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THE EFFECT OF SOME PHENOLIC ACIDS ON INDOLEACETIC ACID-OXIDASE IN PISUM ELATUS (BIEB.)

BY

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ABSTRACT

Indoleacetic acid-oxidase was isolated from Pisum elatus (Bieb.) epicotyls. Its optimal activity was found to be at pH 5.4. Isochlorogenic acid was a competitive inhibitor of the oxidase. Caffeic acid inhibited indoleacetic acid-oxidase but in a noncompetitive fashion and ferulic acid was inactive.

INTRODUCTION

Phenolic acids are important not only as providing the building blocks of lignin [1] but also in relation to growth regulation [2,3] . Interaction between indoleacetic acid (IAA) and various phenols with IAA-oxidase have been studied [4,5]. Some phenolic acids inhibit the oxidation of IAA [6]. Others enhance its oxidation and act as co-factors of IAA-oxidase [7]. Chlorogenic acid was found to be a competitive inhibitor of IAA-oxidase [8].

In this investigation, the effect of isochlorogenic

acid, the isomer of chlorogenic acid as a competitive inhibitor on the activity of IAA-oxidase from Pisum elatus was demonstrated and the effects of caffeic acid and ferulic acid were also studied.

MATERIALS AND METHODS

Seeds of Pisum elatus were soaked in water for 6 hours and grown in the dark for 9 days. The etiolated epicotyls were stored at -15°C [9] then squeezed through cheesecloth and the filtrate was centrifuged at 12,000 Xg for 25 min. Acetone was added to the supernantant to a final concentration of 40%. The precipitate was collected at 600 Xg, taken up in 0.2 M Na_2HPO_4 + 0.1 M citric acid buffer (equal volumes, pH 5.4) and clarified by centrifugation. Each milliliter of this crude enzyme preparation represented 4 g fresh weight of tissue. Enzymatic activity disappeared after 3 months at -15°C [10].

Solutions of IAA and solutions of isochlorogenic acids each containing 100 u/ml were prepared. All reaction mixtures were made up to a total volume of 1.0 ml. Incubation temperature was 30°C. The remaining reaction mixture was analyzed for residual IAA [8].

RESULTS AND DISCUSSION

The optimal pH of a crude oxidase preparation from Avena first internodes (var. Bright) was reported as 6.6 [8].

Since then, various preparative techniques were reported [3,9]. Under the prevailing experimental conitions the optimal pH was 5.4 (fig. 1).

Isochlorogenic acid inhibited the activity of IAA-oxidase (Fig. 2), the degree of inhibition depended on its concentration. From 1.8 to 4.5ugm./ml. there was no oxidation of IAA. Increasing the concentration of isochlorogenic acid resulted in a non-enzymatic destruction of IAA (Fig.2). Diphenolic compounds also showed similar patterns in Impatiens sultani [11].

A lineweaver Burk plot was prepared to determine wheather isochlorogenic acid is, in fact, a competitve inhibitor of IAA-oxidase. Using the first 25 min. of the reaction period, the rate (V) of the reaction was determined [12]. Kinetic analysis of the oxidation of IAA by the IAA-oxidase from Pea showed that the rate of substrate destruction was linear for this first 25 min period. This suggests that isochlorogenic acid is a true competitive inhibitor of IAA-oxidase (Fig. 3).

Caffeic acid was as effective as isochlorogenic acid, but freulic acid did not inhibit IAA-oxidase. However, caffeic acid is not a competitive inhibitor of IAA-oxidase. This may be due to the absence of the active isochlorogenic acid moiety.

The results presented here demonstrate that isochlorogenic acid is equally a competitive inhibitor as well as chlorogenic acid on the activity of IAA-oxidase.

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Table 1: Inhibitory activity of isochlorogenic acid and caffeic acid on IAA-oxidase

Inhibitor	IAA destruction (µgm.)
Control	14
Isochlorogenic acid	
0.4 µg./ml	11
0.9 µg /ml	6.5
1.8 µg /ml	00.0
Caffeic acid	
0.2 µg /ml	12
0.5 µg /ml	6.5
1.0 µg /ml.	00.0

Reaction mixture : 0.5ml. enzyme , 20 µgm IAA, and the acid
Total volume : 1.0 ml,
Incubation time : 1hr at 30°C

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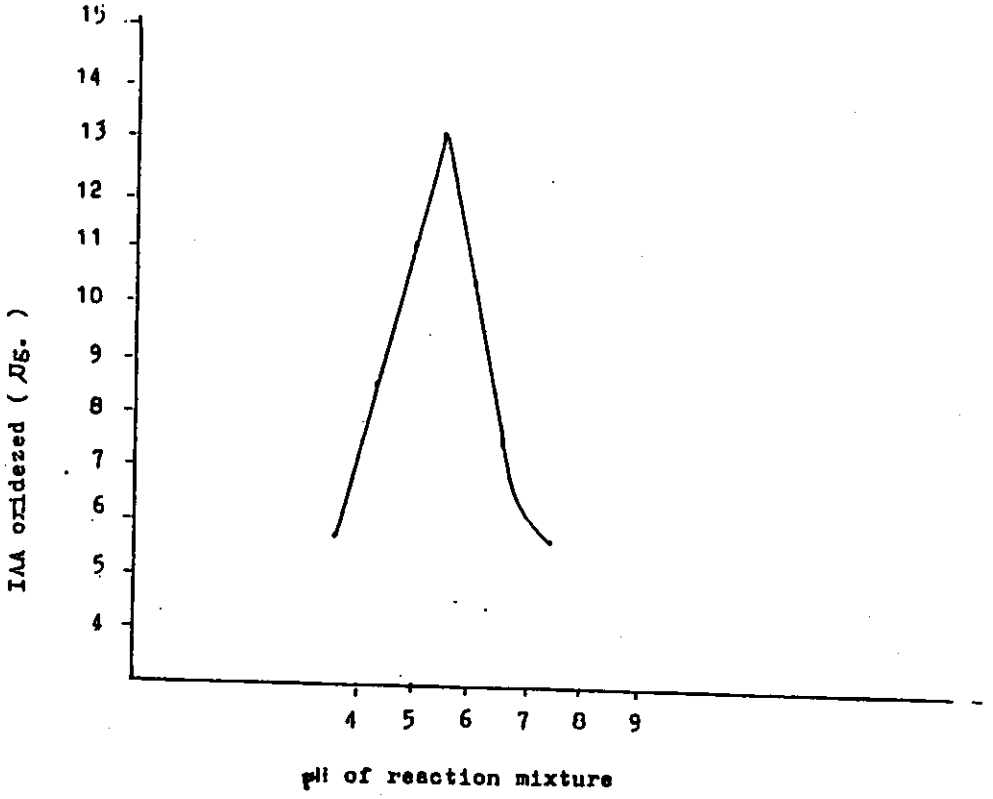

Fig. 1: Optimal pH for the inhibition of IAA by IAA-oxidase from Pisum epicotyls

Reaction mixture: 0.5 ml enzyme and 20 µg IAA in 1.0 ml

Incubation time : 1 hr at 30° C.

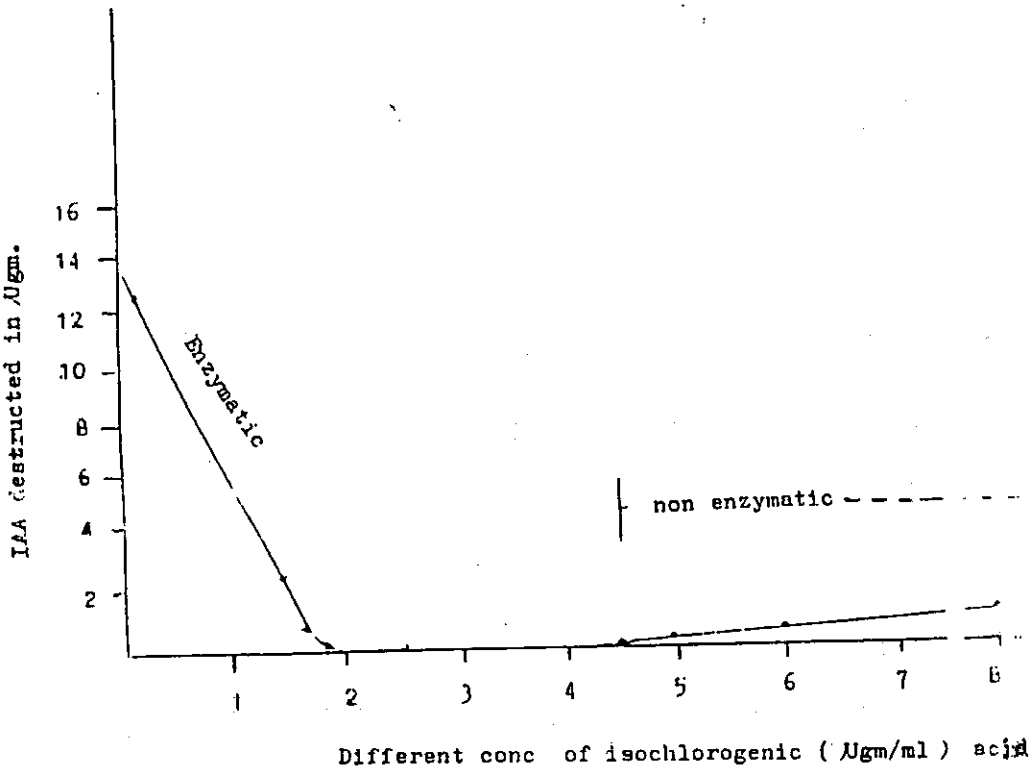

Fig. 2: The inhibitory effect of isochlorogenic acid on IAA-oxidase from Pisum epicotyls
Reaction mixture: 0.5 ml enzyme, 20 Ugm IAA, and different concentrations of isochlorogenic acid
Incubation time: 1hr 30°C.

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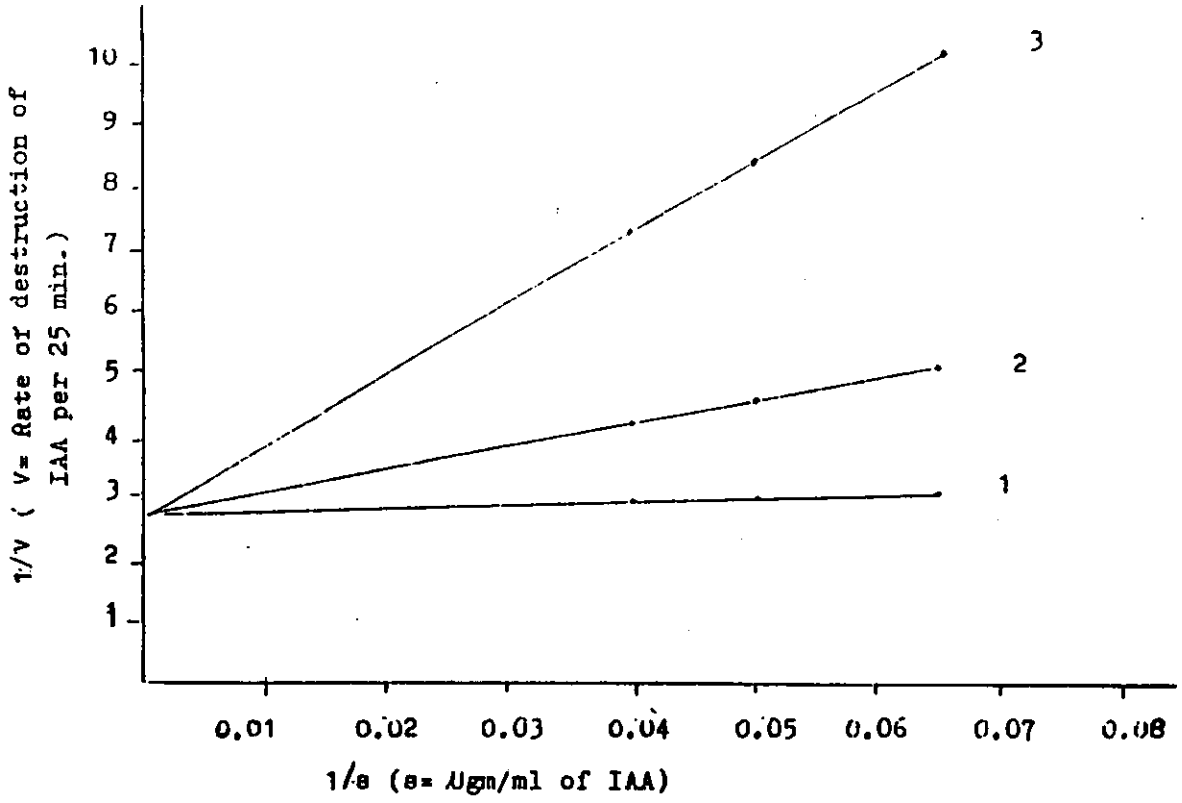

Fig. 3: Effect of isochlorogenic acid on IAA-oxidase from Pisum epicotyls. Disappearance of IAA determined after 25 min. incubation.

Reaction mixture: 0.5ml enzyme and 15, 20, or 25 Ug IAA
(1) no isochlorogenic acid added
(2) + 0.5 Ug isochlorogenic acid
(3) + 1.0 Ug. isochlorogenic acid

تاثير بعض الاحماض الفينولية على انزيم اندول حمض الخليك في نبات بيسم ايلاتس

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يتضمن البحث دراسة تاثير الاحماض الفينولية التالية: ايزوكلورجينيك ، كافييك وفروليك على اوكسيديز اندول حمض الخليك وبالتالى على اندول حمض الخليك. وتوضح النتائج الايزوكلورجينيك والكافييك له تاثير مثبط على اوكسيديز اندول حمض الخليك مما يسبب تنشيط اندول حمض الخليك نفسه. اما حمض الفوروليك فليس له تاثير على اندول حمض الخليك.

والمرجح ان حمض الايزوكلوروجينيك يعمل كمثبط تنافسى للانزيم الموكسد للاندول حمض الخليك وقد يرجع ذلك الى تكوين مركب مع اندول حمض الخليك يكون انشط من الاكسين نفسه.