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BRIEF COMMUNICATION

Plant growth regulating activity of orotic acid and its 1-cyclohexyl and 1-phenyl derivatives

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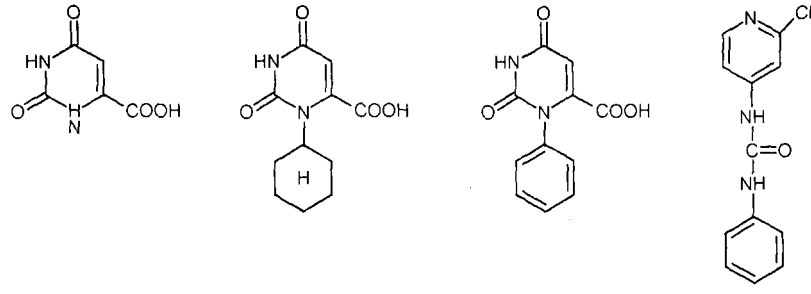
Abstract

Cytokinin-like activity of orotic acid and its 1-cyclohexyl and 1-phenyl derivatives was tested estimating the anthocyanin accumulation and inhibition of root formation in isolated buckwheat cotyledons (*Fagopyrum esculentum* Moench.). The anthocyanin content was stimulated most by 1-phenylorotic acid. Strong synergetic effect of the phenylurea cytokinin 4PU-30 was found.

Additional key words: anthocyanin accumulation, buckwheat, *Fagopyrum esculentum*, 4PU-30, root formation.

Cytokinins, a family of plant hormones which promote division, growth and differentiation in plant cells, have been the subject of numerous studies. Cytokinins can be structurally classified in at least two categories: adenine cytokinins and urea cytokinins. It was recently established that the purine ring is not necessarily required for strong cytokinin activity, and is replaceable by other nitrogen-containing heterocycles (Nishikava *et al.* 1996). One of the strongest synthetic cytokinins has been N-(2-chloro,4-pyridyl)-N'-phenyl urea (forchlorfenuron, 4PU-30) (Shudo 1994).



1 2 3 4

Fig. 1. Structures of the tested compounds: 1 - orotic acid; 2 - N_1-cyclohexylorotic acid; 3 - N_1-phenylorotic acid; 4 - 4PU-30 [N-(2-chloro,4-pyridyl)-N'-phenylurea].

Some studies show that the orotic acid, a natural metabolite and a key precursor in pyrimidine nucleotide biosynthesis, stimulates the growth of animals, plants and microorganisms (Čihak and Reutter 1980, Falk 1985). Therefore, it was interesting to investigate the plant growth regulating activity of orotic acid and of two of its derivatives: cyclohexyl- and phenylorotic acids, and also the combined effect of these compounds with 4PU-30 (Fig. 1). As part of cytokinin action is specific stimulation of anthocyanin biosynthesis in plants (Servettaz *et al.*

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1975, Margna and Vainjarv 1985), we studied anthocyanin accumulation as a criterion of the cytokinin-like activity of the tested compounds. The other criterions chosen were root formation inhibition and the increase of the explants fresh mass (Clemenceau *et al.* 1996).

The orotic acid is a product of the *Sigma Chemical Company* (St. Louis, MO, USA) and 4PU-30 was kindly provided by Prof. Karanov and Prof. Shudo. Orotic acid derivatives were synthesized by authors (Shopova *et al.* 1993, Dodoff and Shopova 1998).

The physiological activity of the compounds studied was tested using the buckwheat (*Fagopyrum esculentum* Moench.) cotyledons (Lejezak *et al.* 1996). After 4 d of dark germination, 15 seedlings were selected for uniformity, the cotyledons were cut and grown in Petri dishes (9 cm) lined with two discs of filter paper wetted with 10 cm^3 of distilled water (control) or solutions of the tested compounds (final concentrations of 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M) either alone or in combination with the synthetic cytokinin 4PU-30 (in concentration 10^{-5} M). The cotyledons were grown at about 20 °C in 12-h photoperiod (irradiance of 100 μmol m^{-2} s^{-1}) for 5 d. Explants of ten cotyledons were then selected and their mass was measured. Rooting ability of the explants was observed visually at the termination of the experiments. After that the explants were quickly ground in a mortar, followed by the addition of 20 cm^3 of 1 % solution of concentrated hydrochloric acid in methanol. The mixture was transferred to an Erlenmeyer flask, and the extraction allowed to continue for 18 h in refrigerator. Extracts were then clarified by filtration, and the absorbance (A) of the samples at 530 and 657 nm was measured using spectrophotometer *Specol 11* (*Carl Zeiss*, Jena, Germany). Anthocyanin content (AC) in the cotyledons (explants) was determined according to Mancinelli and Schwartz (1984) by the equation:

$$AC = (A_{530} - 0.25A_{657}) / m$$

(m designates the mass of the explants) and presented as a percentage of control. Each experiment was replicated four times. The data were processed statistically after Fisher (Steel and Torrie 1960).

Table 1. Effects of orotic acid and its derivatives on the growth of the tested buckwheat cotyledons grown for 5 d from initial explant mass 0.03 g. Means ± SD of at least four experiments.

Compounds	Concentration [M]	Fresh mass [g explant^{-1}]	[% of control]	Anthocyanin content [ΔA g^{-1}(f.m.)]	[% of control]	Number of explants with roots
Control		0.068 ± 0.003	100	0.59 ± 0.06	100	9
1- orotic acid						
1a	10^{-3}	0.062 ± 0.005	91	0.56 ± 0.05	94	7
1b	10^{-4}	0.071 ± 0.006	104	0.70 ± 0.05	118	8
1c	10^{-5}	0.074 ± 0.004	108	0.61 ± 0.04	103	8
1d	10^{-6}	0.070 ± 0.002	103	0.59 ± 0.03	100	9
2-cyclohexylorotic acid						
2a	10^{-3}	0.059 ± 0.006	87	0.61 ± 0.04	102	5
2b	10^{-4}	0.065 ± 0.003	95	0.71 ± 0.07	120	8
2c	10^{-5}	0.075 ± 0.008	110	0.72 ± 0.07	121	8
2d	10^{-6}	0.066 ± 0.006	97	0.60 ± 0.06	101	9
3-phenylorotic acid						
3a	10^{-3}	0.062 ± 0.003	91	0.70 ± 0.05	117	3
3b	10^{-4}	0.067 ± 0.007	97	0.78 ± 0.06	131	5
3c	10^{-5}	0.071 ± 0.006	104	0.72 ± 0.06	120	7
3d	10^{-6}	0.070 ± 0.005	103	0.66 ± 0.07	111	8
4 - 4PU-30	10^{-5}	0.089 ± 0.004	130	0.83 ± 0.04	140	0
1b + 4		0.092 ± 0.008	135	1.16 ± 0.08	196	0
1c + 4		0.093 ± 0.007	137	0.97 ± 0.08	163	0
2b + 4		0.095 ± 0.006	139	1.03 ± 0.06	173	0
2c + 4		0.096 ± 0.008	142	0.89 ± 0.04	149	0
3b + 4		0.095 ± 0.003	139	0.92 ± 0.05	155	0
3c + 4		0.099 ± 0.005	145	1.22 ± 0.05	205	0
3d + 4		0.090 ± 0.006	132	1.01 ± 0.07	170	0

The reference cytokinin 4PU-30 increases the fresh mass of cotyledons by 30 %, and no roots have grown. Only phenylorotic acid inhibited the root formation - an indication of cytokinin-like effect (Margna and Vainjarv

1985). The fresh mass and the root formation diminished at the highest concentration of the tested compounds, which was probably already toxic.

A considerable synergetic effect was observed at combined treatment with the tested orotic acids and 4PU-30. The fresh mass was stimulated by over 30 %. No roots grew in all combinations, therefore the increase of the fresh mass was due to the growth of the cotyledons only. On the contrary, the cotyledons formed roots and hypocotyls in the control and in the presence of the individual tested compounds, so their fresh mass increase was due to the development and regeneration of the whole plant.

The tested compounds, especially phenylorotic acid, stimulate anthocyanin accumulation (Table 1). Synergetic effects of the studied compounds with 4PU-30 were established. The highest activity was achieved by the combinations of 4PU-30 with phenylorotic acid at low concentration ranges. It is necessary to point out that 4PU-30, applied at a concentration of 10^{-8} M, did not cause a full inhibition of root formation (data not shown). However, the combined application of 10^{-8} M 4PU-30 and 10^{-5} M phenylorotic acid inhibited root formation by about 10 %. In addition, this combination led to approximately twice as much anthocyanin accumulation as compared to the individual effect of 10^{-8} M 4PU-30.

Probably the cytokinin-like activity of phenylorotic acid is related to its structure. If the compound is considered as a cyclic ureide, the fragment of phenylurea is evident. It is possible also that the stimulating effects of the tested orotic acids are related to the biosynthesis of the aromatic aminoacids. The assumption that orotic acid increases messenger RNA synthesis has been discussed by Čihak and Reutter (1980). It is also known, that phenylalanine is coded by fragments of polyuridilic acid. Orotic acid, as a precursor of polyuridilic acid, should be able to stimulate the synthesis of phenylalanine, which provokes anthocyanin accumulation. On the other hand, it has already been discussed that the cytokinins influence the permeability of the cell membranes (Margna and Vainjarv 1985). On the basis of the established synergism it may be assumed that phenylurea cytokinin 4PU-30 improves the penetration of orotic acid and its derivatives into the cell. Further biochemical research is necessary for the exact explanation of the mode of action of the tested compounds.

As the orotic acid is natural metabolite, the results obtained have a bearing on environment preservation.

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