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IAA OXIDASE PREPARATIONS FROM SWEET POTATO ROOTS*

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Abstract—IAA oxidase preparations from sweet potato roots have major and minor pH optima, which were related to the pH of phosphate–citrate or phosphate buffers used to solubilize acetone precipitates in the course of enzyme preparation Acetone precipitates were solubilized at five pH's and two representative enzyme types, 7 0 and 4 5 enzymes, with high (pH 5 8–6 8) and low (pH 3 6–4 6) major optima, respectively, further investigated Both 7 0 and 4 5 enzymes had single pH optima at pH 4 5 in scopoletin stimulated IAA oxidase reactions The pH optima and specific activities of IAA oxidase, peroxidase and phenolase were compared in 7 0 and 4 5 enzyme preparations and IAA oxidase inhibition by thiourea and sodium azide reported The occurence of different IAA oxidase pH optima in sweet potato root and other plant preparations is discussed

INTRODUCTION

SEVERAL pH optima at 3 0–3 4,[1] 4 0–4 5[2] and 6 0–7 0[3,4] have been described in IAA oxidase preparations from different plant sources. These pH optima are modified by chelating agents[5,6] and co-factors[7–9] used in enzyme assay. Krupasagar and Sequeira described two pH optima at 3·0 and 6·0 in *Marasmius perniciosus* preparations[10] and concluded that two enzymes, peroxidase and laccase were responsible for IAA oxidation.

In this paper, IAA oxidase preparations from sweet potato roots are shown to oxidize IAA in the pH range 3 0–8·0, in the absence of added co-factors. Such preparations had two pH optima at 3 6–4 6 and 5·8–6·8 depending on the pH of phosphate–citrate or phosphate buffer used in enzyme preparation and assay A single pH optimum was, however, obtained for scopoletin-stimulated IAA oxidase activity. These pH optima are compared with those for peroxidase and phenolase activities present in all enzyme preparations and inhibition of sweet potato root IAA oxidases by thiourea and sodium azide reported.

RESULTS

Crude brei of sweet potato roots prepared either with glass distilled water or with phosphate buffer[11] showed no IAA oxidase activity even after prolonged dialysis, but had considerable peroxidase and phenolase activities Repeated extraction of brei with ethyl acetate and light petroleum resulted in weak IAA oxidase activity.

* Part I in the series "pH Optima of Different Enzyme Preparations".

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Enzyme preparations described below were made by extracting acetone precipitates with phosphate–citrate or phosphate buffer and named after the pH of buffer used in extraction. IAA oxidase activities of 4 5, 5 0, 5 5, 6·5 and 7 0-enzymes thus prepared were assayed both by change in absorptivity at 247 nm and by determining residual IAA using the Salkowski method [12] The latter method was chosen in experiments here reported because it allowed for simultaneous assay of a large number of reaction mixtures All enzymes showed high IAA oxidase activities in the absence of added manganous ions, 2,4-dichlorophenol and hydrogen peroxide and such co-factors were therefore not used Since buffers of different pH's were used in enzyme preparation, the pH's of reaction mixtures for determination of pH optima were adjusted by titration, using a pH meter and checked after each assay.

pH Optima of Different Sweet Potato Root IAA Oxidase Preparations

Typical pH optima for IAA oxidase activities of 5 0-, 6 5- and 7 0-enzymes prepared and assayed in phosphate–citrate buffer are given in Figs. 1 and 2 Since these optima were

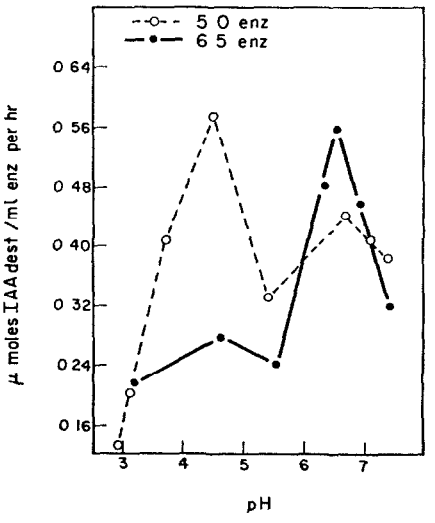

FIG 1.

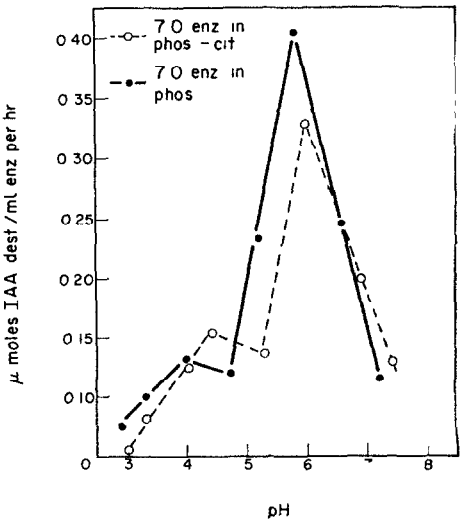

FIG. 2

FIG 1 IAA OXIDASE pH OPTIMA OF 5 0- AND 6 5-ENZYMES IN PHOSPHATE–CITRATE BUFFER.

FIG. 2 IAA OXIDASE pH OPTIMA OF 7 0-ENZYME IN PHOSPHATE–CITRATE AND PHOSPHATE BUFFERS Reaction mixtures in phosphate/HCl contained the following concentrations of chloride (ppm × 10^2) pH 2 8—7 10, pH 3 4—46 2, pH 4 1—2 84, pH 4 7—1 78

determined in enzymes prepared at different times, pH optima of enzymes simultaneously prepared from equal amounts of acetone precipitate are shown in Table 1. Each enzyme preparation had major and minor optima which were related to the pH of extracting buffer. Major and minor optima are further investigated in preparations representative of the two enzyme types recognized, viz. 7 0- and 4 5-enzymes.

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Comparison of pH Optima of Enzyme Prepared in Phosphate–citrate and in Phosphate Buffer

Since chelating effects of citrate at different pH's may be partly responsible for the occurrence of major and minor optima, 7 0- and 4 5-enzymes were simultaneously prepared from equal quantities of acetone precipitate extracted with phosphate–citrate and phosphate buffers, and pH optima of the resulting enzymes determined.

7·0-*Enzyme.* Results (Fig 2) indicated that major and minor pH optima occurred both in phosphate-citrate and in phosphate buffers, but these optima were slightly shifted towards lower pH's in enzyme prepared and assayed in phosphate buffer. IAA oxidase activity was, however, lower in the phosphate reaction mixture at pH 4 5, the minor optimum of the phosphate–citrate prepared enzyme. A small but consistent stimulation of IAA oxidase activities at low pHs (2 9–3·5) in phosphate/HCl compared with phosphate–citrate reaction mixtures occurred in several experiments. Since HCl (see Fig 2) was used to adjust pH's below pH5, the effect of higher concentrations of chloride ($2{\cdot}6 \times 10^3$–$2\,7 \times 10^3$ ppm) on IAA oxidase activity of 7·0 enzyme prepared in phosphate–citrate buffer was

TABLE 1 pH OPTIMA OF SWEET POTATO IAA OXIDASES PREPARED SIMULTANEOUSLY

Enzyme preparation	Major pH optimum	Minor pH optimum
4 5-Enz	3 6	6 3
5 5-Enz	4 7	6 8
6 5-Enz	6 7	4 2
7 0-Enz	6 2	4 5

examined. Reaction mixtures were adjusted either with phosphate–citrate or with 0·1 N HCl in the presence of 0 06 M KCl as described in the experimental section. Results (Table 2) indicated that chloride ion in the concentrations used stimulated IAA oxidase activities below pH 3 4, but enzyme activities were similar both in the presence and absence of chloride in the pH range 5 0–7·1.

The apparent inhibition of IAA oxidase activity at about pH 4 5 (Fig. 2) was examined in a 7·0-enzyme prepared in phosphate buffer to eliminate possible stimulatory effects of citrate at this pH. Reaction mixtures were adjusted to pH 4 5 with citric acid, acetic acid

TABLE 2 EFFECTS OF CHLORIDE ION ON IAA OXIDASE ACTIVITY AT DIFFERENT pH'S IN 7 0-ENZYME PREPARED IN PHOSPHATE-CITRATE BUFFER

pH	IAA oxidase activity (μmole IAA destroyed/hr/ml enz) 7 0-Enz in Phos–cit buffer	*7 0-Enz in presence of HCl/KCl
2 8	0 00	0 06
3 0	0 00	0 10
3 4	0 03	0 11
5 0	0 38	0 40
6 2	0 46	0 46
7 1	0 34	0 36

* Reaction mixtures in phos/HCl/KCl contained the following concentrations of chloride (ppm × 10^3) pH 2 8–2 712, pH 3 0–2 705, pH 3 4–2 613, pH 5 0–2 592, pH 6 2–2 588, pH 7 1–0 0

or 0 1 N HCl, giving IAA oxidase activities of 0 275, 0 280 and 0 056 μmole IAA destroyed/hr/ml enz respectively. The low IAA oxidase activity at pH 4 5 in the presence of chloride is therefore, interpreted as a chloride inhibition of enzyme activity, since similar enzyme activities were obtained in the presence of citrate and acetate

4 5-*Enzyme.* IAA oxidase activity of 4 5-enzymes prepared in phosphate buffer and assayed either in phosphate/HCl or phosphate–citrate buffer, in the pH range 3–8 is shown in Fig 3 At pH 4 5, there was a 56% inhibition of enzyme activity in the presence of

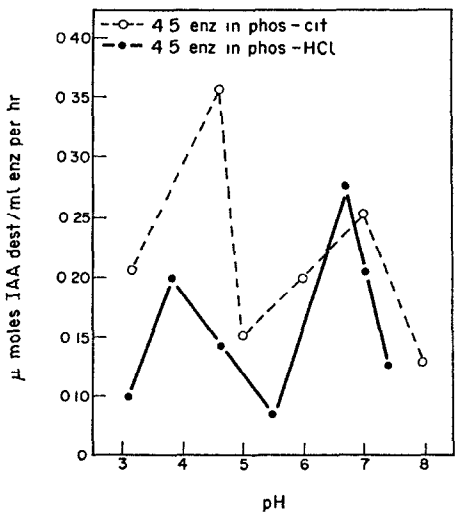

FIG 3

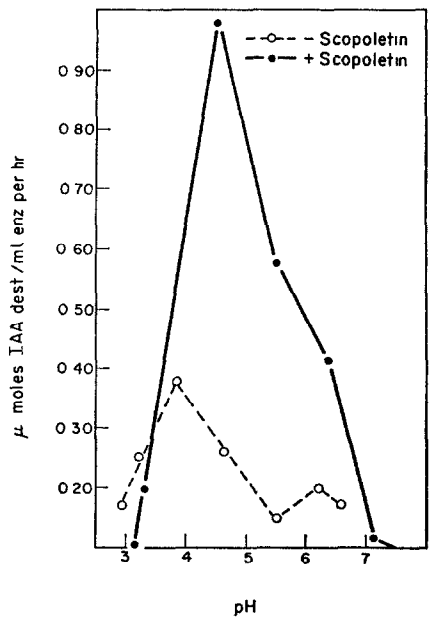

FIG 4

FIG 3 IAA OXIDASE pH OPTIMA OF 4 5-ENZYMES IN PHOSPHATE–CITRATE AND PHOSPHATE BUFFERS

FIG 4. IAA OXIDASE pH OPTIMA OF 4 5-ENZYME IN THE PRESENCE AND ABSENCE OF SCOPOLETIN

chloride (1 88 $\times$ 10^3 ppm) Stimulation of enzyme activity by chloride at pH 3 0, previously recorded in 7·0-enzyme preparations (Fig. 2, Table 2) did not occur in this system. Chloride inhibition of enzyme activity in the pH range 3 0–4·5 (Fig. 3) resulted in a peak of enzyme activity at pH 3 9, the approximate major pH optimum of other 4 5-enzymes (Table 1, Fig. 4) IAA oxidase activity at this peak was, however, 29 per cent less than that at pH 6 8

When reaction mixtures containing 4 5-enzyme prepared in phosphate–citrate buffer were assayed in the presence and absence of chloride, a shift in the pH at which optimal IAA oxidase activity occurred from pH 3 9 to 6 2 was recorded in the presence of chloride. Results (Table 3) showed that, here, chloride stimulated IAA oxidase activity at pH 3 0 and inhibited enzyme activity at pH 4·5 In 4 5-enzyme preparations, therefore, optimal IAA oxidase activity presented at pH 6 2–6 8 in reaction mixtures adjusted with HCl, due apparently to chloride inhibition of IAA oxidase activity in the pH range 3 9–4 5

TABLE 3. IAA OXIDASE ACTIVITIES OF 4 5-ENZYME IN PHOSPHATE–CITRATE BUFFER AT SELECTED pH'S IN THE PRESENCE AND ABSENCE OF CHLORIDE ION

pH	IAA oxidase activity (μmole IAA destroyed/hr/ml enz)	
	4 5-Enz in Phos cit buffer	*4 5-Enz in the presence HCl–KCl
3 0	0 32	0 50
4 5	1 42	0 62
6 2	1·32	1 42

* Reaction mixtures in phosphate–citrate—HCl–KCl contained the following concentrations of chloride (ppm × 10^3) pH 3 0–2·24, pH 4 5–1 88, pH 6 2–1 77

pH Optima of Scopoletin—Stimulated IAA Oxidase Activity

Effects of scopoletin stimulation on the pH optima of 4·5- and 7·0-enzymes, prepared in phosphate–citrate buffer are shown in Figs. 4 and 5 Previously observed double pH optima did not occur in the presence of scopoletin With 4 5-enzyme (Fig 4), there was marked scopoletin-induced stimulation at pH 4·5, resulting in a sharp pH otpimum In contrast, scopoletin-induced stimulation of 7 0-enzyme (Fig 5) showed no such sharp optimum, but

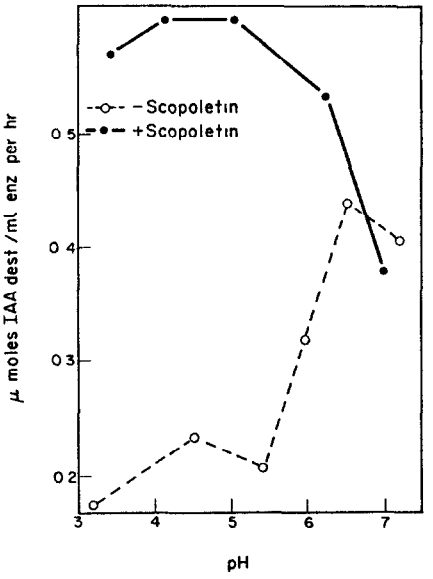

FIG 5

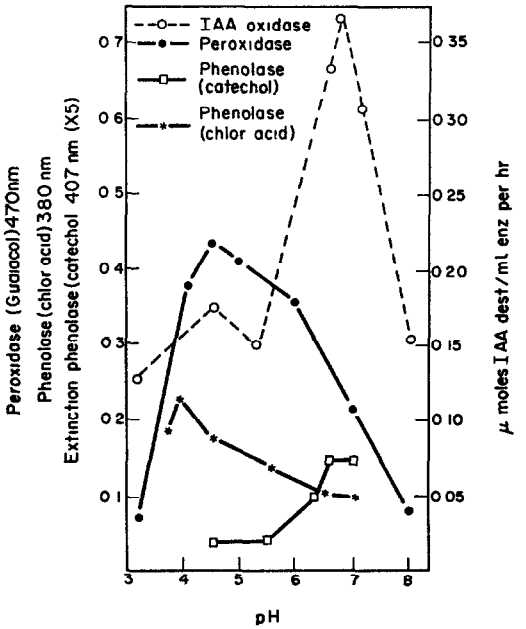

FIG 6

FIG 5 IAA OXIDASE pH OPTIMA OF 7 0-ENZYME IN THE PRESENCE AND ABSENCE OF SCOPOLETIN

FIG 6 pH OPTIMA OF IAA OXIDASE, PEROXIDASE, CATECHOLASE AND CHLOROGENIC ACID OXIDASE OF 7 0-ENZYME

there was a shift in the pH optimum of this enzyme preparation from pH 6 2 in the absence of scopoletin to pH 4 3–5 0 in its presence

Comparison of pH optima of IAA Oxidase with Peroxidase and Phenolase

The pH optima for peroxidase, phenolase and IAA oxidase activities of 7 0-enzyme in phosphate–citrate buffer are shown in Fig 6 Peroxidase activity was assayed with guaiacol, since catechol and pyrogallol were rapidly oxidized by phenolase present in the preparation With this electron donor, the pH optimum of peroxidase occurred at pH 4·5 The phenolase pH optimum was above pH 6 3 when either catechol or pyrogallol was used as substrates Enzyme activity was not assayed above pH 7 0 because of rapid non-enzymic oxidation of substrates in alkaline media Chlorogenic acid oxidase had a pH optimum at pH 3 8 Major and minor optima for IAA oxidase activity occurred at pH 6 8 and 4 5 respectively in this preparation Peroxidase and phenolase pH optima in 4 5-enzyme preparations were similar to those shown in Fig 6

Specific activities for peroxidase, catecholase and IAA oxidase of 7 0- and 4 5-enzyme preparations used in the determination of pH optima are given in Table 4 Although the protein content of the 7 0-enzyme preparation was 60% higher than that of the 4 5-enzyme, the specific activity for IAA oxidase in the 4 5-enzyme was double that of the 7 0-enzyme preparation Catecholase and peroxidase specific activities were, however, 27% and 64% higher in 7·0-enzyme than in 4 5-enzyme preparations

TABLE 4 SPECIFIC ACTIVITIES AT MAJOR pH OPTIMA FOR IAA OXIDASE, PEROXIDASE AND CATECHOLASE IN 7 0- AND 4 5-ENZYME PREPARATIONS

Enzyme	Specific activity (per mg protein) 7 0-enzyme	4 5-enzyme
IAA oxidase μmole destroyed/hr	$1\,43 \times 10^{-3}$	$2\,87 \times 10^{-3}$
Peroxidase ΔA/min	$3\,28 \times 10^{-3}$	$2\,39 \times 10^{-3}$
Catecholase ΔA/min	$0\,177 \times 10^{-4}$	$0\,65 \times 10^{-5}$

Inhibition of IAA Oxidase Activity of 7 0- *and* 4 5-*enzymes by Sodium Azide and Thiourea*

Percentage inhibition of 7 0- and 4 5-enzymes by sodium azide and thiourea, calculated from specific activities of these enzyme preparations are shown in Table 5 Azide inhibition of IAA oxidase activity was greater at pH 4 5 in both enzyme preparations In contrast, thiourea inhibition was greater at the approximate pH optima of each enzyme

TABLE 5 INHIBITION OF IAA OXIDASE ACTIVITY IN 7 0- AND 4 5-ENZYMES AT pH 7 0 AND 4 5

	% Inhibition IAA oxidase activity Azide (10^{-2} M)		Thiourea (10^{-2} M)	
	pH 4 5	pH 7 0	pH 4 5	pH 7 0
4 5-Enzyme	100	75	63	33
7 0-Enzyme	100	74	40	78

DISCUSSION

Single pH optima in the pH range 3–7 have been demonstrated in IAA oxidase preparations from different plant sources These preparations usually contained a single enzyme, variously described as peroxidase,[13,14] laccase,[10,15,16] tyrosinase[17] or chlorogenic acid oxidase,[18] mediating IAA oxidation Krupasagar and Sequeira[10] showed, however, that IAA oxidase preparations from *Marasmus perniciosus* had two pH optima at pH 3 0 and 6 0 These optima were attributed to peroxidase and laccase activities, on the basis of coincidence of IAA oxidase pH optima with those of peroxidase and laccase, respectively. Sweet potato root IAA oxidase preparations were also shown to have two pH optima at pH 3 8–4 6 and pH 5 8–6 8, but enzyme activity at either optimum depended on the pH of buffer used to extract enzyme from acetone precipitates. Thus, enzymes extracted at low pH (4·5–5 5) had greater activity at lower pH optima, whilst major activity occurred at higher pH optima in enzymes extracted with buffer at high pH (6–7). These results suggested that extraction at different pH values resulted in differential solubilization of enzymes and/or co-factors involved in IAA oxidation.

All IAA oxidase preparations examined showed considerable peroxidase and phenolase activities. Since optimal peroxidase activity occurred at pH 4 5, this enzyme might be responsible for the major IAA oxidase pH optimum of preparations extracted at low pH. However, sweet potato IAA oxidases have been shown to be independent of added manganous ions and 2,4-dichlorophenol, generally considered essential for the oxidation of IAA by peroxidase.[19] Phenolase activity in sweet potato root IAA oxidase preparations was specific for orthodihydroxy phenols The pH optimum for catecholase activity which occurred at pH 6–7 might account for the major optimum of IAA oxidases extracted at high pH IAA oxidation by phenolase enzymes is known to be independent of added co-factors [10] The chlorogenic acid oxidase pH optimum at pH 3 8 suggested that this enzyme might also have participated in IAA oxidase activity at low pH Inhibition studies did not clarify the participation of iron and/or copper enzymes in sweet potato root IAA oxidase activity. Sodium azide and thiourea inhibited both peroxidase and phenolase activities in IAA oxidase preparations Azide inhibition of peroxidase was, however, two-fold, that obtained with thiourea and phenolase inhibition by thiourea was three times that obtained with azide.

Janssen[21] demonstrated that co-factors shifted the pH optima of pea and cucumber IAA oxidases and small differences in pH optima found in extracts prepared at different pH might be due to differential solubilization of co-factors, e.g. enzymes prepared at pH 6 5 and 7 0 had optimal activities at pH 6 7 and 6·0–6·2, respectively. Unlike Janssen's pea root enzyme, addition of boiled 7 0-enzyme to 4·5-enzyme preparations did not affect their pH optima, indicating that natural co-factors or inhibitors were probably not responsible for the occurrence of double pH optima in our preparations Accordingly, the single optimum at pH 4 5 in scopoletin-stimulated IAA oxidase activity is interpreted as marked stimulation of a peroxidase mediated reaction, which masked the second optimum at pH

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6–7, probably due to a phenolase enzyme Such stimulation of a peroxidase mediated reaction is in accord with the model for the co-factor effects of scopoletin previously reported by Imbert and Wilson [11]

Demonstration of the existence of double pH optima in sweet potato root IAA oxidase preparations both in the presence of phosphate and phosphate–citrate buffers is thought to exclude the possibility that these optima were due to effects of citrate Apparent stimulation of IAA oxidase activity below pH 3 8 and inhibition at pH 4 5 by chloride, in hydrochloric acid used to extend the pH range of phosphate buffers, is difficult to explain Halogens are, however, known to modify the catalytic activity of several enzymes

In conclusion, it is noted that a protein capable of IAA oxidation has been separated both from commercial horse radish peroxidase and tobacco root IAA oxidase preparations by Sequeira and Mineo.[22] Such a protein might also be involved in the occurrence of double pH optima in sweet potato root IAA oxidase preparations

EXPERIMENTAL

Materials Roots were obtained by growing sweet potato stem cuttings in solution culture as previously described [11]

Enzyme preparation Enzymes were also prepared as previously described[11] except that roots were macerated in distilled H_2O and acetone precipitates extracted with phosphate–citrate or phosphate buffers at different pH's, as explained in Results Simultaneous preparation of enzyme with approximately equal protein contents (Table 1, Figs 2 and 3) was achieved by dividing centrifuged macerates into equal volumes before acetone precipitation, followed by extraction of acetone precipitates under similar conditions with equal volumes of buffer

Adjustment of pH of reaction mixtures In the determination of pH optima, pH's of reaction mixtures were adjusted by titration using acidic and basic components of phosphate–citrate, phosphate or acetate buffers and measured on a pH meter The pH of reaction mixtures in phosphate buffer (Figs 2 and 3) was adjusted below pH 4 7, using 0 1 N HCl, and above this pH with phosphate buffer solutions In experiments to examine the effect of chloride ion on 7 0-enzyme prepared in phosphate–citrate buffer (Tables 2 and 3) pH was adjusted by titration with HCl in the presence of a constant volume of 0 2 M KCl The pH of reaction mixtures containing 4 5-enzyme in phosphate–citrate buffer was adjusted below pH 4 7 using 0 1 N HCl and above this pH with phosphate buffer, in the presence of a constant volume of 0 2 M KCl

Assay of enzyme activity IAA oxidase was assayed by determining residual IAA using the Salkowski reagent as previously described [11] The reaction mixture used was as follows IAA—2 μmoles, buffer—500 μmoles, inhibitors—4 μmoles, or scopoletin—5 nmoles, enzyme—1 ml in 8 0 ml total vol Reaction time was 120 min Peroxidase was assayed by measuring the formation of the guaiacol oxidation product colorimetrically at 470 nm, in the presence of hydrogen peroxide Phenolase activities were assayed by measuring the formation of catechol, pyrogallol and chlorogenic acid oxidation products colorimetrically at 407, 440 and 380 nm, respectively Protein contents were determined by the Folin method [23]

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Key Word Index—Ipomoea batatas, Convolvulaceae, indolyl-3-acetic acid oxidase