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PLANT GROWTH REGULATING ACTIVITY OF SOME NOVEL 1,1¢-POLYMETHYLENEBIS(3-ARYLSUBSTITUTED)-THIOUREAS

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Summary. The plant growth regulating activity of some novel 1,1′-polymethylenebis(3-arylsubstituted)thioureas 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 inhibited root growth in intact wheat seedlings. Some plant growth regulating structure–activity relationships have been established.

Key words: bioassays, bis-thioureas, 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

Introduction

Derivatives of aryl-disubstituted ureas and thioureas provide a rich source of candidates for development as agrochemical and pharmaceutical products. On the other hand, urea and thiourea derivatives are the most important groups of non-purine cytokinins (Takahashi et al., 1978; Armstrong et al., 1981; Mok et al., 1982; Yonova et al., 1989).

Polyamines are widely distributed in plants and are known to be present in monocots (wheat, barley, rice etc.), in dicots (pea, tobacco, sunflower, mung bean etc.) and in perennial plants (apple, citrus etc.). Recent reports show that polyamines have an important regulatory role in plant growth and development (Galston and Kaur-Sawhney, 1980; Altman and Bachrach, 1981).

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Similar effects of cytokinins and polyamines have been reported in various physiological phenomena including retardation of leaf senescence (Altman, 1982). These provide an evidence that polyamines have some relationship to cytokinins (Galston, 1983).

In view of the above facts and of our desire to develop agricultural substances of high potency, we have fused the arylthiourea moiety and diamines with 1,1′-polymethylenebis(3-arylsubstituted)thioureas structure to study how far this combination could contribute to the plant growth regulating activity.

Several compounds with similar structure have been reported to act as inhibitors of guinea pig liver transglutaminase and plasma transglutaminase (Lee et al., 1985) and as antihypertensive agents (Tilley et al., 1980). Vassilev and Mashev (1974) reported the cytokinin-like activity of 1,1′-ethylene (and hexylene) bis(3-phenyl)-thioureas in relation to senescence delay of radish leaf discs and barley seedlings.

In our earlier paper we showed that some novel bis-arylureas containing different number of methylene groups between both urea bridges possess plant growth regulating activity, and that halogenation of the aromatic rings increases the activity of the compounds and modifies the influence of the polymethylene chain (Yonova et al., 1997).

The aim of present study was to investigate the plant growth regulating activity of 1,1′-polymethylenebis(3-arylsubstituted)thioureas and to compare it with that of the parent compounds (diphenylurea and diaminoalkanes). The effect of halogen substituents on the phenyl rings and the length of polymethylene chain on plant growth regulating activity were also discussed.

Materials and Methods

Chemicals

1,1′-Polymethylenebis(3-arylsubstituted)thioureas, subject of the present study, were synthesized earlier by the authors. The synthesis and physicochemical characteristics of the compounds were described (Yonova and Ionov, 1997). Compounds with the following common formula were investigated:

$$\textit{para}\text{-R-}C_6H_4NHCSNH(CH_2)_nNHCSNHC_6H_4\text{-R-}\textit{para}$$

(1) – (13)

(1) R= -H, n=2	(6) R= -H, n=4	(10) R= -H, n=6
(2) R= -F, n=2	(7) R= -F, n=4	(11) R= -F, n=6
(3) R= -Cl, n=2	(8) R= -Cl, n=4	(12) R= -Cl, n=6
(4) R= -Br, n=2	(9) R= -Br, n=4	(13) R= -Br, n=6
(5) R= -H, n=3		

Among the 13 compounds only four – **1, 5, 6** and **10** have been described in the available references (Lee et al., 1985; Vassilev and Mashev, 1974). However, there are no data about their plant growth regulating activity.

Biological evaluations

The influence of the compounds tested on betacyanin synthesis in *Amaranthus caudatus* cotyledons was investigated after the method of Biddington and Thomas (1973).

The effect of bis-thioureas on growth of excised radish cotyledons was studied according to Green and Muir (1978).

The action of the compounds on root growth of wheat seedlings in the dark was determined according to Stenlid (1982).

The compounds were tested in a concentration range of 1 µM – 5 mM. We selected this wide concentration range because the obtained compounds are derivatives of diamines which have optimal concentration about 7 mM and of phenylurea cytokinins with optimal concentration of 0.01 mM. The data presented are means from two experiments, each in five or six replications. Student–Fisher's procedures were used for statistical calculation.

Results and Discussion

In this paper we report the plant growth regulating activity of some bis-arylthiourea 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 – diphenylurea (DPU) and aliphatic diamines, stimulated amaranthin synthesis stronger than putrescine (PUT) and weaker than DPU and the corresponding bis-arylureas (Table 1). Compound **2** was an exception; it displayed higher betacyanin-promoting activity (131% at 3 mM) as compared to its oxygen analogue (Yonova et al., 1997). *Para* bromine did not activate betacyanin synthesis, and compound **4** showed the opposite activity, inhibiting betacyanin synthesis with 44%. This action of compound **4** (n=2) is probably due to the nearness of both larger bromine atoms and their steric effects on the receptor site.

The compounds tested exhibited moderate stimulating effect on the growth of excised radish cotyledons in darkness (Table 2). In general, bis-arylthioureas were less active than bis-arylureas. The growth stimulating activity of 1,1′-polymethylene-bis(3-phenyl)thioureas increased with increasing the number of methylene groups in the aliphatic chain, the most effective being substance **10** (n=6) (125% at 0.01 mM of the control). Similar tendency was also found for the action of bis-(phenyl)thioureas on the root growth of intact wheat seedlings in the dark – with an increment of the

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

Compds	.	% betacyanin at concentrations of mM				
No	0.001	0.01	0.1	0.5	1	3
1	97±3.3	109±2.4	107±1.4	106±4.3	105±5.1	118±2.9
2	89±4.8	88±6.9	109±4.2	–	102±2.7	131±3.3
3	91±2.8	112±2.3	107±5.0	108±2.7	107±3.0	120±8.1
4	58±1.7	57±1.9	56±2.1	69±2.5	65±3.0	65±3.1
5	101±6.0	82±2.8	72±5.9	99±6.0	109±5.0	138±2.7
6	103±3.3	98±3.5	109±4.9	102±4.2	103±4.2	89±7.1
7	89±2.1	79±2.4	88±1.5	81±3.0	92±4.2	89±3.9
8	98±2.8	100±2.2	96±2.5	95±2.2	106±5.2	107±4.5
9	93±2.9	77±3.3	64±2.7	60±2.2	61±0.9	53±0.9
10	105±4.5	106±4.6	91±2.8	103±1.0	106±2.4	96±2.3
11	118±3.1	113±3.6	118±4.0	–	116±2.1	111±4.8
12	120±1.7	121±3.0	108±3.2	102±2.0	108±5.8	105±3.8
13	88±2.4	102±2.1	100±1.5	102±1.3	93±1.4	90±2.0
DPU	106±2.8	112±1.7	121±2.1	143±3.8	152±2.9	129±1.8
PUT	–	–	–	–	88±2.1	89±3.3

$E_{542\text{-}620}$ for control (buffer) 0.1314±0.0045 (100%);
LSD 5% = 0.020780; LSD 1% = 0.028030

lipophylity the inhibiting effect of the compounds augmented (Table 3). The effect of reactive halogen atom(s) in bis-(phenyl)thioureas depended on the length of polymethylene chain: the inhibiting effect of 1,1′-polymethylenebis-[3-(4-fluorophenyl)]-thioureas decreased with increasing the number of methylene groups while that of bis-(4-bromophenyl)thioureas increased.

The results of this study demonstrated the plant growth regulating activity of some bis-arylthiourea derivatives of aliphatic diamines containing 2–6 methylene groups in the aliphatic chain. Investigations on the structure–activity relationships showed that the plant growth regulating activity depended on the length of polymethylene chain as well as on aromatic phenyl ring’s substituents. These compounds were slightly effective in stimulating betacyanin synthesis and cell enlargement and in inhibiting cell elongation as well. It was evident that the effectiveness of application increased with an augmentation of the aliphatic chain. The same tendency has been found for some naturally occurring and synthetic aliphatic diamines in the other biological systems (Alexieva, 1994). Generally we noted a negative effect of a larger bromine atom on the *para* position of bis-(phenyl)thioureas particularly of those with short polymethylene chain. In this case an antagonistic action could be hypothesized (Iwamura et al., 1979). *Para* fluorine atom (an electronegative atom smaller than other halogens)

Table 2. Effect of some bis-arylthiourea derivatives of diaminoalkanes on expansion of etiolated *Raphanus sativus* L. cotyledons. Values given as percentage of the control ± SD.

Compds	Weight/10 units at concentrations of mM								
No	0.001	0.005	0.01	0.05	0.1	0.5	1	3	5
1	93±1.1	109±3.9	88±3.2	95±1.7	81±2.3	92±1.6	87±3.0	86±2.2	89±3.3
2	108±5.2	111±4.8	109±3.4	107±1.8	105±0.9	113±3.1	110±3.7	103±3.2	108±4.0
3	109±6.0	93±4.6	91±1.7	98±4.8	97±1.6	94±1.9	91±6.2	85±1.9	85±4.9
4	105±1.4	106±2.6	106±2.5	112±4.0	107±4.2	93±2.1	98±2.5	105±1.3	91±4.6
5	86±1.5	84±2.4	84±2.9	85±2.6	85±4.1	86±3.1	81±3.0	94±2.2	–
6	83±0.9	96±1.8	89±0.8	92±4.0	85±2.8	83±3.0	88±5.3	86±3.3	105±1.7
7	107±4.3	100±3.7	96±1.9	93±3.3	87±2.7	100±2.1	96±2.8	107±2.3	102±0.9
8	114±2.1	107±3.9	108±3.3	110±4.4	105±2.5	109±1.9	106±1.6	111±2.9	110±1.2
9	89±1.2	97±2.6	95±1.8	97±1.3	94±0.7	96±1.9	96±1.8	96±2.4	106±1.3
10	108±2.4	95±2.7	125±4.4	113±4.2	104±1.2	111±1.0	103±3.7	104±3.3	103±1.1
11	118±3.8	117±4.5	110±2.2	111±3.5	100±4.0	107±3.1	107±3.0	105±2.4	103±2.0
12	97±1.7	97±1.8	103±0.9	97±1.2	96±1.7	102±0.6	95±1.6	94±2.1	97±3.5
13	102±1.1	97±1.1	97±2.0	102±1.7	100±1.7	98±1.4	101±1.0	100±2.1	94±1.7
DPU	97±3.5	–	111±5.1	–	90±4.0	102±2.4	100±4.0	90±0.6	91±2.8
PUT	103±1.3	–	94±1.2	–	95±0.7	100±2.1	105±2.5	108±2.2	94±1.2

Control (6.7 mM K-Na Pi buffer) 0.1255±0.0040 g (100%);
LSD 5 % = 0.01726; LSD 1 % = 0.02289

Table 3. Effect of some bis-arylthiourea derivatives of diaminoalkanes on root growth of young *Triticum aestivum* L. seedlings. Values given as percentage of the control ± SD.

Compds	Root growth at concentrations of mM										
No	0.001	0.005	0.01	0.05	0.1	0.5	1	3	5	8	10
1	98±1.5	101±0.9	105±1.9	107±2.5	102±3.6	107±2.3	100±4.2	97±1.7	102±3.6	106±5.7	98±0.8
2	90±2.5	73±1.2	80±1.9	88±3.6	89±3.2	87±0.5	82±1.2	85±2.0	91±1.6	–	–
3	96±2.1	96±4.3	84±2.7	93±3.5	96±3.5	99±4.6	92±3.9	88±2.8	78±3.6	–	–
4	114±4.4	125±1.8	127±2.0	119±3.6	116±2.9	112±2.2	121±2.3	112±2.5	108±1.3	–	–
6	100±1.4	109±3.3	108±2.0	103±4.8	95±2.1	100±1.8	99±1.7	106±2.8	115±1.7	–	–
7	91±3.6	89±2.9	91±2.3	93±2.2	89±2.5	89±1.1	96±2.7	95±2.2	86±3.2	–	–
9	102±2.3	106±1.7	105±0.8	108±2.1	103±2.9	105±2.4	81±3.8	82±2.2	88±3.3	–	–
10	114±2.5	107±3.0	101±1.4	95±2.6	96±2.2	93±1.9	90±2.7	80±1.3	79±1.5	–	–
11	115±1.0	119±2.7	108±0.7	112±0.8	101±2.0	99±0.7	106±2.0	91±2.5	103±1.6	–	–
13	92±2.0	105±2.3	82±1.9	85±1.4	85±2.2	81±2.4	70±3.8	69±1.8	70±2.0	–	–
DPU	102±2.2	–	96±2.7	–	93±1.0	–	93±1.3	95±1.3	93±1.4	–	99±6.1
DAE	98±4.1	–	104±2.4	–	112±1.3	–	96±4.9	86±3.1	90±0.7	–	81±1.2
PUT	108±2.6	–	114±2.5	–	110±0.6	–	108±2.9	109±0.9	98±1.3	–	94±3.0
DAH	103±0.6	–	112±2.8	–	112±3.9	–	105±4.6	109±5.7	105±0.8	–	102±2.0

Control (6.7 mM K-Na Pi buffer) 29.52 ± 0.56 (100%); lo » 10 mm
LSD5% =1.7447;LSD1% = 2.315

substituent on the phenyl ring greatly enhanced plant growth regulating activity. For all compounds tested, the dose–effect relationship was linear, excepting compound **2** in inhibiting root growth of intact wheat seedlings. 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 activity. The low activity of almost all bis-thioureas indicates the possibility of designing compounds having antagonistic action. This might be proven by investigations on the competitive binding affinity of the bis-thioureas at the receptor site when mixed with a stronger plant growth regulator in specific bioassay systems.

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