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SYNTHESIS AND PLANT GROWTH REGULATING ACTIVITY OF SOME NOVEL 2-METHOXY-4-(1- OR 2-PROPENYL)-6-SUBSTITUTED PHENOLS

Emanuil Karanov[1], Ljubomir Iliev[1], Vera Alexieva*[1], Georgi Ts. Georgiev[1], Nguyen T. Thang[2], Liliana Natova[2]

[1]Acad. M. Popov Institute of Plant Physiology, Acad. G. Bonchev Str., Bl. 21, Sofia 1113, Bulgaria
[2]Higher Institute of Chemical Technology, Sofia, Bulgaria

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Summary. The synthesis and plant growth regulating activity of some novel 2-methoxy-4-(1- or 2-propenyl)-6-substituted phenols are described. Most of the compounds possessed cytokinin like activity – they stimulated betacyanin synthesis in *A. caudatus* cotyledons, growth of excised radish cotyledons and induced retardation of chlorophyll disappearance in radish leaf discs. Some chemical structure – plant growth regulating activity relationships have been established.

Introduction

Modification of plant growth and development through the use of plant growth regulators is becoming an increasingly important aspect of modern agricultural practice (Nickell, 1994). The availability of synthetic regulators that mimic the effect of plant hormones has greatly facilitated this practice. Synthetic analogues of the naturally occurring auxins, cytokinins and ethylene have been particularly useful in this regard. However, most of the known plant growth regulators have comparatively low physiological activity. The increase of the doses applied is undesirable because of ecological reasons. All this imposes the search of new high physiologically active substances.

One of the modern approaches of the design and development of new xenobiotics includes a chemical modification of endogenous plant products with moieties

*Corresponding author, Fax 359-(2)-739952

typical for some high active plant growth regulators. We designed and synthesised some novel derivatives of eugenol [2-methoxy-4-(2-propenyl)-phenol] and isoeugenol [2-methoxy-4-(1-propenyl)-phenol]. Eugenol is widely spread in bay, myrtle and carnation oils, and N-containing substituents on 6-position are structural elements of many high active plant growth regulators (Alexieva et al., 1994) and pesticides (Melnikov, 1987).

The aim of this article was to describe the synthesis, physiological activity, and structure-activity relationship of some novel 2-methoxy-4-(1- or 2-propenyl)-6-substituted phenols. The structures of the compounds are presented in Table 1. Only four of them – **5**, **6**, **9** and **12** are described in the literature (Sen and Arora, 1958; Bhaduri and Khana, 1964). However, there are no data available about their plant growth regulating activity.

Materials and Methods

Synthesis of the compounds

Synthesis of 2-methoxy-4-(1- or 2-propenyl)-6-substituted phenols was performed by reacting eugenol (resp. isoeugenol) with a corresponding amine in appropriate organic solvent. When the reaction mixture boiled HCHO was added. After boiling (1 to 13 hours) and filtering the products obtained were purified by recristalization.

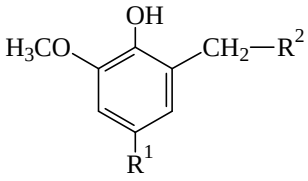
Bioassay Procedures

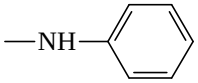
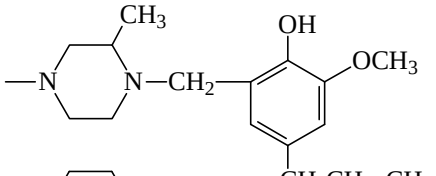
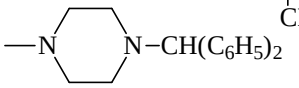
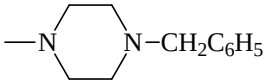
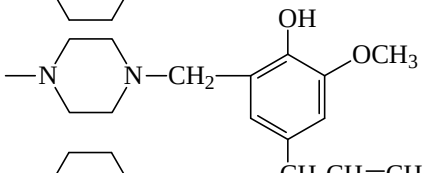
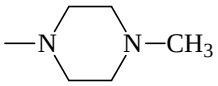
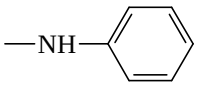
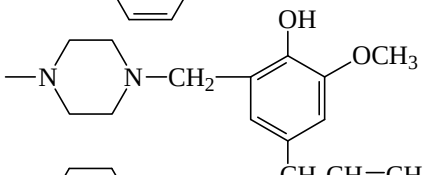
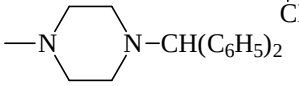
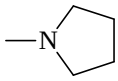
Synthesis of betacyanins in *Amaranthus caudatus* M. Ten *A. caudatus* explants for each replicate, consisting of the upper portion of the hypocotyl plus cotyledons, were placed in Petri dishes on filter paper, moistened with 2 ml test solutions. The betacyanins were determined by the procedure of Biddington and Thomas (1973). Optical density was measured at 542 nm and 620 nm.

Growth of isolated radish cotyledons. Cytokinin-stimulated growth of excised radish cotyledons was measured according to Letham (1971). Seeds of radish (*Raphanus sativus* L., cv. Red) were germinated for 72 h in darkness at 25 °C on filter paper. Ten detached cotyledons per replicate were placed in Petri dishes on filter paper wetted with 3 ml test solutions. The cotyledons were blotted dry and weighed after 72 h on incubation in a growth chamber (25 °C, 12 h photoperiod).

Cytokinin-promoted chlorophyll retention. The inhibition of chlorophyll degradation was tested with radish (Bruce et al., 1965) and barley (*Hordeum vulgare* L., cv. Alfa) (Kende, 1965) leaf explants incubated on test solution in darkness (96 h, radish discs, and 72 h, barley segments). Eight radish discs or four barley segments from each replicate were blotted dry and boiled in 10 ml 80% ethanol for 5 min. The optical density of the cooled, decanted solution was measured at 665 nm.

Table 1. Structural formulas of the compounds



No.	R^1	R^2
1.	—$CH_2CH{=}CH_2$	
2.	—$CH_2CH{=}CH_2$	
3.	—$CH_2CH{=}CH_2$	
4.	—$CH_2CH{=}CH_2$	
5.	—$CH_2CH{=}CH_2$	
6.	—$CH_2CH{=}CH_2$	
7.	—$CH_2CH{=}CH_2$	—$N(CH_3)_2$
8.	—$CH{=}CHCH_3$	
9.	—$CH{=}CHCH_3$	
10.	—$CH{=}CHCH_3$	
11.	—$CH{=}CHCH_3$	—$N(CH_3)_2$
12.	—$CH{=}CHCH_3$	

Growth of etiolated wheat (*Triticum aestivum* L., cv. Zlatoklas) **segments**. Coleoptile segments were isolated from 5-day-old wheat seedlings which had been grown in the dark at 25 °C. Ten segments were placed in Petri dishes on filter paper. After 20 h incubation in darkness the length of the segments was measured.

All compounds were tested at concentrations 0.1–0.001 mM. The data presented are means from at least three experiments, each in three or four replications. Fisher's procedure was used for statistical calculation.

Results and Discussion

Synthesis of the compounds shown in Table 1, was carried out using the well known Manih's reaction (Blik, 1948). According to the literature data interaction between eugenol (resp. isoeugenol), corresponding amine and HCHO is realized at molar ratio 1:1:1 (US Pat.), 1:1:1.5 (Hankovszky et al., 1966) or 1:1:1.75 (Bhaduri et al., 1964). Varying this ratio, time of the reaction duration and temperature of the reaction mixture we obtained comparatively high yields and purity of the derivatives. The formulas, eugenol (isoeugenol)/amine/HCHO ratio, time and temperature of boiling duration, % yield, melting point (resp. refraction coefficient) and R_f values are presented in Table 2.

Table 2. Formulas, solvents, starting compounds ratio, time of duration, temperature, yields, melting points m.p. (refraction coefficients, n_D) and R_f values for the synthesized 6-substituted-2-methoxy-4-(1- or 2-propenyl) phenols. The structure formulas of the compounds are presented in Table 1

No.	Formula	Solvent	eugenol/ amine/ HCHO	React. time (min)	T°C	Yield (%)	m.p. (n_D)	R_f value – system
1	$C_{13}H_{19}NO_2$	toluene	1.2:1:1	600	90-95	54	(1.5361)	0.38 - A
2	$C_{17}H_{19}NO_2$	heptane	1:1:1	90	95-100	55	132-5	0.89 - B
3	$C_{16}H_{24}N_2O_2$	toluene	1:1.2:1	360	90-95	62	86-7	0.45 - A
4	$C_{22}H_{28}N_2O_2$	toluene	1.2:1:1	600	90-95	67	84-5	0.78 - B
5	$C_{28}H_{32}N_2O_2$	ethanol 96%	1:1:1	780	90-95	66	159-160	0.90 - C
6	$C_{26}H_{34}N_2O_4$	toluene	2:1:2.13	360	90-95	94	149-150	0.89 - A
7	$C_{27}H_{35}N_2O_4.2HCl$	toluene	2:1:2.13	120	90-95	84	167-9	0.82 - B
8	$C_{13}H_{19}NO_2$	heptane	1.2:1:1	600	95-100	68	(1.5540)	0.76 - A
9	$C_{17}H_{19}NO_2$	toluene	1:1:1	90	90-95	60	135-6	0.91 - B
10	$C_{15}H_{21}NO_2$	toluene	1:1:1	600	90-95	58	(1.5658)	0.38 - A
11	$C_{28}H_{32}N_2O_2$	toluene	1:1:1	360	90-95	79	1.5750)	0.87 - A
12	$C_{26}H_{34}N_2O_4$	toluene	1.78:1:1.78	360	90-95	64	180-2	0.91 - A

A – chloroform:methanol (9:1); B – *n*-butanol:acetic acid:H_2O (5:1:4); C – chloroform:methanol:benzene (9:1:5)

The growth regulating activities of 6-substituted 2-methoxy-4-(1- or 2-propenyl) phenols were tested by using several bioassay systems, and the results of these experiments are shown in Tables 3–6.

Table 3. Influence of the compounds tested on betacyanin synthesis in *Amaranthus caudatus* M. cotyledons

Compounds	Conc. (mM)	$E_{542-620}$	% to the control	Compounds	Conc. (mM)	$E_{542-620}$	% to the control
1	0.1	0.138	94	7	0.1	0.150	102
	0.01	0.166	113		0.01	0.170	116
	0.001	0.190	129		0.001	0.194	132
2	0.1	0.175	119	8	0.1	0.161	110
	0.01	0.183	124		0.01	0.178	121
	0.001	0.198	185		0.001	0.201	137
3	0.1	0.197	134	9	0.1	0.149	101
	0.01	0.200	136		0.01	0.163	111
	0.001	0.186	126		0.001	0.173	118
4	0.1	0.187	127	10	0.1	0.141	96
	0.01	0.199	135		0.01	0.154	105
	0.001	0.184	125		0.001	0.186	126
5	0.1	0.140	95	11	0.1	0.214	146
	0.01	0.162	110		0.01	0.218	148
	0.001	0.180	122		0.001	0.202	137
6	0.1	0.212	144	12	0.1	0.135	92
	0.01	0.200	136		0.01	0.159	108
	0.001	0.193	131		0.001	0.211	144
LSD 5%						0.012	
LSD 1%						0.027	

Control (buffer) 0.147 (100%); BA 0.01 mM 0.266 (181%); 4-PU-30 0.01 mM 0.200 (136%)

Initially, the growth stimulating activity of the novel compounds was examined in three bioassays which respond to cytokinins, since they possess structural moieties typical for some novel compounds, classified as cytokinin antagonists (Alexieva et al., 1994). Surprisingly, in terms of betacyanin synthesis in *A. caudatus* M. cotyledons, almost all the derivatives tested showed high stimulating activity (Table 3). Among the eugenols, compounds **2** at concentration 0.001 mM exceeded the effects of both standards. High activity showed also **3**, **4**, **6** and **7** – their stimulating action was kept in the whole concentration range. In general, isoeugenols gave up to the eugenols, with exception of the compound **11**, which stimulated betacyanin synthesis with 37–48% (to the control) and its effect was similar to that of the standard 4-

PU-30 (136% to the control). Generally, the derivatives with smaller molecules possessed higher activity due probably to facilitated penetration.

Table 4. Influence of the compounds tested on the growth of *Raphanus sativus* L. cotyledons

Compounds	Conc. (mM)	Weight/ 10 units (g)	% to the control	Compounds	Conc. (mM)	Weight/ 10 units (g)	% to the control
1	0.1	0.254	121	7	0.1	0.271	129
	0.01	0.304	145		0.01	0.277	132
	0.001	0.294	140		0.001	0.300	143
2	0.1	0.220	105	8	0.1	0.223	106
	0.01	0.229	109		0.01	0.234	111
	0.001	0.248	118		0.001	0.254	121
3	0.1	0.251	120	9	0.1	0.226	107
	0.01	0.250	119		0.01	0.229	109
	0.001	0.254	121		0.001	0.231	110
4	0.1	0.254	121	10	0.1	0.216	103
	0.01	0.257	122		0.01	0.227	108
	0.001	0.272	130		0.001	0.222	106
5	0.1	0.238	113	11	0.1	0.216	103
	0.01	0.234	111		0.01	0.230	110
	0.001	0.247	118		0.001	0.238	113
6	0.1	0.222	106	12	0.1	0.224	107
	0.01	0.233	111		0.01	0.233	111
	0.001	0.267	127		0.001	0.238	113
LSD 5%						0.014	
LSD 1%						0.023	

Control (H_2O) 0.210 (100%); BA 0.01 mM 0.337 (160%); 4-PU-30 0.01 mM 0.316 (150%)

The derivatives from both groups (substituted eugenols and isoeugenols) stimulated growth of excised radish cotyledons, the most effective being **1**, **4** and **7** (from 30% to 43% to the control) (Table 4). However, in this model system all the derivatives were less active than the standards (160% and 150% to the control).

Another physiological activity due to cytokinins is the delay of senescence of leaf explants. The ability of some of the novel compounds to retard senescence is shown in Table 5. In concentration 0.01 mM the derivatives induced significant retardation of chlorophyll disappearance in radish leaf discs (dicotyledonous plant). Greatest effect was produced by compound **2** at concentration 0.1 mM – 260% to the control, followed by 6-diphenylamino- (compound **1**), N-methylpiperazino- (compound **5**) and 6-dimethylamino-substituted (compound **7**) eugenols.

Table 5. Influence of the compounds tested on chlorophyll degradation in radish (*Raphanus sativus* L.) and barley (*Hordeum vulgare* L.) leaf segments

Compound	Conc. (mM)	Radish leaf discs E_{665}	Radish leaf discs %	Barley leaf segments E_{665}	Barley leaf segments %
Control	–	0.170	100	0.129	100
BA	0.01	0.508	299	0.207	160
4-PU-30	0.01	0.564	332	0.170	132
1	0.1	0.310	182	0.139	108
	0.01	0.302	178	0.134	104
2	0.1	0.443	260	0.143	111
	0.01	0.210	124	0.150	116
5	0.1	0.231	136	0.124	96
	0.01	0.285	168	0.140	108
7	0.1	0.266	156	0.159	123
	0.01	0.278	164	0.170	132
9	0.1	0.263	155	0.131	102
	0.01	0.272	160	0.137	106
11	0.1	0.238	140	0.134	104
	0.01	0.245	144	0.145	112
12	0.1	0.182	107	0.120	93
	0.01	0.191	112	0.136	105
LSD 5%		0.014		0.010	
LSD 1%		0.021		0.017	

Control values: Radish leaf discs – initial state – 5.30 mg chlorophyll ($a+b$).dm^{-2}
after 96 h ageing – 1.21 mg chlorophyll ($a+b$).dm^{-2}
Barley leaf segments – initial state – 3.96 mg chlorophyll ($a+b$).dm^{-2}
after 96 h ageing – 0.92 mg chlorophyll ($a+b$).dm^{-2}

The failure of all the derivatives to elicit a cytokinin-like response in barley leaf segment bioassay (monocotyledonous plant) is not puzzling. It could be due to their poor penetration or metabolic inactivation in this model system.

As shown in Tables 3–5, the compounds tested possessed cytokinin-like activity – they mimicked the effect of exogenous applied cytokinins and, in some cases, their stimulating activity was similar to those of BA (benzyladenine) and 4-PU-30 [N^1-(2-chloro-4-pyridyl)-N^2-phenylurea]. However, on a base of the close structural analogy between these derivatives and some native and synthetic phenolic inhibitors we tested the compounds in a typical for auxins physiological reaction – coleoptile elongation of dark incubated *Triticum aestivum* L. segments. The results presented in Table 6 show that high concentrations (0.1 mM) provoked an inhibition of excised coleoptile growth. However, the lower concentrations (0.01–0.001 mM) stimulated segment elongation with 15–40 % to the control. In this bioassay system isoeugenols

Table 6. Influence of the compounds tested on the elongation of *Triticum aestivum* L. coleoptile segments

Compounds	Conc. (mM)	Elongation (mm)	% to the control	Compounds	Conc. (mM)	Elongation (mm)	% to the control
1	0.1	2.2	110	7	0.1	1.8	90
	0.01	2.4	120		0.01	2.2	110
	0.001	2.5	125		0.001	2.3	115
2	0.1	1.6	80	8	0.1	1.8	90
	0.01	1.9	95		0.01	2.0	100
	0.001	2.2	110		0.001	2.4	120
3	0.1	1.6	80	9	0.1	2.4	120
	0.01	2.0	100		0.01	2.8	140
	0.001	2.2	110		0.001	2.2	110
4	0.1	1.6	80	10	0.1	1.8	90
	0.01	2.4	120		0.01	2.0	100
	0.001	2.6	130		0.001	2.4	120
5	0.1	1.9	95	11	0.1	2.4	120
	0.01	2.0	100		0.01	2.4	120
	0.001	2.2	110		0.001	2.6	130
6	0.1	1.7	85	12	0.1	2.8	140
	0.01	2.0	100		0.01	2.4	120
	0.001	2.3	115		0.001	2.2	110
LSD 5%						0.1	
LSD 1%						0.3	

Control 2.0 mm (100%); IAA 0.01 mM 5.0 mm (250%)

exceeded the activity of eugenols. Growth stimulating action on coleoptile growth of the eugenols and isoeugenols could be explained with their influence on IAA metabolism. There are certain data concerning the promotion or inhibition of IAA-oxidation by phenolic derivatives (Letham, 1978). It is generally accepted that phenolic compounds with two or three adjacent hydroxylic groups (e.g. caffeic, chlorogenic and gallic acids) enhance the action of endogenous IAA, i.e. they act as IAA-oxidase inhibitors, while the phenols with a single hydroxyl group (e.g. *p*-coumaric and ferulic acids) antagonize IAA (Letham et al., 1978). However, the validity of this conclusion has been questioned. In the list of our compounds only **2**, **5** and **9** possess 2 hydroxylic groups, at that long distance, but almost all the derivatives at concentration 0.001 mM stimulated segment growth. Therefore, control of IAA-oxidation by the compounds tested could be suggested as a regulatory mechanisms in plant growth. However, an other mode of action is also possible.

Results described in this paper clearly demonstrated growth stimulating activity of the novel 2-methoxy-4-(1- or 2-propenyl)-6-substituted phenols (derivatives of the

eugenol and isoeugenol). However, on the base of data presented here, it is difficult to explain the observed effects. Additional investigations using intact plants will verify the ability of the newly synthesised compounds to stimulate plant growth. Such experiments will also throw light on the possible mode of their plant growth regulating action.

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