Phytochemistry, 1976, Vol. 15, pp. 661–663. Pergamon Press. Printed in England.

PHYSIOLOGICAL EFFECTS OF BETALAINS UPON HIGHER PLANTS

GÖRAN STENLID
Department of Plant Physiology, Agricultural College of Sweden, S-750 07 Uppsala 7, Sweden

(*Received* 31 *December* 1975)

Key Word Index—*Beta vulgaris*; Chenopodiaceae; betalain; betanin; indole-3-acetic acid; ATP formation; IAA oxidase.

Abstract—Red and yellow betalains isolated from red beetroots by means of gel filtration were strong inhibitors of indole-3-acetic acid oxidase; 50% inhibition was obtained at 5×10^{-7} M and 3×10^{-7} M respectively. Concentrations of 10^{-4} M betanin had no effect upon ATP production in mitochondria. The red pigment relieved the inhibitory effects upon wheat root elongation caused by indole-3-acetic acid but not the inhibition caused by 2,4-dichlorophenoxyacetic acid.

INTRODUCTION

The betalains have been frequently studied both from a chemical point of view and as a chemotaxonomic character of the order Centrospermae [1, 2]. Their possible physiological role has not aroused the same interest [2, p. 131] and it seemed worthwhile to study their effects upon some plant processes. Since they substitute as pigments for the anthocyanidins, it is of special interest to test them upon processes known to be affected by anthocyanidins. Such processes are root growth, interactions with auxins (including effects upon IAA oxidase) and ATP formation in mitochondria [3, 4].

RESULTS AND DISCUSSION

The destruction of indole-3-acetic acid (IAA) was drastically inhibited by betalains from red beet tissue (Fig. 1). Both the yellow and the red fractions produced almost total inhibition at concentrations between 10^{-6} M and 10^{-5} M and 50% inhibition was obtained at 3×10^{-7} to 5×10^{-7} M. Quercetin and caffeic acid, two well known inhibitors of IAA oxidase, give 50% inhibition at *ca* 2×10^{-6} M, and the betalains must be classified among the most efficient inhibitors known for the enzymatic destruction of IAA.

For phenolics to inhibit IAA oxidase, an *o*-dihydroxylic group is a normal prerequisite [5]. In betanin one of the two hydroxyls is masked by glucose (which normally reduces the activity) and in vulgaxanthin there are no hydroxyl groups at all. Therefore some other structural detail must be responsible for the inhibitory activity in the case of betalains. Betanin, being an indole derivative, might act as a structural analogue competing with IAA but such an explanation is not possible for the yellow vulgaxanthin. Probably some other characteristic, such as the carboxyl groups, leads to the inhibitory activity.

There are no conspicuous effects of the beet pigments upon the elongation of wheat seedling roots grown in a nutrient solution (Table 1). The red pigments had no effects and the yellow fractions were inhibitory at higher concentrations. When betanin was tested in combination with IAA (Table 1), the pigment counteracted IAA giving a distinct stimulation of wheat root elongation in the presence of IAA in the surrounding solution. No such antagonistic effect was observed when root elongation was inhibited with the synthetic auxin 2,4-D. As auxins inhibit root elongation it could be expected that substances inhibiting IAA oxidase should be synergistic to IAA and increase its inhibitory activity. It has also been shown that some inhibitors of IAA oxidase (such as caffeic acid) may act in this way [6–8]. The deviating behaviour of betanin can be related to the fact that it is an indole derivative and therefore inhibits not only IAA oxidase but possibly also the effect of IAA itself at some site necessary for its effect upon root elongation. The latter effect may be predominant in wheat roots, giving

Table 1. Effect of beet pigments on root elongation of young wheat seedlings

Beet pigment added	Auxin added —	IAA 3×10^{-8} M	2,4-D, 4×10^{-7} M
—	100	31	48
Betanin, 10^{-6} M	99	—	—
Betanin, 3×10^{-6} M	102	42	44
Betanin, 10^{-5} M	103	52	51
Vulgaxanthin, 10^{-6} M	97	30	45
Vulgaxanthin, 3×10^{-6} M	70	32	38

Values expressed as % of elongation in control medium without extra additions.

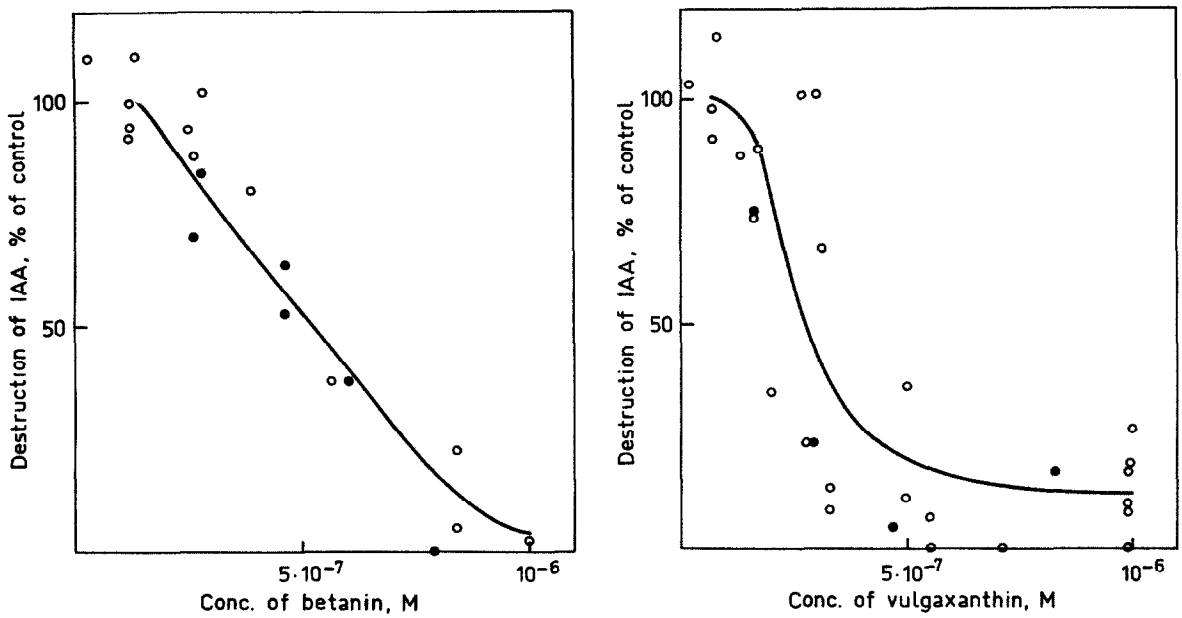

Fig. 1. Effect of red and yellow betalains on the destruction of IAA. IAA oxidase prepared from wheat roots (○) or barley roots (●). Phloridzin or 2,4-dichlorophenol (10^{-5}–3×10^{-5} M) added as a stimulator in control solns.

rise to a stimulation of root growth. The concentrations necessary for the effect upon root growth are higher than those giving total inhibition of IAA oxidase. The penetration to the site of action is certainly slower in the case of root growth.

The betalains do not give any effect upon ATP production in mitochondria. Thus, ATP production in cucumber mitochondria treated with betanin at 3×10^{-5} M and 10^{-4} M was 94 and 85% respectively and with vulgaxanthin at 3×10^{-5} M was 93% of controls. In this respect the betalains differ from many of the flavonoids (especially the aglycones) [4]. The hydrophilic properties of the betalains may decrease the possibilities to act at the mitochondrial membranes.

There are great similarities in the ecological role of anthocyanins and betanin [9]. However, the change from anthocyanins to betalains in the Centrospermae has the following physiological consequences: (1) The possible effects upon IAA oxidase are more decidedly inhibitory; and (2) There is a possibility of structural competition between betanin and IAA.

There are, however, no indications that the betalains play any role *in vivo* as regulators of IAA activity. But as with the flavonoids, they must be considered as potential modifiers of auxin metabolism. An effect in this respect may have some bearing on the fact that many families containing betalains have accessory cambia, as the formation of these cambia certainly to some extent is regulated by auxins.

EXPERIMENTAL

Separation of pigments. Roots (hypocotyls) of red beets (*Beta vulgaris* L. var. *rubra*) were cut into pieces and extracted with boiling H_2O. 50 ml portions of the aq extract were pptd with 500 ml 99.5% EtOH and filtered. The ppt. was dissolved in Pi buffer (pH 6.1, 0.01 M). The soln was passed through a column of Sephadex G25 and eluated with the same buffer. A good separation was obtained with one dominating red fraction and two yellow fractions (one minor one before and one major one after the red fraction). The absorption spectra showed maxima at 535 nm (the red pigment) and at 474 nm (the major yellow pigment). This agrees quite well with the values reported in literature [1] for betanin and vulgaxanthin. Concentrations were estimated from the absorbance at 535 and 474 nm using the molar extinction coefficients 60500 for betanin [10] and 25300 for vulgaxanthin [11].

Root growth. Young wheat seedlings (*Triticum aestivum* L. cv Svalöf's Diamant II) were used for the experiments which were performed as described elsewhere [12]. The root elongation during 18–20 hr at 23° in the dark was used as a measure of root growth. *Mitochondria* were prepared from darkgrown cucumber hypocotyls and ATP was determined with the firefly-luciferase method as described previously [4]. *IAA oxidase preparations* were obtained from young roots of wheat seedlings (cv Svalöf's Diamant II) which were ground with Pi buffer (pH 6.1; 0.02 M) at 2°, pressed through cheese cloth and centrifuged at 20000 × g for 15 min. The supernatant was passed through Sephadex G25 and the turbid fractions showing activity in destroying IAA were combined to give the "IAA oxidase" preparations. They were stored frozen at −25° and used immediately after thawing. The destruction of IAA was followed by means of the method of Gordon and Weber as described previously [13]. For the demonstration of the inhibitory action of the betalains it was necessary to add a stimulator of IAA oxidase (phloridzin or 2,4-dichlorophenol). Some experiments were also performed with enzyme preparations from barley roots.

Acknowledgement—I am indebted to Miss Margaretha Melin for technical assistance.

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