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PLANT GROWTH REGULATING ACTIVITY OF SOME PHOSPHORUS DERIVATIVES OF COUMARIN

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Summary. Plant growth regulating activity of some new synthesised phosphorus containing derivatives of coumarin was investigated. It was shown that some of the compounds inhibited the stem growth of intact pea plants, growth of wheat and cucumber seedlings as well as elongation of excised wheat coleoptile segments. Chemical structure – plant growth regulating activity relationship was also established.

Key words: coumarin, phosphorus containing coumarin derivatives, chemical structure – plant growth activity relationship

Introduction

Coumarin is a native inhibitor and its plant growth regulating activity after exogenous application is well known (Karanov, 1972; Letham, 1978). In a number of model systems using plant organs (or explants) direct application of coumarin has been found to inhibit auxin-induced enhancement of intact seedlings growth, to decrease root growth and in the same time to decline cell wall elasticity (Letham, 1978).

In order to clarify chemical structure – physiological activity relationship a number of coumarin derivatives were tested. Coumarin, 8-methyl coumarin, daphnetin, and 4-methyl-daphnetin all exhibited similar activity in inhibition of *Avena* roots growth; 3-, 4-, 5-, 6- and 7-coumarin, umbelliferone, esculetin, scopoletin and herniarin were much lesser effective. Methoxylation at 4- and 8- position of coumarin did

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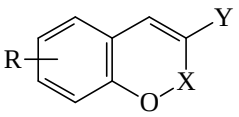
not change the activity in inhibiting seed germination. Hydroxylation and other substitutions usually markedly reduced the activity. Coumarin had higher efficiency than umbelliferone, esculetin, herniarin and scopoletin in inhibiting barley and lettuce seed germination. Only tetrahydrocoumarin appears to be more effective than coumarin (Letham, 1978).

Synthetic analogues of coumarin are insufficiently investigated. Some coumarin derivatives (4-hydroxy-3-substituted coumarins) were found to act as anticoagulants in clinical practice (Manolov, 1989). These compounds possessed also plant growth regulating activity – they inhibited stem and root growth of wheat and cucumber, but in a lower degree than those of coumarin (Alexieva et al., 1992).

In our paper the plant growth regulating activity of some phosphorus-containing derivatives of coumarin was studied. The aim of this research was to find new highly physiological effective compounds as well as to clarify structure-activity relationship.

Materials and Methods

Synthesis of the novel phosphorus containing derivatives of coumarin was described previously (Rodious et al., 1994). Plant growth regulating activity of the compounds (Table 1) was determined in accordance with their influence on the growth of 2-week-old pea plants grown as a water culture in a growth chamber (12 h photoperiod; 25 °C±1).

Table 1. Coumarine derivatives studied 

No.	X	Y	R
1	C=O	H	H
2	C=O	$P(O)(OEt)_2$	H
3	C=O	$P(O)(OEt)_2$	6-Cl
4	C=O	$P(O)(OEt)_2$	6-Br
5	C=O	$P(O)(OEt)_2$	$7\text{-}N(Et)_2$
6	P(O)(OMe)	C(O)OEt	H
7	P(O)(OEt)	C(O)OEt	H
8	P(O)(OEt)	C(O)OEt	$7\text{-}N(Et)_2$
9	P(O)(OEt)	C≡N	H

The influence of the compounds tested on the growth of intact seedlings was determined by measuring the hypocotyl (coleoptile respectively) and root length of 4-

day-old cucumber (*Cucumis sativa*, cv. Pobeda), and wheat (*Triticum aestivum*, cv. Sadovo) seedlings, grown in the dark (25 °C±1).

The excised wheat (*Triticum aestivum*, cv. Zlatoklas) coleoptile segments (1 cm) was used to estimate the auxin-like activity of the compounds tested.

The action of the coumarin derivatives on the growth of pea (*Pisum sativum*, cv. Koray) axillary buds was determined after Wickon and Thimann (1956). The increment of buds length was measured during a 13 day period.

All the compounds were tested at 1, 0.1, and 0.01 mM. The data presented are averaged from 3– 4 independent experiments. The results were processed statistically after Fisher.

Results and Discussion

In experiments aiming to study the effect of the compounds on the growth of pea plants, the most effective derivatives were **3** and **4** which inhibited the second internode of the plants with about 30–35% at concentration 1mM. Their effect was kept also at the lower concentrations (at 0.1 and 0.01mM) (Table 2). As known most of the chemical substances possessing $P(O)(OEt)_2$ – group act as herbicides (Lachkova et al., 1989; Melnikov, 1987). Surprisingly, no diminution of both root length and root fresh weight was observed. On the contrary, almost all compounds at concentrations 0.1 and 0.01mM caused an stimulation of the pea plant root fresh weight and the stimulating effect reached 40–49% (compounds **7** and **8**) and 62% (compound **3**).

The influence of the compounds on the growth of intact cucumber and wheat seedlings is presented in Table 3. All derivatives at 1mM inhibited cucumber root and hypocotyl growth with more than 95%, but the effect on wheat roots was weaker – growth reduction was of about 80%. Inhibitory activity declined with the decrease of the concentration applied. At concentrations 1 and 0.1mM all the compounds exceeded the effect of the standard coumarin (compound **1**). Derivatives containing phosphorus in the ring (**6**, **7**, **8** and **9**) possessed strong inhibitory activity at lower concentrations also (0.01mM). These data confirmed known results about the growth inhibitory action of some coumarin derivatives on wheat coleoptile, maize roots, *Plenum pratense* roots, rise, mung bean, lettuce and clover seedlings (Alexieva et al., 1992; Karanov, 1972; Letham, 1978).

In case excised wheat coleoptile segments were used as a test-object (Table 4), an inhibition was evident at all concentration range tested (1–0.01 mM). However, in this model system 3-[-$P(O)(OEt)_2$] substituted coumarins were more effective than the compounds with phosphorus incorporated in the cycle. Thus, compound **5** inhibited coleoptile segments growth with 87% at 1mM and 65% at 0.1mM, while the compound **8** expressed lower activity – at 1mM it inhibited coleoptile elongation only with 65% and at 0.01mM its action was found to be stimulative – 120% to the con-

Table 2. Influence of coumarin and its phosphorus-containing derivatives on the growth of intact pea plants (Knight et al., 1969)

		2nd Internode		Stem				Root			
No.	Conc.	Length		Length		Weight		Length		Weight	
	(mM)	(mm)	%	(mm)	%	(g)	%	(mm)	%	(g)	%
Control		14.8	100.0	105.8	100.0	408	100.0	132.0	100.0	139	100.0
1	1	11.3	76.4	89.6	84.7	399	97.8	111.0	84.0	88	63.3
	0.1	13.0	87.3	89.3	84.4	406	99.5	107.3	81.3	97	69.8
	0.01	14.9	100.6	102.4	96.8	410	100.4	121.9	92.3	112	80.6
2	1	13.0	87.8	100.6	95.1	383	93.9	89.2	67.6	174	125.2
	0.1	12.8	86.5	93.2	88.1	364	89.2	111.2	84.2	134	96.4
	0.01	13.6	91.9	103.6	97.9	433	106.1	128.0	97.0	132	95.0
3	1	9.8	66.2	74.2	70.1	218	53.4	75.6	57.3	124	89.2
	0.1	12.5	84.4	96.5	91.2	424	103.9	109.1	82.6	226	162.6
	0.01	13.2	89.2	98.3	92.9	416	102.0	124.5	94.3	217	156.1
4	1	10.4	70.3	84.7	80.0	317	77.7	79.3	60.1	128	92.1
	0.1	11.8	79.7	96.8	91.5	398	97.5	112.9	85.5	192	138.1
	0.01	12.9	87.2	94.2	89.0	437	107.1	129.9	98.4	160	115.1
5	1	12.6	85.1	94.8	89.6	376	92.2	81.8	62.0	141	101.4
	0.1	11.5	77.7	88.5	83.6	380	93.1	103.1	78.1	160	115.1
	0.01	13.8	93.2	106.7	100.8	432	105.9	130.0	98.5	168	120.9
6	1	11.8	79.7	89.6	84.7	326	79.9	88.8	67.3	88	63.3
	0.1	12.0	81.8	88.0	83.2	378	92.6	117.2	88.8	162	116.5
	0.01	11.6	78.4	90.0	85.1	426	104.4	133.3	101.0	164	118.0
7	1	10.4	70.3	83.6	79.0	290	71.1	88.0	66.7	98	70.5
	0.1	11.4	77.0	89.3	84.4	419	102.7	122.5	92.8	196	141.0
	0.01	12.0	81.0	93.4	88.3	429	105.1	129.5	98.1	198	142.4
8	1	11.0	74.3	86.2	81.5	270	66.2	89.0	67.4	124	89.2
	0.1	11.5	77.7	90.2	85.2	372	91.2	112.8	85.4	208	149.6
	0.01	12.0	81.1	93.1	88.0	392	96.1	126.1	95.5	150	107.9
9	1	11.7	79.0	80.4	76.0	277	67.9	74.4	56.4	128	92.1
	0.1	12.6	85.1	86.4	81.7	432	105.9	112.1	84.9	178	128.0
	0.01	12.2	82.4	93.8	88.6	408	100.0	124.3	94.2	142	102.2
	LSD 5%	1.1		5.6		24		12.6		14	
	LSD 1%	1.9		10.3		35		19.3		29	

trol, similarly to exogenous applied IAA. Karanov (1972) noted that some coumarin derivatives stimulated growth of barley coleoptile and pea epicotyl segments, sunflower and bean hypocotyl segments. The results presented in this paper showed that the compounds tested strongly inhibited elongation of wheat coleoptile segments which was due probably to the phosphorus containing moieties in their structures.

Table 3. Influence of coumarin and its phosphorus-containing derivatives on the growth of cucumber and wheat seedlings, grown in the dark

Variant	Conc. (mM)	Cucumber		Wheat	
		Root (mm)	Hypocotyl (mm)	Root (mm)	Coleoptile (mm)
Control		75.6	32.8	75.4	46.4
1	1	10.1	6.7	15.8	7.8
	0.1	69.2	17.4	65.8	36.9
	0.01	90.5	34.4	77.0	45.8
2	1	2.4	1.4	10.4	5.0
	0.1	49.6	15.7	58.9	33.3
	0.01	73.4	31.3	75.8	44.6
3	1	1.4	1.1	4.9	2.6
	0.1	47.6	15.4	44.3	22.2
	0.01	71.5	29.6	64.1	39.7
4	1	6.4	1.8	11.8	6.2
	0.1	63.5	23.8	58.4	34.9
	0.01	70.0	32.0	70.1	38.9
5	1	2.4	1.4	10.0	4.4
	0.1	58.0	19.3	54.7	39.3
	0.01	72.7	31.4	73.6	42.2
6	1	1.5	1.0	3.1	1.8
	0.1	53.5	10.4	52.5	33.2
	0.01	63.1	24.0	66.1	40.7
7	1	1.9	1.4	8.9	4.4
	0.1	35.8	12.2	56.3	35.3
	0.01	66.0	26.9	67.6	40.4
8	1	6.1	1.9	7.2	3.6
	0.1	34.9	12.7	46.9	26.8
	0.01	57.9	27.4	65.0	41.2
9	1	3.0	1.8	7.4	3.2
	0.1	35.0	10.8	45.8	22.6
	0.01	59.9	22.1	64.8	39.1
LSD 5%		0.6	0.5	1.4	1.3
LSD 1%		1.1	0.9	2.9	2.3

The foregoing results prompted us to study the growth regulating effect of the substances on axillary bud growth using isolated pea stem buds. All 3-[-P(O)$(OEt)_2$] substituted coumarins were able to repress axillary bud enhancement during the whole period (13 days). However, their action was modest in comparison with the IAA (0.01 mM) (data not shown).

In view of the facts presented in this paper, it could be supposed that compounds with such a chemical structure influenced IAA-metabolism. There are certain data

Table 4. Influence of coumarin and its phosphorus-containing derivatives on the growth of excised wheat coleoptile segments

Variant	Concentration (mM)	Length (mm)	%
Control		5.8	100.0
1	1	2.2	37.9
	0.1	2.9	50.0
	0.01	4.1	70.7
2	1	1.6	27.6
	0.1	3.1	53.4
	0.01	3.7	63.8
3	1	1.0	17.2
	0.1	2.9	50.0
	0.01	3.2	55.2
4	1	0.8	13.8
	0.1	2.8	48.3
	0.01	4.0	69.0
5	1	0.7	12.1
	0.1	2.0	34.5
	0.01	3.6	62.1
6	1	1.8	31.0
	0.1	4.3	74.1
	0.01	4.8	82.8
7	1	1.9	32.8
	0.1	3.8	65.5
	0.01	4.4	75.9
8	1	2.0	34.5
	0.1	3.6	62.1
	0.01	7.0	120.7
9	1	1.8	31.0
	0.1	2.4	41.4
	0.01	4.8	82.8
IAA	0.01	7.2	124.1
LSD 5%		0.6	
LSD 1%		1.3	

concerning the promotion or inhibition of oxidation by coumarin derivatives (Letham, 1978). Control of IAA-oxidation by the compounds tested could be suggested as a regulatory mechanism in plant growth. However, some other mode of action could not be excluded.

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