	94 \pm 2.2	-	91 \pm 3.1	98 \pm 3.8	86 \pm 2.7	102 \pm 2.5	-	-	-
5	106 \pm 3.2	107 \pm 2.9	113 \pm 2.3	104 \pm 0.9	101 \pm 1.2	111 \pm 1.7	121 \pm 2.2	135 \pm 3.0	130 \pm 3.3	112 \pm 1.8	110 \pm 0.9
6	124 \pm 1.6	-	114 \pm 1.2	-	117 \pm 2.7	120 \pm 4.2	114 \pm 3.1	127 \pm 2.9	130 \pm 4.1	-	-
7	113 \pm 4.2	-	96 \pm 3.0	-	110 \pm 1.4	111 \pm 2.8	117 \pm 4.0	120 \pm 3.6	128 \pm 3.1	-	-
8	110 \pm 1.8	112 \pm 2.4	115 \pm 2.5	116 \pm 1.1	121 \pm 3.7	136 \pm 2.2	150 \pm 3.2	145 \pm 4.4	137 \pm 4.1	130 \pm 3.6	137 \pm 2.9
9	98 \pm 5.1	-	94 \pm 3.3	-	113 \pm 2.2	127 \pm 3.0	114 \pm 5.0	127 \pm 3.2	-	-	-
10	113 \pm 5.3	120 \pm 3.4	135 \pm 2.9	131 \pm 3.8	110 \pm 2.8	92 \pm 4.7	92 \pm 5.2	111 \pm 3.4	-	-	-
11	99 \pm 3.3	-	103 \pm 5.2	-	124 \pm 3.5	136 \pm 3.6	138 \pm 3.4	133 \pm 5.5	126 \pm 6.5	120 \pm 4.3	115 \pm 3.2
12	111 \pm 7.5	-	108 \pm 4.3	-	116 \pm 2.7	113 \pm 4.3	127 \pm 5.7	115 \pm 4.6	114 \pm 4.1	-	-
13	110 \pm 3.9	-	90 \pm 3.9	-	102 \pm 1.4	111 \pm 5.6	107 \pm 3.3	107 \pm 5.8	110 \pm 3.8	-	-
14	101 \pm 1.8	-	115 \pm 4.0	-	124 \pm 4.5	126 \pm 1.9	136 \pm 2.5	125 \pm 1.3	116 \pm 2.1	117 \pm 2.8	110 \pm 1.9
15	121 \pm 0.9	123 \pm 1.4	126 \pm 0.9	122 \pm 2.7	129 \pm 2.7	142 \pm 6.4	157 \pm 3.4	161 \pm 3.9	150 \pm 3.5	-	-
16	90 \pm 3.1	-	81 \pm 3.6	-	91 \pm 3.0	100 \pm 2.7	96 \pm 1.5	93 \pm 4.3	-	-	-
17	98 \pm 3.2	-	102 \pm 3.7	140 \pm 4.4	134 \pm 1.7	122 \pm 5.8	103 \pm 3.9	92 \pm 2.1	-	-	-
18	104 \pm 3.5	-	115 \pm 3.6	-	106 \pm 2.0	106 \pm 3.5	105 \pm 2.9	89 \pm 5.1	-	-	-
19	94 \pm 1.8	-	109 \pm 5.2	-	86 \pm 5.8	91 \pm 7.1	83 \pm 3.8	87 \pm 5.6	80 \pm 4.3	-	-
DPU	106 \pm 2.8	-	112 \pm 1.7	-	121 \pm 3.1	143 \pm 3.8	152 \pm 2.9	129 \pm 1.8	117 \pm 2.2	123 \pm 1.4	121 \pm 2.1
PUT	-	-	-	-	-	-	88 \pm 2.1	89 \pm 3.3	93 \pm 2.6	93 \pm 1.8	94 \pm 3.0

E₅₄₂₋₆₂₀ for control (buffer) 0.125 \pm 0.0023 (100%);

LSD 5% = 0.0181; LSD 1% = 0.0241

Results and Discussion

Here we report the plant growth regulating activity of some bis-urea derivatives of biogenic and non-biogenic diamines containing different number of methylene groups in the aliphatic chain.

The investigated compounds which are derivatives of two active structures – di-phenylurea (DPU) and aliphatic diamines (DA) stimulated betacyanin synthesis in *Amaranthus* cotyledons more than DPU (from 15 to 47%) and putrescine (PUT) (2-fold) (Table 1). The optimal concentration for almost all test compounds was 1 mM similarly to DPU. The bis-phenylureas containing from 2 to 6 methylene groups between both urea bridges showed high stimulating activity in a wide concentration range 0.01–10 mM, the most effective being bis-phenylurea with 4 methylene groups – compound **8** (150% at 1 mM). The compounds containing even number of methylene groups were more active than those with odd ones. Substituents with electron withdrawing properties (fluorine and chlorine) on the benzene rings affected the betacyanin promoting activity to a various degree. *Para* fluorine obviously increased the activity of 1,1'-hexamethylenebis(3-phenyl)urea – compound **15** (157% and 161% at 1 mM and 3 mM, respectively). *Meta* chlorine had stronger stimulating effect than its *para* isomer, what was in agreement with our earlier conclusion that position-type isomers differ in their activities (Yonova et al., 1989). 1,1'-Ethylenebis[3-(3-chlorophenyl)]urea – compound **3**, stimulated considerably amaranthin synthesis in the whole concentration range (max. 198% at 1 mM).

It is known that replacing the phenyl ring in DPU with heterocyclic ring structures results in increased activity (Mok et al., 1982). However, compound **17** showed only a slightly higher effect than DPU at concentrations 0.05 and 0.1 mM (140% and 134%, respectively) while compounds **18** and **19** were less active. Since the molecular requirement for high cytokinin activity in aromatic urea derivatives includes the presence of a 4-pyridyl moiety (Okamoto et al., 1981), we suggested that the presence of six methylene groups between both 4-pyridyl rings could be a probable cause for the low activity of the latter two substances.

Table 2 illustrates results from simultaneous application of some bis-ureas with the parent compounds (DPU and PUT) and the purine cytokinin benzyladenine (BA) in the same model system. In general, the bis-ureas increased the effects of DPU and PUT (10–140%) and decreased that of BA (with 14–35%). The observed antagonism of BA-induced betacyanin synthesis was in accordance with the results of other authors (Ueda and Kato, 1982) and supports the hypothesis about cytokinin antagonistic action (Iwamura et al., 1979). Thus, a compound with low cytokinin activity hinders by itself the binding of high active cytokinin at the receptor site. By contrast, compounds **5** (1 and 5 mM) and **8** (1 mM) generated a slight increase in the amount of pigment produced (2–14%) compared to that found in BA alone. This effect of compounds **5** and **8** suggests a possibility for involvement in benzyladenine-dependent

Table 2. Effect of some bis-arylurea derivatives of diaminoalkanes on the betacyanin synthesis induced by 1 mM DPU, 1 mM PUT and 4 μ M BA. Results are expressed as % induction of the control.

Comps No	Conc. (mM)	% Betacyanin					
		+DPU	% Δ	+PUT	% Δ	+BA	% Δ
1	1.0	(148.7)		(96.5)		(220.2)	
		176.3	+27.6	117.0	+20.5	194.4	-25.8
3	1.0	(168.9)		(108.8)		(277.5)	
		182.6	+13.7	139.9	+31.1	256.5	-21.0
5	1.0	(147.0)		(86.8)		(266.0)	
		153.6	+6.6	121.0	+34.2	267.8	+1.8
5	5.0	(160.5)		(92.2)		(250.3)	
		172.1	+11.6	116.0	+23.8	252.8	+2.5
8	1.0	(147.0)		(86.8)		(266.0)	
		155.3	+8.3	143.0	+56.2	280.2	+14.2
8	5.0	(160.5)		(92.2)		(250.3)	
		150.9	-9.6	141.2	+49.0	236.7	-13.6
11	1.0	(147.0)		(86.8)		(257.7)	
		172.3	+25.3	222.1	+135.3	122.7	-135.0
14	1.0	(147.0)		(86.8)		(257.7)	
		154.3	+7.3	227.2	+140.4	133.1	-124.6

The values in parenthesis are % betacyanin in *Amaranthus* cotyledons with DPU, PUT and BA only. LSD 5% = 0.0193; LSD 1% = 0.0255

betacyanin synthesis (Feray et al., 1992). We found that with increasing the length of polymethylene chain (2 \rightarrow 3 \rightarrow 4), the inhibiting effect of bis-phenylureas on BA-induced betacyanin production became stimulating while the stimulating effect of these compounds on DPU-induced pigment synthesis decreased in the same order. At n=5 or 6, there was again negative effect on BA-induced and strong positive effect on DPU- and PUT-induced pigment synthesis.

The compounds studied had moderate effect on the growth of excised radish cotyledons in darkness, the most effective being **2** and **4** (from 11% to 27% compared to the control) (Table 3). The latter two compounds were also more active than the standards (DPU 111% and PUT 108% to the control). The growth stimulating activity of bis(3-halogenophenyl)urea's derivatives dropped with increasing the number of methylene groups in the aliphatic chain. On the basis of these results, it seems clear that the compounds possess physiological effects similar to the di- and polyamines rather than the active cytokinins in this model system. Exogenous native and synthetic mono-, di- and polyamines have also been shown to stimulate the growth of detached radish and cucumber cotyledons in light but all the compounds possessed lower effects than that of kinetin (Alexieva, 1994).

Table 3. Effect of some bis-urea derivatives of diaminoalkanes on the expansion of etiolated *Raphanus sativus* L. cotyledons. Values given as percentage of the control \pm SD.

Comps No	Weight/10 units at concentrations of mM									
	0.001	0.005	0.01	0.05	0.1	0.5	1	3	5	
1	87 \pm 1.2	76 \pm 3.8	80 \pm 2.1	76 \pm 1.6	73 \pm 1.0	78 \pm 2.8	82 \pm 2.6	79 \pm 1.5	81 \pm 2.5	
2	121 \pm 5.6	115 \pm 7.9	109 \pm 4.0	112 \pm 5.1	112 \pm 2.7	95 \pm 4.6	109 \pm 2.3	111 \pm 4.1	106 \pm 1.4	
4	122 \pm 4.0	127 \pm 3.6	114 \pm 4.3	116 \pm 3.5	118 \pm 4.7	122 \pm 6.5	107 \pm 5.7	116 \pm 5.0	104 \pm 2.7	
5	80 \pm 3.6	71 \pm 1.8	89 \pm 4.2	76 \pm 2.2	87 \pm 4.3	84 \pm 1.1	79 \pm 4.8	85 \pm 3.0	85 \pm 3.2	
7	102 \pm 3.0	110 \pm 1.7	99 \pm 5.1	102 \pm 3.1	101 \pm 4.0	97 \pm 3.1	94 \pm 2.1	89 \pm 3.3	–	
8	89 \pm 3.7	98 \pm 4.1	79 \pm 3.0	98 \pm 4.6	90 \pm 4.5	79 \pm 5.4	83 \pm 2.2	88 \pm 3.4	92 \pm 3.2	
10	93 \pm 1.2	99 \pm 2.3	89 \pm 2.0	99 \pm 1.8	96 \pm 1.8	95 \pm 1.8	95 \pm 4.0	94 \pm 1.8	–	
14	77 \pm 1.6	72 \pm 1.3	83 \pm 1.4	95 \pm 3.2	94 \pm 3.5	84 \pm 3.4	79 \pm 1.6	84 \pm 3.4	80 \pm 1.6	
16	86 \pm 2.5	91 \pm 2.9	90 \pm 2.6	82 \pm 3.0	92 \pm 3.2	92 \pm 2.5	78 \pm 2.1	83 \pm 1.8	–	
17	98 \pm 2.0	103 \pm 2.1	101 \pm 1.7	105 \pm 0.3	104 \pm 0.8	103 \pm 1.3	100 \pm 1.3	101 \pm 1.2	100 \pm 0.8	
19	88 \pm 3.3	94 \pm 2.9	95 \pm 2.2	85 \pm 1.1	83 \pm 1.7	97 \pm 1.1	81 \pm 2.0	75 \pm 2.3	81 \pm 1.2	
DPU	97 \pm 3.5	–	111 \pm 5.1	–	90 \pm 4.0	102 \pm 2.4	100 \pm 4.0	90 \pm 0.6	91 \pm 2.8	
PUT	103 \pm 1.3	–	94 \pm 1.2	–	95 \pm 0.7	100 \pm 2.1	105 \pm 2.5	108 \pm 2.2	94 \pm 1.2	

Control (6.7 mM K-Na Pi buffer) 0.1233 \pm 0.0035 g (100%);
 LSD 5 % = 0.015; LSD 1% = 0.020

Table 4. Effect of some bis-urea derivatives of diaminoalkanes on the root growth of young *Triticum aestivum* L. seedlings. Values given as percentage of the control \pm SD.

Compd's No	Root growth at concentrations of mM										
	0.001	0.005	0.01	0.05	0.1	0.5	1	3	5	8	10
1	102 \pm 3.60	106 \pm 3.80	99 \pm 2.30	92 \pm 1.50	90 \pm 1.80	92 \pm 1.50	96 \pm 2.30	100 \pm 6.20	108 \pm 2.60	94 \pm 1.20	94 \pm 1.75
3	90 \pm 1.30	93 \pm 1.46	89 \pm 0.50	87 \pm 0.60	89 \pm 0.70	92 \pm 0.40	94 \pm 1.10	90 \pm 1.90	94 \pm 0.70	94 \pm 0.90	94 \pm 0.30
5	101 \pm 1.31	104 \pm 1.20	96 \pm 0.75	93 \pm 1.04	90 \pm 0.83	98 \pm 1.52	104 \pm 2.16	100 \pm 1.13	94 \pm 2.24	-	-
7	91 \pm 1.03	94 \pm 2.12	89 \pm 1.85	85 \pm 2.11	88 \pm 0.74	90 \pm 1.31	92 \pm 3.15	89 \pm 0.93	87 \pm 2.63	-	-
8	104 \pm 2.86	98 \pm 1.74	100 \pm 4.21	94 \pm 1.58	92 \pm 2.03	96 \pm 2.81	93 \pm 1.92	92 \pm 2.23	89 \pm 0.83	-	-
10	93 \pm 1.64	98 \pm 1.28	91 \pm 2.81	88 \pm 2.56	89 \pm 1.73	90 \pm 1.94	87 \pm 0.68	84 \pm 0.56	88 \pm 1.25	-	-
14	115 \pm 3.10	106 \pm 2.43	101 \pm 1.79	95 \pm 1.30	99 \pm 3.43	93 \pm 2.67	91 \pm 1.92	85 \pm 0.84	82 \pm 1.18	-	-
16	98 \pm 2.16	100 \pm 3.47	97 \pm 3.13	102 \pm 2.81	95 \pm 2.48	89 \pm 1.73	88 \pm 0.87	81 \pm 1.06	80 \pm 1.33	-	-
17	109 \pm 1.20	106 \pm 1.47	109 \pm 2.00	105 \pm 1.47	103 \pm 2.20	104 \pm 1.80	95 \pm 3.30	91 \pm 2.00	69 \pm 1.40	-	-
19	104 \pm 1.30	80 \pm 2.80	82 \pm 2.07	98 \pm 3.40	85 \pm 2.80	56 \pm 0.40	56 \pm 1.50	55 \pm 1.30	49 \pm 1.03	-	-
DPU	102 \pm 2.17	-	96 \pm 2.70	-	93 \pm 1.01	-	93 \pm 1.27	95 \pm 1.30	93 \pm 1.40	-	99 \pm 6.10
DAE	98 \pm 4.10	-	104 \pm 2.40	-	112 \pm 1.31	-	96 \pm 4.86	86 \pm 3.07	90 \pm 0.74	-	81 \pm 1.16
PUT	108 \pm 2.62	-	114 \pm 2.50	-	110 \pm 0.56	-	108 \pm 2.92	109 \pm 0.93	98 \pm 1.27	-	94 \pm 2.95
DAH	103 \pm 0.56	-	112 \pm 2.80	-	112 \pm 3.90	-	105 \pm 4.60	109 \pm 5.70	105 \pm 0.82	-	102 \pm 2.02

Control (6.7 mM K-Na Pi buffer) 21.68 \pm 0.34 mm (100%); $l_0 \approx$ 10 mm
 LSD 5% = 1.7447; LSD 1% = 2.3156

Auxins and cytokinins can be considered as possible natural inhibitors and regulators of root growth. The elongation of roots of wheat and cucumber seedlings in the dark has been strongly inhibited by various native and synthetic cytokinins (Stenlid, 1982). The inhibitory action of both auxins and cytokinins is related to the synthesis and action of ethylene. The inhibiting activity of the tested compounds on the root growth of intact wheat seedlings was higher than that of the parent compounds (DAE exhibited low activity at 10mM, DPU and PUT were very slowly active and DAH

Table 5. Effect of some bis-urea derivatives of diaminoalkanes on the growth of *Nicotiana tabacum* CMS/81 callus tissues. Values given as mean average \pm SD.

Compounds No	Concentration (mg.l ⁻¹)	Tissue weight (g/flask)		
		FW	DW	DW/FW \times 100
Control		1.631 \pm 0.225	0.059 \pm 0.008	3.62
Standard		17.279 \pm 1.005	0.287 \pm 0.018	1.66
1	0.5	15.785 \pm 0.705	0.272 \pm 0.007	1.71
–	1.0	15.335 \pm 1.073	0.272 \pm 0.009	1.77
–	5.0	14.508 \pm 1.328	0.254 \pm 0.019	1.75
–	10.0	11.496 \pm 1.060	0.226 \pm 0.010	1.97
3	0.5	16.100 \pm 1.34	0.317 \pm 0.019	1.97
–	1.0	11.578 \pm 2.06	0.264 \pm 0.003	2.28
–	3.0	11.898 \pm 1.10	0.245 \pm 0.002	2.06
–	5.0	8.908 \pm 0.26	0.202 \pm 0.002	2.27
–	10.0	10.514 \pm 0.83	0.251 \pm 0.003	2.39
–	20.0	8.553 \pm 0.80	0.172 \pm 0.002	2.01
19	0.5	5.453 \pm 1.34	0.151 \pm 0.030	2.77
–	1.0	13.631 \pm 1.02	0.262 \pm 0.015	1.92
–	2.0	10.168 \pm 2.31	0.221 \pm 0.004	2.17
–	2.5	6.336 \pm 1.04	0.173 \pm 0.002	2.73
–	5.0	11.880 \pm 2.37	0.225 \pm 0.003	1.89
–	10.0	11.523 \pm 1.22	0.394 \pm 0.045	3.42
–	25.0	1.685 \pm 0.24	0.055 \pm 0.008	3.26
–	40.0	1.652 \pm 0.11	0.052 \pm 0.0006	3.15
–	50.0	1.353 \pm 0.13	0.037 \pm 0.0007	2.73
–	100.0	0.767 \pm 0.04	0.021 \pm 0.0004	2.74
DPU	0.05	18.880 \pm 0.643	0.301 \pm 0.010	1.59
–	0.1	19.679 \pm 1.035	0.308 \pm 0.016	1.57
–	0.5	18.165 \pm 0.953	0.288 \pm 0.015	1.59
–	1.0	18.158 \pm 0.483	0.284 \pm 0.007	1.56
PUT	1.0	19.939 \pm 0.894	0.324 \pm 0.013	1.62
–	5.0	20.600 \pm 1.074	0.331 \pm 0.009	1.60
LSD 5%		3.0724	0.045	
LSD 1%		4.1107	0.060	

was inactive) (Table 4). Compound **17** gave 31% inhibition at 5 mM, whereas compound **19** was found to possess the highest inhibitory effect. A plateau was attained with rising concentrations (0.5–5 mM) of the latter compound, with a maximum inhibition of about 50% (Fig. 1). From the present experiments it cannot be concluded whether this was due to a lag phase before the plant growth regulator reached the critical site or part of the growth mechanism was really insensitive.

The parent compounds, DPU and PUT, induced higher accumulation of fresh biomass as compared to the standard (0.05 mg.l⁻¹ kinetin) and the other studied compounds (Table 5). However, the maximum optimal concentrations of DPU and PUT were higher (2-fold and 100-fold, respectively) than that of kinetin. Differences in the callus tissue dry matter were observed. The dry/fresh weight ratios revealed that the greatest dry matter accumulation occurred in callus cultures grown on nutrient medium with the three studied compounds. Compound **19** was by far the most active, it induced more than a 2-fold increase in dry matter in wide concentrations range (maximum at 10 mg.l⁻¹ or 20 µM) compared to the standard and the parent compounds.

Conclusions

The results of this study have demonstrated the plant growth regulating activity of some bis-urea derivatives of aliphatic diamines containing 2-6 methylene groups in the hydrophobic moiety. The investigations on the structure–activity relationships confirmed the importance of the length of polymethylene chain and the nature of 3-substituent (ring type and phenyl ring's substituents) in the activity of the urea/diamine-like plant growth regulator group. The compounds containing an even number of meth-

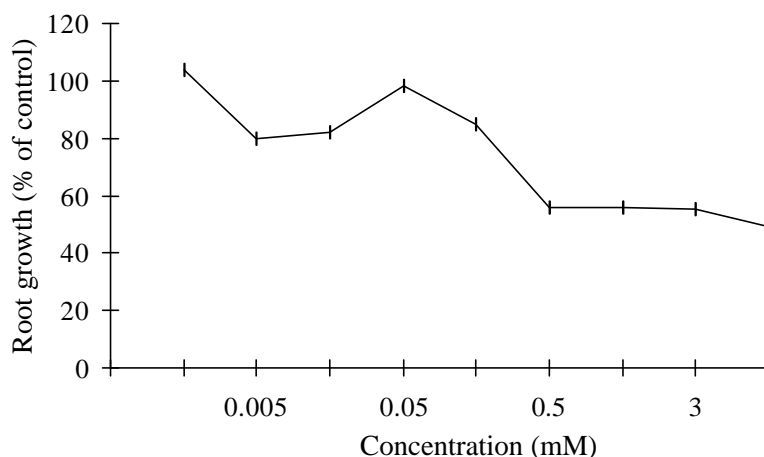


Fig. 1. Dose-response curve of 1,1'-hexamethylenebis[3-(3,5-dichloro-4-pyridyl)]urea (compound **19**) for the inhibition of root growth in intact wheat seedlings

ylene groups were more active than those with odd ones. Halogenation of the aromatic rings increased the activity of the compounds and modified the influence of polymethylene chain. Among the halogen substituents, fluorine (an electronegative atom smaller than other halogens) enhanced to a great extent the activity particularly with increasing the number of methylene groups in the alkylene chain. The integrity of the heterocyclic rings in 1,1'-polymethylenebis-ureas was an essential feature for providing activity in the regulation of root growth in intact wheat seedlings and the stimulation of dry matter accumulation in tobacco callus tissues.

However, on the basis of data presented here, it is difficult to explain the observed effects. Additional investigations are required to elucidate the possible way of their plant growth regulating action.

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