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EFFECT OF *p*-HYDROXYBENZOIC ACID ON GROWTH, AUXIN CONTENT AND AUXIN CATABOLISM

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Abstract—*p*-Hydroxybenzoic acid at low concentrations causes a stimulation of the growth of stem sections, whereas at higher concentrations the elongation may be inhibited. The compound acts as an activator of the IAA-oxidizing system and produces a decrease in the endogenous level of auxins (IAA-like substances) which can be extracted from *Lens* stem and separated by paper chromatography.

INTRODUCTION

MANY phenolic compounds inhibit the elongation of plant sections when used in high concentration, whereas at lower level the growth may be stimulated.[1,2] It was established earlier that the phenols act as inhibitors or activators of the destruction of β-indolylacetic acid *in vitro*,[3–5] and more recently *in vivo*,[6] thus influencing growth.[7] If the phenolic substances—which were considered as cofactor of the IAA oxidizing system[8]—change the *in vivo* activity of the enzymes controlling the IAA catabolism, it can be assumed that the endogenous auxin level will also be modified.[9] This hypothesis was recently confirmed: chlorogenic acid, which inhibits the IAA oxidase, causes an increase of the IAA-like substances extracted from *Lens* root.[10] On the other hand, it has been frequently reported that monophenols usually reduce the level of IAA, while polyphenols have a synergetic effect.[11–13]

These facts can be related to *in vivo* auxin metabolism,[14,15] but the significance of the action of endogenous phenolic substances *in vivo* is not yet clearly established.[16] This paper presents some results on the effect of *p*-hydroxybenzoic acid (*p*-HBA), on the growth, the auxin content and the auxin catabolism of the stem of *Lens culinaris*. The presence of *p*-HBA, in several plant tissues, was reported[17] and its biosynthesis has been studied.[18]

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RESULTS

Growth

The effect of *p*-HBA on the elongation of the 10 mm sections of the stem of *Lens* (12 hr of incubation) is shown in Fig. 1. There is a maximum stimulation in the growth response at 10^{-6} M. However, when *p*-HBA was used in higher concentrations (10^{-5} –10^{-3} M), there is an inhibition of the elongation which increases with increasing concentrations. These results are in agreement with those of Vieitez and Coll.[2] It can be also noted that the growth properties of *p*-HBA were quite different than those of IAA. Consequently, this observation

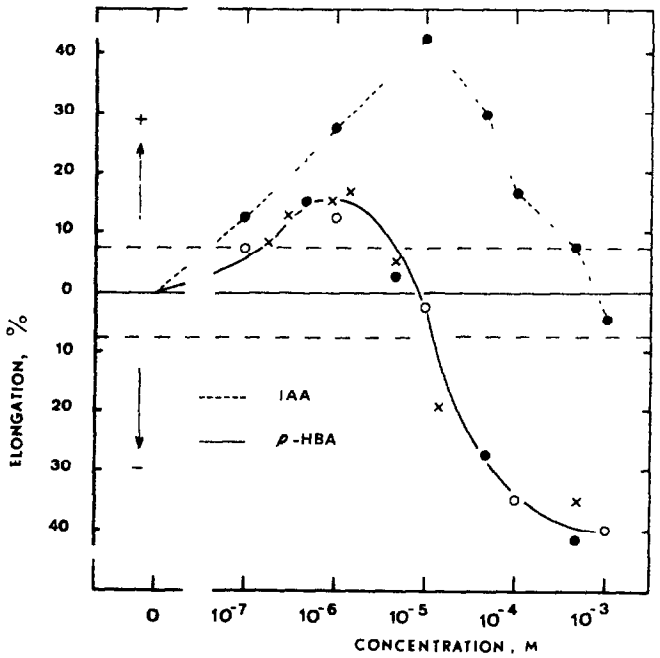

FIG. 1. EFFECT OF *p*-HBA AND IAA ON THE GROWTH.

The growth is expressed in terms of per cent elongation of the 10-mm sections of the stem of *Lens*. Incubation time: 12 hr. Buffered solution + 2% sucrose (pH 6·1).

seems to indicate that *p*-HBA cannot be considered as a growth hormone as it was previously stated for certain naturally occurring phenolic acids.[16]

Auxin Content

To study the action of *p*-HBA on the concentration of endogenic growth substances (IAA-like substances) of *Lens*, a series of experiments was made using 10-mm stem sections. These sections were treated with *p*-HBA at different concentrations (10^{-7}–10^{-3} M) for 12 hr (25 ; dark) and the results are shown in Table 1. As it can be observed, the level of the IAA-like substances, extracted from the stems, decreases with increasing concentrations of *p*-HBA.

TABLE 1. CHANGES IN THE ENDOGENOUS GROWTH-SUBSTANCES (IAA-LIKE SUBSTANCES) IN 10 mm SECTIONS OF THE STEM OF *Lens culinaris* SEEDLINGS TREATED WITH *p*-HYDROXYBENZOIC ACID DURING 12 hr

Concentration of *p*-HBA (M)	Auxin content (μg/100 g fresh wt.)	Change %
O (control)	8·07±0·92	—
10^{-7}	8·49±0·85	+5·2
10^{-6}	7·16±0·98	−11·2
10^{-5}	8·18±1·04	+1·3
10^{-4}	5·61±1·34	−30·4
10^{-3}	4·12±1·07	−48·9

Auxin Catabolism

As can be seen in Fig. 2, *p*-HBA causes, at a concentration of 10^{-4} M a stimulation of the activity of IAA-oxidase prepared from *Lens* root. The activation increases with time of incubation (from 0 to 60 min), then remains approximately constant. It can also be observed (Fig. 3) that the intensity of the reaction is a function of the concentration of the accelerator; from 10^{-7}–10^{-5} M, the stimulation increases with increasing concentration, then from 10^{-5}–10^{-4} M, the activation is optimum. Consequently, *p*-HBA, for each concentration

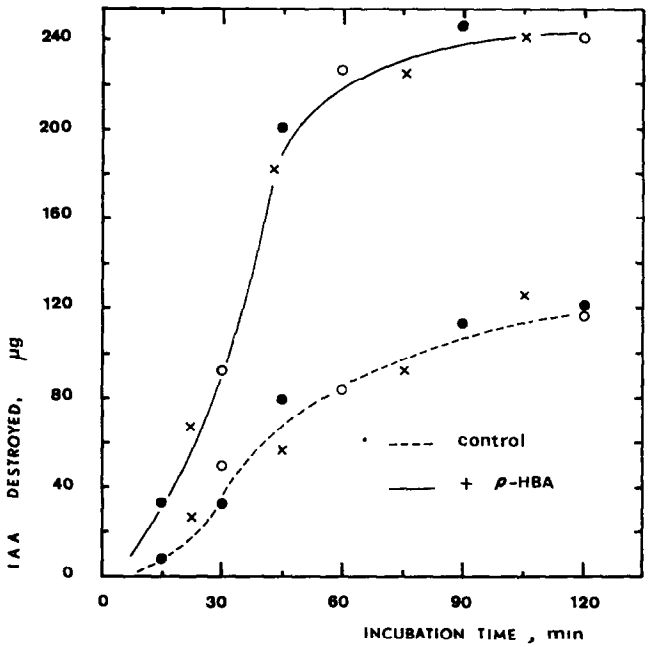

FIG. 2. EFFECT OF IAA-OXIDASE SYSTEM FROM *Lens* ROOT.

The enzyme activity is expressed in terms of IAA destroyed.

Incubation time from 15 to 120 min. Reaction mixtures contained 2 ml enzyme, 2 ml of IAA (175 μg/ml) in the presence or absence of *p*-HBA (10^{-4} M).

tested, can be considered to positively affect[19] the IAA oxidizing enzymes. These observations are in agreement with those of Zenk and Müller.[6]

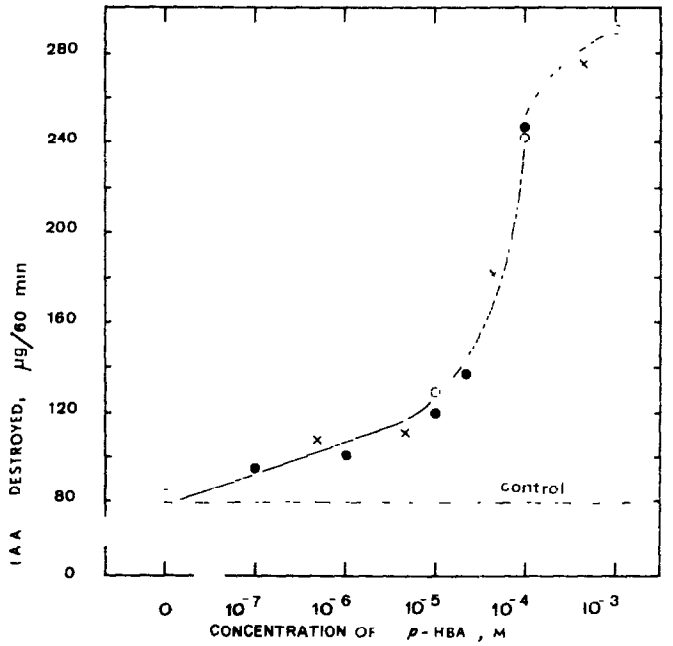

FIG. 3. ACTIVATION OF ACTIVITY OF IAA-OXIDASE SYSTEM FROM *Lens* ROOT BY *p*-HBA.
Active mixture contained 2 ml enzyme, 350 µg IAA and various concentrations of *p*-HBA (from 10^{-7} to 10^{-3} M).

DISCUSSION

One possible explanation of the interaction between *p*-HBA and endogenous auxin in the elongation of plant sections might be the formation of some phenol-auxin complexes.[20] But the fact that the phenols influence the destruction of IAA indicates that the growth action of these compounds should be related to the auxin catabolism. On the other hand, similar effects on growth and IAA-oxidase were obtained for the three mono-hydroxybenzoic acids (*o*-, *m*- and *p*-);[21] for instance *o*-hydroxybenzoic acid, which has no action on the IAA destruction,[22] has no effect on growth. It was previously observed[10] that chlorogenic acid, a competitive inhibitor of the IAA-oxidase, causes an increase in the auxin content. The present results show that *p*-HBA, which is an accelerator of the IAA oxidizing system, produces a decrease in the endogenous level of growth substances. Consequently, according to the observations of Zenk and Müller,[6] phenols can be divided into three groups: (1) synergistic acids (e.g. chlorogenic acid) which inhibit IAA destruction, (2) growth-inhibiting acids (e.g. *p*-HBA) which increase IAA destruction, and (3) acids which influence neither growth nor destruction.

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Therefore, as was previously discussed[23] a variation of the IAA oxidase activity which regulates the endogenous auxin level (high enzyme activity leading to low auxin content and vice versa), causes a change in the growth reactions.[9] Consequently, it is possible to explain the decrease of the relative elongation produced by *p*-HBA, when added at high concentration, by the fact that this phenolic acid causes a stimulation of the IAA-oxidase and a decrease of the endogenous auxin level. But it seems difficult at the moment to understand the growth activation observed for lower concentrations of *p*-HBA. Therefore, it appears that *p*-HBA acts on several other biochemical process which are connected with growth.[24] It was observed for instance that monohydroxy-derivatives of benzoic acid changed the rate and the nature of the respiration,[25–27] reacted *in vivo* with coenzyme A[28] and strongly modified sucrose uptake.[27,29]

It may be concluded that the relative growth curve which expressed the action of *p*-HBA on the stem elongation could be considered as the resultant of two curves: one related to a positive growth effect caused by the possible action of *p*-HBA on several biochemical process; and the second to a negative growth effect due to the stimulatory action of *p*-HBA on IAA-oxidase and consequently on auxin content and growth.

EXPERIMENTAL

Biological Material

The seeds of *Lens culinaris* were first soaked in deionized water for 12 hr, washed and finally placed in vermiculite in darkness ($22° \pm 0{\cdot}5$).[30] After seven days, epicotyls were approximately 40 mm long.

Growth

The stems were cut (second internode) in 10-mm sections which were placed (20 sections for 4 ml) in buffered solution (KH_2PO_4–Na_2PO_4–Na_2HPO_4, 0·1 M, pH 6·1) with sucrose (2%) on a shaking incubator in darkness ($25° \pm 0{\cdot}5°$) for 12 hr.[31]

Auxin Content

The techniques used for extracting and separating the growth substances have already been described in detail.[32] The method for analysing the biological activity of the different regions of the chromatogram have been also discussed.[33] Attention has so far been confined to the acid fraction of the ethyl acetate extract[34] and the chromatographic solvent used was isopropanol:28% ammonia:water (8:1:1).

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Auxin Catabolism

The method has been described previously.[35] *In vitro* IAA oxidase analysis were performed by first converting the *Lens* root (~ 100) to a brei. The tissues were removed from the medium by decantation onto a wire gauze, rinsed with 0·1 M pH 6·1 phosphate buffer and transferred to a previously chilled mortar containing a little sand and cold buffer. The mixture was subjected to a first centrifugation (15 min, 3500 *g*), the extract was added to the buffer for a second centrifugation (10 min, 8000 *g*) and the supernatant made up to 15 ml with buffer. 2 ml of the solution were mixed with 4 ml of water and 2 ml of cold buffer. At zero time, 2 ml of IAA (10^{-3} M) were added. The mixture was incubated in a metabolic shaking incubator (28) in complete darkness. Initial and residual IAA were determined by colorimetric analysis (modified Salkowski reaction).

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