The Oxidation of β -(3-Indolyl)propionic Acid and γ -(3-Indolyl)-*n*-butyric Acid by Peroxidase and Mn^{2+}

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Wagenknecht & Burris (1950) have shown that both β -(3-indolyl)propionic acid (IPA) and γ -(3-indolyl)-*n*-butyric acid (IBA) are oxidized by extracts of waxpod bean root (*Phaseolus vulgaris* L.) and of pea epicotyls (*Pisum sativum* L.), though the rate of oxidation of these compounds was less than that of 3-indolylacetic acid (IAA). With waxpod bean-root extracts the presence of small amounts of Mn^{2+} stimulated the oxidation of IPA and IBA, but with pea epicotyl extracts this effect of Mn^{2+} was observed only after the extract had been dialysed. Kenten (1955) reported that the direct oxidase action of peroxidase preparations on IAA was increased in the presence of certain monophenols, maleic hydrazide or Mn^{2+} . During this work it was found that horseradish peroxidase preparations also catalyse the oxidation of IPA and IBA by oxygen, but only in the presence of Mn^{2+} . These observations are recorded here, partly on account of the interest in systems which may be responsible for the metabolism of substances with growth hormone properties, and partly because they suggest that the oxidation of IPA and IBA by the plant extracts used by Wagenknecht & Burris (1950) may have been due to the presence of peroxidase and Mn^{2+} in the extracts.

MATERIALS AND METHODS

Glucose oxidase. A commercial preparation obtained from the Sigma Chemical Co., St Louis, U.S.A. 'Glucose oxidase, crude on inert base', was suspended in 0.2M phosphate pH 6 (20 mg./ml.) and centrifuged. The clear yellow supernatant was used without further purification.

Peroxidase and catalase preparations. These were as described by Kenten (1955). In the text the peroxidase activity of some preparations is given as enzyme units (E.U.) (Keilin & Hartree, 1951). With a dry preparation the number of E.U. = P.N. $\times$ wt. of the preparation in g., where P.N. = purpurogallin number. For each experiment the catalase preparation ($k_1 = 2.2 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$) was suspended (10 mg./ml.) in 0.02M phosphate pH 6 and the insoluble material removed by centrifuging.

IPA and IBA solutions. Unless otherwise stated, IPA or IBA was brought into solution by warming and adding 5% (w/v) NH_3 . After cooling, the solutions were adjusted to pH 6-7 with NH_3 and stored in the dark at 0°. Fresh solutions were prepared weekly.

Buffers. Phosphate buffers were prepared from solutions of KH_2PO_4 and KOH; pyrophosphate buffers from solutions of $Na_2H_2P_2O_7$ and $K_4P_2O_7$; citrate buffers from solutions of citric acid and sodium citrate.

Manometric experiments. These were carried out with Warburg apparatus at 25°. The volume of the reaction mixture was 3 ml. and KOH was present in the centre well unless otherwise stated. The formation of CO_2 was measured by Warburg's direct method (Dixon, 1943).

RESULTS

Manometric studies of the oxidation of IPA by horseradish-peroxidase preparations and manous ions

In phosphate at pH 6 horseradish-peroxidase preparations catalyse the oxidation of IPA by oxygen provided that Mn^{2+} is present. Before the oxidation begins there is often a lag period. The lag period is variable and is at least partially dependent on the age of the IPA solution and the light intensity prevailing at the time the experiment is carried out.

The lag period. For most of the present work warm water and NH_3 were used to neutralize and assist solution of the IPA. A lag period was usually found when such solutions were used immediately after preparation. With one single exception among a number of experiments a lag period was obtained when neutral solutions of IPA were prepared by using NH_3 and keeping the temperature below 5°. A typical experiment with an IPA solution prepared in this way is shown in Fig. 1. Reaction mixtures consisted of: in the main vessel 3.3×10^{-3} M IPA and 3.3×10^{-5} M- $MnSO_4$ in 6.7×10^{-2} M phosphate at pH 6, and where present 1.3×10^{-5} M- H_2O_2 . After equilibration 100 μ g. horseradish peroxidase P.N. 940 was added from the side arm. One experiment was carried out immediately the IPA solution was prepared and a second after storing the solution at 0° in darkness for 24 hr. In the absence of Mn^{2+} or peroxidase the oxygen uptake was negligible, but where both these factors were present a rapid uptake commenced after a lag period. The lag period was reduced by keeping the IPA solution at 0° for 24 hr. It was abolished when hydrogen peroxide was added to the reaction

mixtures, suggesting that the reaction depends on the formation of peroxide. In the absence of added H_2O_2 , once the oxygen uptake began it proceeded at the same rate as that found in the presence of added H_2O_2 . Working under conditions similar to those of Fig. 1, lag periods of up to 2.5 hr. have been observed, but 2-3 days storage of the IPA

Fig. 1. The oxidation of IPA by a horseradish-peroxidase preparation P.N. 940 plus Mn^{2+} . Peroxidase and Mn^{2+} with: ○, freshly prepared IPA; Δ, IPA kept at 0° for 24 hr.; ●, freshly prepared IPA + H_2O_2 ; ▲, IPA kept at 0° for 24 hr. + H_2O_2 . Control experiments: from the complete system of IPA, Mn^{2+} , peroxidase and H_2O_2 , the following were omitted: x, Mn^{2+} ; □, peroxidase + H_2O_2 , or Mn^{2+} + H_2O_2 ; ■, peroxidase.

solution at 0° often considerably shortened the lag period. It was also found that exposure of the IPA solution to bright light reduced the lag period. In one experiment the Warburg vessels containing the reaction mixtures were exposed to sunlight for 5 min. before equilibration. No lag was observed with these reaction mixtures, whereas a lag period of 2 hr. was found with control mixtures protected from sunlight. Further control experiments showed that a 5 min. exposure to sunlight of the IPA solution in phosphate at pH 6 was sufficient to

abolish or bring about a large reduction in the lag period.

Unless otherwise stated, most of the present work was carried out under conditions where only brief lag periods were obtained. This was achieved either by using IPA solutions which had been aged at 0° in darkness for 3-4 days, or by adding small amounts of hydrogen peroxide to the reaction mixtures. In no case was any significant difference in the rate of oxygen uptake observed whether added hydrogen peroxide, light or ageing of the IPA solution was used to reduce the lag period.

Fig. 2. Effect of variation in Mn^{2+} concentration on the rate of oxidation of IPA by horseradish peroxidase P.N. 940. Experiments in $6.7 \times 10^{-2} \text{M}$ phosphate at pH 6, $6.7 \times 10^{-3} \text{M}$ IPA, 30 μg . horseradish peroxidase P.N. 940 and varying amounts of MnSO_4 , ○, $5 \times 10^{-6} \text{M}$; Δ, 10^{-5}M ; ⊕, $3 \times 10^{-5} \text{M}$; ×, 10^{-4}M ; □, $3 \times 10^{-4} \text{M}$; ◇, 10^{-3}M . Control experiments omitting peroxidase, Mn^{2+} or IPA gave O_2 uptakes less than that shown by the dotted line.

Effect of varying the concentration of Mn^{2+} . The effect of varying the concentration of Mn^{2+} on the rate of oxidation of IPA solutions, previously aged at 0° , by horseradish peroxidase is shown in Fig. 2. With increasing amounts of Mn^{2+} up to about 10^{-4}M the initial rate of oxygen uptake increases, but with further increases in Mn^{2+} there is a lag period before the oxidation begins. The length of the lag period varied in different experiments; there was a tendency for the lag period to increase with increase in Mn^{2+} from 3×10^{-4} to 10^{-3}M , but

such a regular effect was not always found. At the end of the experiment the complete reaction mixtures were coloured brownish yellow, the most intense coloration being found in the mixtures with the largest oxygen uptake. The results show that measurable rates of oxidation of IPA by peroxidase are obtained in the presence of amounts of Mn^{2+} as low as $5 \times 10^{-6} M$ (i.e. $0.25 \mu g./ml.$). In order to avoid a lag period induced by the presence of too high a concentration of Mn^{2+} , most of the present work has been carried out with Mn^{2+} concentrations of $10^{-4} M$.

Effect of various metals. The cations Co^{2+} , Ni^{2+} , Al^{3+} , Zn^{2+} , Cu^{2+} , Fe^{2+} and Fe^{3+} were tested at concentrations of 10^{-3} and $10^{-4} M$ in reaction mixtures consisting of $6.7 \times 10^{-3} M$ IPA, $30 \mu g.$ horseradish peroxidase P.N. 940 and $6.7 \times 10^{-2} M$ phosphate at pH 6. No significant oxygen uptake was obtained in these experiments in a 1 hr. period. Control experiments made simultaneously with mixtures containing $10^{-4} M$ - $MnSO_4$ gave uptakes of 134–142 $\mu l.$ oxygen in 1 hr.

Effect of pH. This was tested in reaction mixtures consisting of: in the main vessel $6.7 \times 10^{-3} M$ IPA, $10^{-4} M$ - $MnSO_4$ and $10^{-4} M$ - H_2O_2 in buffer $3.3 \times 10^{-2} M$ with respect to acetate and phosphate; after equilibration $30 \mu g.$ horseradish peroxidase P.N. 940 were added from the side arm. The maximum rate of oxygen uptake was obtained at pH 6, the rates at pH 5 and 7 being respectively 80 and 65 % of that at pH 6, while at pH 4 no oxygen uptake was observed in 1 hr. The oxygen uptakes obtained with reaction mixtures, in the range pH 4–7, from which either Mn^{2+} or peroxidase was omitted were negligible. When freshly prepared IPA was used and hydrogen peroxide omitted from the reaction mixtures it was found that the lag period decreased with increase in pH value. In one typical experiment the length of the lag period was 30 min. at pH 7 and 100 min. at pH 6, but at pH 5 the reaction had not started after 180 min.

Effect of catalase. When horseradish peroxidase ($30 \mu g.$ P.N. 940) was added to reaction mixtures containing $6.7 \times 10^{-3} M$ IPA, $10^{-4} M$ - $MnSO_4$ and $6.7 \times 10^{-2} M$ phosphate at pH 6, the presence of 0.1–0.5 mg. catalase preparation caused an increase in the lag period, after which the oxygen uptake commenced, though with the larger amounts of catalase at a reduced rate. By the use of flasks with double side arms it was shown that the addition of larger amounts of catalase (2 mg.), after the reaction had started, brought about a total inhibition (Fig. 3).

Effect of varying the concentration of peroxidase. The effect of varying the amount of a horseradish-peroxidase preparation of P.N. 500 on the rate of oxidation of IPA in the presence of Mn^{2+} is shown in Table 1. The results show that the rate of uptake

increases with increase in amount of peroxidase until a maximum rate is reached at about $20 \mu g.$ peroxidase preparation/ml.

Experiments with peroxidase preparations of different purity. A number of peroxidase preparations of varying degrees of purity were tested for their capacity to catalyse the oxidation of IPA by

Fig. 3. Effect of catalase on the oxidation of IPA by horseradish peroxidase P.N. 940 plus Mn^{2+} . Reaction mixtures consisted of; in the main vessel $6.7 \times 10^{-3} M$ IPA, $10^{-4} M$ - $MnSO_4$ in $6.7 \times 10^{-2} M$ phosphate at pH 6 and where present 2 mg. catalase preparation. After equilibration $30 \mu g.$ horseradish peroxidase was added from the side arm and 21 min. later 2 mg. catalase, or 2 mg. catalase previously heated 10 min. at 100° , was added from the second side arm. $\circ$, Catalase omitted; $\square$, catalase present initially; $\times$, catalase added at $\uparrow$; $\triangle$, heated catalase added at $\uparrow$.

Table 1. Effect of varying the concentration of peroxidase

Reaction mixtures consisted of: in the main vessel $6.7 \times 10^{-3} M$ IPA and $10^{-4} M$ - $MnSO_4$ in $6.7 \times 10^{-2} M$ phosphate at pH 6; after equilibration various amounts of horseradish peroxidase P.N. 500 were added from the side arm.

Peroxidase added ($\mu g.$)	Uptake of O_2 ($\mu l.$ in 1 hr.)
2	27
4	50.5
8	87.5
16	106.5
32	122
64	145
128	143.5

oxygen in the presence of Mn^{2+} . The results (Table 2) show that the various preparations do not differ appreciably in activity, suggesting that the observed oxidation of IPA is due to peroxidase.

Table 2. *Oxidation of IPA by different peroxidase preparations in the presence of Mn^{2+}*

Reaction mixtures consisted of: peroxidase preparation equivalent to 0.0025, 0.005 or 0.05 E.U., $6.7 \times 10^{-3}M$ IPA and $10^{-4}M$ - $MnSO_4$ in phosphate at pH 6; after equilibration the peroxidase was added from the side arm. Blank uptakes when IPA or Mn^{2+} was omitted were less than 4 μ l. in 1 hr.

E.U. present ...	Preparation	P.N.	Uptake of O_2 (μ l. in 1 hr.)		
			0.0025	0.005	0.05
	Turnip root	70	51.5	96	155
	Horseradish	500	52	95	138
	Horseradish	940	52	84	144
	Horseradish	1040*	42	80	134

* The purity of this preparation is about 90% (Kenten & Mann, 1953).

Table 3. *Effect of heating the horseradish-peroxidase preparation of P.N. 500 on (i) the oxidation of IPA by oxygen in the presence of Mn^{2+} , and (ii) the oxidation of a pyrogallol by hydrogen peroxide*

Peroxidase (P.N. 500) solutions were heated in a boiling-water bath for 0, 5, 10, and 15 min. The activity of these solutions was tested with pyrogallol and H_2O_2 under the fixed conditions of the purpurogallin test and manometrically with IPA in reaction mixtures consisting of $6.7 \times 10^{-3}M$ IPA, $10^{-4}M$ - $MnSO_4$ and $10^{-5}M$ - H_2O_2 in $6.7 \times 10^{-2}M$ phosphate at pH 6 with 10 μ g. of the heated peroxidase.

Time of heating (min.)	Loss in activity (%)	
	IPA	Pyrogallol
5	44	50
10	68	75
15	81.5	89

Effect of heating the peroxidase preparation of P.N. 500. The results of Table 3 show that rather prolonged heating in the neighbourhood of 100° is necessary to inactivate the peroxidase. The fall in activity towards both IPA and pyrogallol brought about by heating the preparation is of the same order and supports the previous suggestion that the oxidation of IPA is due to peroxidase.

Effect of phenols and maleic hydrazide. Certain monophenols, resorcinol and maleic hydrazide stimulate, but polyphenols such as pyrogallol and catechol inhibit, the peroxidase-catalysed oxidation of IAA by oxygen (Kenten, 1955). Accordingly the effect of these substances was tested in reaction mixtures consisting of the phenol or maleic hydra-

zide, $6.7 \times 10^{-3}M$ IPA, 30 μ g. horseradish peroxidase P.N. 940 and $6.7 \times 10^{-2}M$ phosphate at pH 6. The peroxidase was added from the side arm after equilibration. No significant oxygen uptake was obtained in 1 hr. with the phenols at 10^{-3} , 10^{-4} or $10^{-5}M$ or with maleic hydrazide at $5 \times 10^{-3}M$. When the reaction mixtures also contained $10^{-4}M$ - $MnSO_4$ no oxygen uptake was obtained in the presence of $10^{-4}M$ pyrogallol or catechol. However, in the presence of Mn^{2+} , although 10^{-2} and $10^{-3}M$ phenol inhibited, a slight stimulation of the oxygen uptake was observed with $10^{-4}M$ phenol (Table 4). By the use of reaction mixtures and conditions similar to those of Fig. 3, it was shown that the addition of catechol or pyrogallol ($10^{-4}M$ final concentration) from the second side arm, 20 min. after the reaction between IPA, Mn^{2+} and peroxidase had started, brought about complete inhibition (Table 4).

Table 4. *Effect of phenols on the oxidation of IPA by peroxidase plus Mn^{2+}*

Reaction mixtures were similar to those of Fig. 3 except that catalase was replaced by catechol, or pyrogallol, or phenol.

Components present with IPA and peroxidase	Uptake of O_2 (μ l.)	
	20 min.	60 min.
Mn^{2+}	58.5	133
Mn^{2+} + phenol $10^{-2}M$	—	21
Mn^{2+} + phenol $10^{-3}M$	—	92.5
Mn^{2+} + phenol $10^{-4}M$	—	163
Mn^{2+} + phenol $10^{-5}M$	—	137
Mn^{2+} , catechol $10^{-4}M$ added at 20 min.	58.5	61.5
Mn^{2+} , pyrogallol $10^{-4}M$ added at 20 min.	54	59

Effect of pyrophosphate and citrate. It has been shown previously (Kenten, 1955) that citrate and pyrophosphate strongly inhibit the activating effect of Mn^{2+} on the peroxidase catalysed oxidation of IAA by oxygen. It has been found that citrate and pyrophosphate at pH 6 also inhibit the reaction between IPA, Mn^{2+} and peroxidase. By the use of reaction mixtures and conditions similar to those of Fig. 3, except that the initial phosphate concentration was $3.3 \times 10^{-2}M$, it was shown that an addition of pyrophosphate or citrate at pH 6 ($3.3 \times 10^{-2}M$ final concentration) from the second side arm reduced the subsequent rate of oxygen uptake by 95 and 75% respectively. Control experiments showed that an addition of phosphate was without effect. The effect of pyrophosphate and citrate is presumably due to their capacity of forming complex ions with Mn^{2+} .

Course of the oxidation. When reaction mixtures containing 200 μ g. horseradish peroxidase P.N. 500, $10^{-4}M$ - $MnSO_4$, $3.3 \times 10^{-5}M$ - H_2O_2 (0.1 μ mole) and varying amounts of IPA in $6.7 \times 10^{-2}M$

phosphate at pH 6 were used, it was found that the total oxygen uptake varied with the initial IPA concentration. Thus in one experiment with 2.5, 5 and 10 μ moles IPA, the consumption of oxygen ceased after 4 hr. when the total uptake had reached values of 0.70, 0.67 and 0.55 μ mole oxygen/mole IPA. With 5 μ moles IPA, increasing the peroxidase concentration sixfold increased the total oxygen uptake by only 8%. In these experiments, using Warburg's direct method, no carbon dioxide formation was detected.

Oxidation of IPA by horseradish-peroxidase preparations plus hydrogen peroxide. Colorimetric tests suggested that the oxidation of IPA by hydrogen peroxide was catalysed by horseradish peroxidase, with the formation of a yellow oxidation product. This was proved manometrically by using the glucose-oxidase system as a source of hydrogen peroxide:

In the presence of catalase the hydrogen peroxide formed is decomposed and an apparent oxygen uptake of 0.5 mole oxygen/mole glucose is observed. If, however, a peroxidase system is present which uses the hydrogen peroxide for a secondary or coupled oxidation then an increase in the oxygen

uptake is observed which may reach 1 mole oxygen/mole glucose if all the hydrogen peroxide produced is used in the coupled oxidation.

Basal reaction mixtures consisted of: in the main vessel, 0.3 ml. glucose oxidase, 30 μ g. catalase and 0.2 ml. 0.05 M glucose in 0.067 M phosphate at pH 6. The complete reaction mixtures contained, in addition, 30 μ g. horseradish peroxidase P.N. 940 and 0.75 ml. 0.02 M IPA. The glucose was added from the side arm after equilibration. Control reaction mixtures were similar to the complete mixtures except that glucose, IPA or peroxidase was omitted.

The results (Fig. 4) show that in the absence of peroxidase or IPA the total oxygen uptake corresponded to 0.5 mole oxygen/mole glucose. In the presence of both peroxidase and IPA the total oxygen uptake was slightly greater than double this value, being about 1.1–1.2 mole oxygen/mole glucose. The 0.1–0.2 mole oxygen in excess of that theoretically required could be accounted for if peroxidase has a slight direct oxidase action under the conditions used. At the end of the experiment the complete reaction mixture was brownish yellow, indicating that oxidation of the IPA had taken place. The results suggest that the hydrogen peroxide produced by the glucose-oxidase system is used by the peroxidase to oxidize IPA.

Manometric studies of the oxidation of IBA by horseradish-peroxidase preparations and manganous ions. The few experiments which have been carried out with IBA suggest that, as with IPA, it is directly oxidized by the horseradish-peroxidase preparations only when Mn^{2+} is also present, and that monophenols cannot replace Mn^{2+} in this reaction. With reaction mixtures consisting of 6.7×10^{-3} M IBA, 10^{-4} M- MnSO_4 and 30 μ g. horseradish peroxidase P.N. 940 in 6.7×10^{-2} M phosphate at pH 6, oxygen uptakes of 80–88 μ l. in 1 hr. were obtained. The rate of oxidation of IBA is therefore less than that of IPA, since with this latter substance under similar conditions oxygen uptakes of about 140 μ l. in 1 hr. were obtained. When either peroxidase or Mn^{2+} was omitted the oxygen uptake was negligible. In contrast to IPA, the oxidation of freshly prepared IBA solutions was found to proceed after only a brief lag period.

DISCUSSION

The results of the present work show that highly purified horseradish-peroxidase preparations catalyse the oxidation of IPA by oxygen in the presence of Mn^{2+} . With freshly prepared neutral IPA solutions the reaction is usually preceded by a lag period whose length may vary from a few minutes to 2.5 hr. The lag period can be reduced by ageing the IPA solution at 0°, exposing it to bright

Fig. 4. The oxidation of IPA by peroxidase plus H_2O_2 . Reaction mixtures as described in the text. Δ , Glucose + glucose oxidase + IPA + peroxidase + catalase; $\times$, peroxidase omitted; $\circ$, IPA, or IPA + peroxidase, omitted; $\bullet$, glucose omitted.

light, or by adding very small amounts of hydrogen peroxide. Thus the variability of the length of the lag period could be partly due to differences in the light intensity in the laboratory when the different experiments were carried out. Since small amounts of added hydrogen peroxide abolish the lag period it is suggested that peroxides arise in IPA solutions when they are stored or exposed to light by autoxidation or photo-oxidation. Since IPA solutions absorb ultraviolet but not visible light such photo-oxidation is presumably brought about by ultraviolet light. The addition of catalase stops the reaction, suggesting that the reaction is accompanied by and depends on the formation of hydrogen peroxide. Although, under the conditions used, the direct oxidation of IPA by peroxidase depends on the presence of Mn^{2+} , it has also been shown that peroxidase readily catalyses the oxidation of IPA by hydrogen peroxide in the absence of Mn^{2+} . It would appear, therefore, that in the direct oxidase reaction Mn^{2+} may play a part in the production of hydrogen peroxide. Since at pH 6 IPA solutions consumed negligible amounts of oxygen whether Mn^{2+} was present or not it is clear that Mn^{2+} does not act by catalysing the autoxidation of IPA with the production of hydrogen peroxide.

Similar direct oxidase reactions have been described in which the oxidations of dihydroxymaleic acid (Swedin & Theorell, 1940), certain other dicarboxylic acids (Kenten & Mann, 1953), phenylacetaldehyde (Kenten, 1953) and IAA (Kenten, 1955) by oxygen are catalysed by peroxidase. The mechanism of this type of reaction is still largely a matter of discussion. Lemberg & Legge (1949) suggested a tentative mechanism for the dihydroxymaleic acid reaction, but it has been criticized by Theorell (1951) and Chance (1951) on the grounds that it does not account for the fact that 1 mole oxygen is consumed per mole of dihydroxymaleic acid. Chance (1952) has suggested that a Mn^{2+} -activated peroxidase-peroxide complex is the catalyst for the oxidase reaction with dihydroxymaleic acid, peroxidase and Mn^{2+} , while Kenten & Mann (1953) obtained evidence that the oxidation of certain dicarboxylic acids by peroxidase and Mn^{2+} was dependent on manganese oxidation.

In the present work it was found that neither hydrated MnO_2 nor manganipyrophosphate oxidized IPA rapidly at pH 6, and, if a manganese oxidation-reduction cycle plays a part in the oxidation of IPA, the oxidized manganese must react with an intermediate product.

In contrast to the results obtained by Kenten (1955), who found that the peroxidase-catalysed oxidation of IAA by oxygen was increased in the presence of certain monophenols, maleic hydrazide or Mn^{2+} , in the present work peroxidase did not

catalyse the oxidation of IPA unless Mn^{2+} was also present, and monophenols, or maleic hydrazide, were not capable of replacing Mn^{2+} in this reaction. A stimulation of the oxidation of IPA by peroxidase and Mn^{2+} was observed when $10^{-4}M$ phenol was also present but the effect was small, and lower ($10^{-5}M$) or higher (10^{-3} – $10^{-2}M$) concentrations of phenol were without significant effect or were inhibitory. It has been demonstrated that polyphenols (Kenten, 1955), when present in low concentration, strongly inhibit the peroxidase-catalysed oxidation of IAA by oxygen. In the present work it has been shown that pyrogallol and catechol have a similar effect on the oxidation of IPA by peroxidase and Mn^{2+} . The inhibiting effect of the polyphenols appears to be most easily explained by assuming that they are more readily oxidized by peroxidase than is IPA and that they therefore compete successfully for the hydrogen peroxide formed.

Although comparatively few experiments were carried out with IBA the results obtained were essentially similar to those found with IPA, except that the oxidation proceeded with only a brief lag period.

Since the oxidation of IPA and IBA proceed at concentrations of peroxidase and Mn^{2+} commonly encountered in plant extracts, it is probable that the oxidation of these compounds observed by Wagenknecht & Burris (1950) was due to the presence of peroxidase and Mn^{2+} in the plant extracts. In support of this suggestion it has been found that the oxidation of IBA by peroxidase and Mn^{2+} proceeds at a slower rate than that of IPA, and the oxidation of this latter compound takes place without the formation of significant amounts of carbon dioxide. These properties agree with those reported for bean root and pea epicotyl preparations by Wagenknecht & Burris (1950). In this connexion it may be noted that citrate inhibits the peroxidase-catalysed oxidation of IPA by oxygen in the presence of Mn^{2+} , and although Tang & Bonner (1947), working in citrate-phosphate, failed to demonstrate the oxidation of IPA by pea-epicotyl preparations, Wagenknecht & Burris (1950), using phosphate buffers and similar preparations, found that IPA was readily oxidized.

Since neither IPA nor IBA is a known plant metabolite the present reaction is probably without physiological significance. It is of interest, however, that peroxidase systems are connected with the metabolism of other indole compounds, namely tryptophan (Wiltshire, 1953) and IAA (e.g. Galston, Bonner & Baker, 1953; Kenten, 1955), which are known to be plant metabolites. Since Mann & Smithies (1955) have shown that peroxidase catalyses the oxidation of indole by hydrogen peroxide, it is likely that with the other compounds the indole nucleus is the site of peroxidase attack.

SUMMARY

1. The oxidation of IPA and IBA by oxygen is catalysed by highly purified horseradish peroxidase preparations in the presence of Mn^{2+} .

2. The mechanism of the reaction has not been elucidated but the formation of hydrogen peroxide as an obligatory intermediate is suggested by the inhibiting effect of catalase.

3. It is suggested that the previously reported oxidation of IPA and IBA by plant extracts may have been due to the presence of peroxidase and Mn^{2+} in the extracts.

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Absorption and Metabolism of [^{14}C]Pyrethroids by the Adult Housefly, *Musca domestica* L., in vivo

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The pyrethrins represent the most important group of pyrethroid insecticides of natural origin and are widely used for the control of various insect pests, particularly houseflies. The flowers of the plant *Chrysanthemum cinerariaefolium* provide a rich source of the natural insecticide, e.g. by extraction with light petroleum (LaForge & Haller, 1935). The natural pyrethrins are a mixture of the four principal esters (indicated in Fig. 1) and their stereoisomers (Harper, 1949).

Fig. 1. Pyrethroids of natural origin.

Ester	Structure
Pyrethrin I	$R = CH_3$; $R' = -CH_2 \cdot CH \cdot CH \cdot CH_2$
Cinerin I	$R = CH_3$; $R' = -CH_2 \cdot CH \cdot CH \cdot CH_3$
Pyrethrin II	$R = CO \cdot O \cdot CH_3$; $R' = -CH_2 \cdot CH \cdot CH \cdot CH_2$
Cinerin II	$R = CO \cdot O \cdot CH_3$; $R' = -CH_2 \cdot CH \cdot CH \cdot CH_3$

In this paper the esters derived from the cyclopropanemonocarboxylic acid (chrysanthemic acid, $R = CH_3$) and the cyclopentenone alcohol are called chrysanthemic esters, whereas those derived from the cyclopropanedicarboxylic acid monomethyl ester (pyrethric acid, $R = CO_2Me$) are called the pyrethric esters. Several insecticidally active analogues and homologues of the chrysanthemic esters have been synthesized in recent years (Schechter, Green & LaForge, 1949; Crombie, Edgar, Harper, Lowe & Thompson, 1950). Allethrin is the name approved by the U.S. Interdepartmental Committee on Pest Control for the synthetic allyl homologue ($R = CH_3$; $R' = CH_2 \cdot CH \cdot CH_2$) of cinerin I and is an economically important ester. It is more or less toxic than the natural esters to various insect species (Gersdorff, 1949; Bottger, 1951) and appears to have greater stability (Blackith, 1952). The toxicity of the natural pyrethroids to insects is enhanced by the prior or simultaneous application of suitable synergists, particularly certain compounds containing the methylenedioxyphenyl group (Schroeder, Jones & Lindquist, 1948; Chamberlain, 1950; Kerr, 1951). The synergists are themselves relatively non-insecticidal. The toxicity of allethrin