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EFFECT OF CHLOROGENIC ACID ON THE AUXIN CATABOLISM AND THE AUXIN CONTENT OF ROOT TISSUES

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Abstract—Chlorogenic acid inhibits the IAA-oxidase system extracted from *Lens* root, and appears to be an active competitive inhibitor of these enzymes. The compound causes an increase in the endogenous level of growth substances (IAA-like substances) extracted from *Lens* root and separated by paper chromatography.

INTRODUCTION

STUDIES of the catabolism of β-indolylacetic acid (IAA) have shown that phenolic compounds can produce an inhibition of the activity of the oxidizing enzyme.[1] Gortner and Kent[2] found a natural inhibitor of the IAA-oxidase system in pineapple tissue, whose biological properties and absorption spectrum were similar to that of chlorogenic acid. Rabin and Klein[3] studied the effect of the latter compound on the activity of relatively crude IAA-oxidase from peas. They observed that chlorogenic acid was a competitive inhibitor for the oxidase; caffeic acid inhibited in a non-competitive fashion, and quinic acid, the other moiety of chlorogenic acid, was inactive. With an *Avena*-test, Tomaszewski[4] observed that IAA is strongly inactivated in the presence of chlorogenic acid. Zenk and Müller,[5] studying the kinetics of the decarboxylation of IAA by *Avena* coleoptile sections *in vivo*, observed that chlorogenic acid inhibited this process and the inhibition increased with increasing time of incubation. On the other hand, Gordon and Paleg[6] observed that the presence of chlorogenic acid augmented the concentration of IAA by enhancing its biosynthesis from tryptophan. Several reports in the literature[7] have pointed out the wide distribution of chlorogenic acid exists in plants.

This paper presents some results on the effects of chlorogenic acid on the activity of IAA-oxidase system from *Lens* root, and on the endogenous IAA concentration of this biological material.

RESULTS

IAA–Oxidase System Activity

As can be seen in Fig. 1 chlorogenic acid inhibits the activity of IAA-oxidase, the inhibition increasing with time of incubation. It can also be observed (Fig. 2) that the intensity of the reaction is a function of the concentration of the inhibitor. These results are in agreement with those of Zenk and Müller[5] discussed above.

[1] P. E. PILET, *Les Phytohormones de croissance*, p. 328, Masson, Paris (1961).
[2] W. A. GORTNER and M. J. KENT, *J. Biol. Chem.* **204**, 593 (1953).
[3] R. S. RABIN and R. M. KLEIN, *Arch. Biochem. Biophys.* **70**, 11 (1957).
[4] M. TOMASZEWSKI, *Bull. Acad. Pol. Sci.* **7**, 127 (1959).
[5] M. H. ZENK and G. MÜLLER, *Nature* **200**, 761 (1963).
[6] S. A. GORDON and L. G. PALEG, *Plant Physiol.* **36**, 838 (1961).
[7] See M. SHIROYA, T. SHIROYA and D. HATTORI, *Physiol. Plantarum* **8**, 594 (1955).

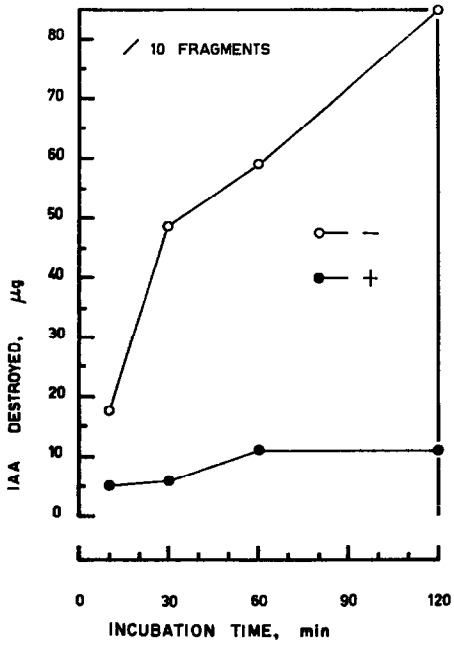

FIG. 1. ACTIVITY OF IAA-OXIDASE SYSTEM FROM *Lens* ROOT.
The enzyme activity is expressed in terms of IAA destroyed per 10 fragments (approx. 150 mg). Incubation time from 10 to 120 min. Reaction mixtures contained 2 ml enzyme, 2 ml of IAA (50 μg/ml) in the absence (−) and in the presence (+) of chlorogenic acid (10^{-5} M).

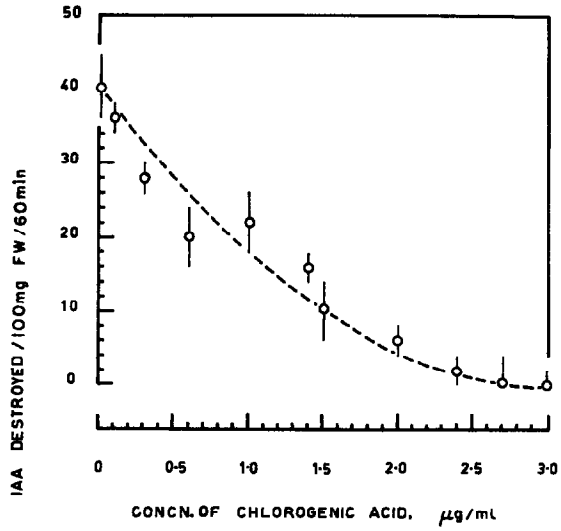

FIG. 2. INHIBITION OF ACTIVITY OF IAA-OXIDASE SYSTEM FROM *Lens* ROOT BY CHLOROGENIC ACID.
Active mixture contained 2 ml enzyme, 50 μg IAA and various concentrations of chlorogenic acid (from 0 to 3·0 μg/ml). Incubation period 60 min at 25°.

Kinetic Analysis

Kinetic analyses (Fig. 3) of the disappearance of IAA controlled by *Lens* root enzyme, clearly show that chlorogenic acid can be considered as a competitive inhibitor of the IAA-oxidase system prepared from *Lens* root. These observations are in agreement with those of Rabin and Klein.[3]

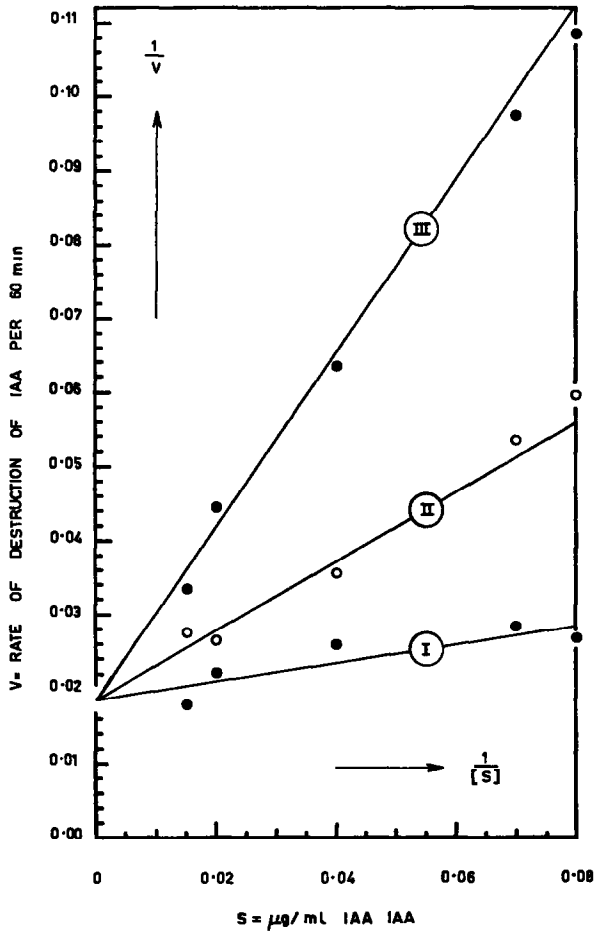

FIG. 3. CHLOROGENIC ACID AS A COMPETITIVE INHIBITOR OF IAA-OXIDASE SYSTEM FROM *Lens* ROOT. Destruction of IAA determined after 60 min incubation. Active mixtures contained 2 ml enzyme in the absence (I) and in the presence of 0·5 µg (II), and 1·0 µg (III) of chlorogenic acid.

IAA-Like Substances

To study the action of chlorogenic acid on the concentration of endogenous growth substances (IAA-like substances) of *Lens* seedlings, a series of experiments was made using 18-mm root sections. The roots were treated with chlorogenic acid at different concentrations in Petri dishes on filter paper for 12 hr (25°; dark) and the results are shown in Fig. 4. As it can be observed, the level of the IAA-like substances extracted from the roots, increases with increasing concentration of chlorogenic acid.

DISCUSSION

Chlorogenic acid produced an inhibition of the activity of an IAA-oxidase system prepared from roots of *Lens*, and an increase of the level of IAA-like substances extracted from the same material. It can be concluded that the accumulation of the endogenous growth-substances is a result of the inhibition of the IAA-oxidase system. These observations confirm those already published.[8] It is clear that IAA-oxidase activity must determine the endogenous level: high enzyme activity leading to low auxin content and vice versa. The increase of IAA-

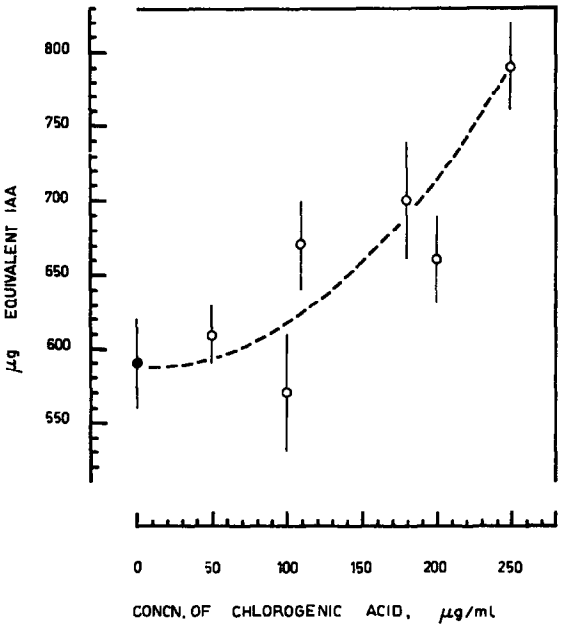

FIG. 4. CHANGES IN THE GROWTH-SUBSTANCE (IAA-LIKE SUBSTANCES: EXPRESSED IN TERMS OF µg EQUIVALENT OF IAA) IN THE 18-mm ROOT OF *Lens culinaris* SEEDLINGS TREATED WITH CHLOROGENIC ACID (0–250 µg/ml), DURING 12 hr.

like substances obtained from roots treated with chlorogenic acid can be explained in terms of its competitive inhibition of the IAA-oxidasic system. However, it has also been shown that chlorogenic acid might increase the concentration of IAA by enhancing the biosynthesis of the latter substance.[6] Recently it was observed that more IAA is present in a medium which contains chlorogenic acid and L-tryptophan, then in the medium with L-tryptophan alone.[9] Consequently, chlorogenic acid can be considered as being effective, both of the catabolism and the anabolism of the auxin.

EXPERIMENTAL

Biological Material

The seeds of *Lens culinaris* were first soaked in deionized water for 12 hr, washed and finally placed on wet filter paper in Petri dishes in darkness (22° ± 0·5°). After 24 hr only seedlings with ca. 1 mm root were kept in the above conditions; they were removed for

[8] P. E. PILET, *Experientia* **13**, 35 (1957); P. E. PILET et A. W. GALSTON, *Physiol. Plantarum.* **8**, 888 (1955).
[9] J. H. M. HENDERSON and J. P. NITSCH, *Nature* **195**, 780 (1962).

treatment when their roots had reached a length of 18 ± 2 mm, for this has been found to be a period of optimal growth.[10]

Measurement of IAA-Oxidase

The essential part of the technique has been presented previously.[11,12] *In-vitro* IAA destruction experiments were carried out with ground tissues. The roots (ca. 150 mg) were removed from the medium, rinsed in a few ml of buffer (0·1 M, pH 6·1) and transferred to a previously chilled mortar and ground. The mixture was subjected to a first centrifugation (3500 *g*, 15 min), the extract was added to the buffer for a second centrifugation (8000 *g*, 10 min), and the supernatants made up to 10 ml with buffer.

2 ml of the active solution were mixed with 2 ml of deionized water and 4 ml of cold buffer. At zero time, 2 ml of IAA (50 μg/ml) 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).

Analysis of IAA-Like Substances

The method used for extracting and separating the growth substance components by paper partition chromatography, and the technique for analysing the biological activity of the different regions of the chromatogram have already been described in detail.[13] Attention has so far been confined to the acid fraction of the ethyl acetate extract, according to the technique of Thurmann and Street[14] and the chromatographic running solvent has been isopropanol: ammonia 28%: water (8:1:1). Bio-assays has been the *Lens* root section test.[15]

[10] P. E. Pilet, *Experientia* 7, 262 (1951); P. E. Pilet and F. W. Went, *Am. J. Botany* 43, 190 (1956).
[11] P. E. Pilet, *Rev. gén. botan.* 64, 106 (1957).
[12] P. E. Pilet and G. Collet, *Méthode d'analyse du catabolisme auxinique*, Ch. Zwahlen, Lausanne (1962).
[13] P. E. Pilet, *Rev. gén. botan.* 65, 605 (1958); P. E. Pilet, *Bull. soc. vaudoise sci. nat.* 67, 525 (1961); P. E. Pilet, *Physiol. vég.* 1, 171 (1963).
[14] D. A. Thurmann and H. E. Street, *J. Exp. Botany*, 11, 187 (1960).
[15] P. E. Pilet, M. Kobr and P. A. Siegenthaler, *Rev. gén. botan.* 67, 573 (1960).