ENZYMATIC FORMATION OF INDOLE-3-CARBOXALDEHYDE
FROM INDOLE-3-ACETIC ACID

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It was first suggested by Tang and Bonner (14) that indole-3-carboxaldehyde is the product of the oxidative degradation of indole-3-acetic acid (IAA) by crude enzyme preparations from etiolated pea seedlings. Using the more active bean root enzyme, Wagenknecht and Burris (15) attempted without success to obtain the 2,4-dinitrophenylhydrazone of the aldehyde. On the basis of chromatographic data, Racusen (9) reports that the pea enzyme forms small amounts of the aldehyde. More recently, Wagenknecht (personal communication) has made similar observations with the bean root system. Ray and Thimmann (10) and Ray (11) have recently reported that the enzyme from *Omphalia flavida* is unable to form the aldehyde. The recent work of MacEachlan and Waygood (6) likewise has left unsettled the nature of the end product.

Manning and Galston (7) have reported the formation in the pea system of two products having high Rf values and giving qualitative color tests which indicate a similarity to our products #4 and #5 (see below). They were unable to demonstrate the formation of indole-3-carboxaldehyde, and also ruled out ortho-formamidoacetophenone, ortho-aminoacetophenone, and 4-hydroxyquinoline as major end products, although they believe that the indole ring is ruptured.

Chromatographic separation and spectrophotometric examination of the products of the IAA oxidase-peroxidase system from *Lupinus albus* L. (13) gave no indication that the aldehyde was formed; likewise, the results obtained when the chromatograms were tested with 2,4-dinitrophenylhydrazine hydrochloride, modified Salkowski (3), and Ehrlich reagent suggested the absence of the aldehyde. In view of the conflicting results, attempts were made to supplement the purified IAA oxidase with the auxiliary enzyme systems presumed to be present in the crude enzyme preparations employed in the early investigations. The present report is concerned with the coupling of the oxidase system to the cytochrome oxidase system. Under these conditions the formation of indole-3-carboxaldehyde as a major reaction product was observed.

Subsequent reexamination of the reaction products from the oxidase-peroxidase system, particularly those freshly prepared from etiolated lupine hypocotyls, indicated the normal formation of the aldehyde in small amounts. Therefore, in order to obtain data on the oxidation mechanism, indole-3-carboxylic acid and a number of substituted indole-3-acetic acid derivatives were tested both with the oxidase-peroxidase system alone and with the added cytochrome system. It is of interest to note that since this paper was submitted, Jones and Taylor (5) have reported the isolation of both indole-3-carboxaldehyde and indole-3-carboxylic acid from cabbage extracts.

**Materials and Methods**

Preparation and purification of the IAA oxidase from *Lupinus albus* L. has been previously described (13). The course of the oxidation of IAA was followed with the Warburg manometric apparatus under the conditions outlined earlier (13) except where 0.4 M tris-(hydroxymethyl)-aminomethane buffer at pH 7.2 was used in an attempt to approach the pH optimum of the cytochrome oxidase system and still maintain IAA oxidase activity. Under these conditions a small amount of phosphate, 4 micromoles per flask, was added for optimum IAA oxidase activity. The normal 3-ml reaction mixture was enzyme; 1 ml 0.4 M orthophosphate buffer, pH 6.3; 0.3 micromole MnCl2; 0.3 micromole sodium-2,4-dichlorophenolate; (in the main compartment) 0.1 ml M KOH with a standard wick in the center well; and 30 micromoles of sodium indole-3-acetate in the side arm. When the cytochrome system was to be coupled to the oxidase, cytochrome oxidase was added to each flask in the form of 0.2 ml of a 5% suspension of homogenized rat liver, together with cytochrome c, usually 2 x 10⁻⁶ M, and 0.3 micromoles of Al⁺⁺⁺.

Ascending chromatography on Whatman 3-mm filter paper was carried out with a solvent mixture consisting of 28% NH₃OH, water, and isopropyl alcohol in proportions ranging from 1:1:6 to 1:1:12, depending on whether it was desired to obtain greater resolution of the more acidic or the neutral fraction of the products. The reaction products to be discussed have been designated by numbers 1 to 5 in order of increasing Rf value (13). This has been done in preference to Rf designations alone because the relative positions of the components were retained in the various solvent mixtures used. The products (13) which appeared important in this study were the apparently large #2 (Rf 0.6) group of non-indolyl fluorescent compounds and the #4 and #5 (Rf 0.8 and 0.9), presumably polymeric indolyl products which bracket the aldehyde. The #1 (Rf 0.45) indolyl component (13) was not encountered in this study—presumably because the reaction with crude enzyme was not permitted to go to exhaustion.

In some experiments, the substrate was IAA, labeled with C¹⁴ either in the methylene (12) or in the carboxyl position (13). In order to obtain suffi-
cient activity on the chromatograms to permit reasonable counting time and accuracy, it was found necessary to label the substrate in each flask with approximately 30 microcuries of the methylene-labeled IAA or 3 microcuries of carboxyl-labeled IAA. C\textsuperscript{14} in the reaction products was measured by counting the chromatograms directly. Sixth-tenths of a milliliter of the enzyme reaction mixture was applied to a 4-inch segment of the chromatograph sheet. After chromatographic separation, sections 6 cm wide were counted by advancing the strip 1 cm at a time through the masked chamber of a modified PCC-10 proportional counter (Nuclear Measurements Corp., Indianapolis, Ind.).

Qualitative survey of the products on the chromatograms from all of the Warburg experiments was made by ultraviolet examination (Mineralight SL 2537) and by swabbing the strips with Ehrlich's reagent, 2,4-dinitrophenylhydrazine hydrochloride, and modified Salkowski reagent (3). The sensitivity of the ultraviolet scanning was greatly enhanced by illuminating the chromatograms from the back. Quantities of the indole-3-carboxaldehyde which were normally undiscernible were thus rendered detectable.

Fractionation and colorimetric determination of indole-3-carboxaldehyde and other indole products in the reaction mixture was carried out by the following procedure. One ml of the reaction mixture from the Warburg flask was added to 9 ml of buffer containing 7.5 % NaHCO\textsubscript{3} and 2.5 % Na\textsubscript{2}CO\textsubscript{3}, and extracted with three 15-ml portions of peroxide-free ether. IAA was estimated in the aqueous phase with the modified Salkowski reagent (3) after the bulk of the dissolved ether had evaporated. Although the inadequacies of the Salkowski method have been well documented by previous investigators (8), the procedure was used in an attempt to obtain an evaluation of the residual IAA and the IAA equivalents of some of the products. It had been hoped that the Salkowski data along with the aldehyde and oxygen uptake values would permit an approximation of the reaction balance.

For the determination of indole-3-carboxaldehyde, the ether phase was evaporated to dryness in an air stream and the residue was taken up in 5 ml of absolute ethanol. A suitable aliquot of the ethanol solution was made up to 5 ml with ethanol, and to this was added 0.5 ml of 2,4-dinitrophenylhydrazine hydrochloride reagent (100 mg of 2,4-dinitrophenylhydrazine hydrochloride in 100 ml of 6 N HCl). The colored solution was read in the Klett photoelectric colorimeter, using the No. 54 filter. Color development is instantaneous; the color is stable for at least an hour and apparently is unaffected by the presence of other indolyl compounds. The standard curve for indole-3-carboxaldehyde has a range of 0 to 200 micrograms over 340 Klett units. Recovery values in samples added to the Warburg reaction mixture substantiated the reliability of the extraction method.

Purification of the indole-3-carboxaldehyde for identification was carried out by strip chromatography of the fraction extractable from the reaction mixture by ether at pH 10. The aldehyde fraction was eluted with acetone, concentrated, and the chromatography repeated. A small amount was taken up in ethanol for the ultraviolet absorption measurement; another aliquot was mixed with powdered KBr and a disk was prepared for infrared spectrophotometry. The invaluable help of Dr. J. H. Pomeroy and Mrs. C. A. Craig in this phase of the work is gratefully acknowledged.

Since indole-3-carboxaldehyde does not react appreciably with modified Salkowski reagent, the latter was used to determine the non-aldehyde, Salkowski-positive, indolyl compounds soluble in ether at pH 10 (the # 4 and # 5 products on the chromatograms), and a survey was made by comparison with the IAA standard curve.

The author wishes to acknowledge the provision of the substituted IAAs so generously made available for this investigation. Na-indole-3-glycolate was supplied by Dr. Marvin D. Armstrong of the University of Utah. Ethylindole-3-glyoxylate was obtained from Dr. Bruce Stowe of Harvard University; the ester was hydrolyzed by the method of Elks et al (1). Ethylindole-3-isobutyrate was provided by Dr. H. Erdtman and Dr. A. Jönsson of the Tekniska Högskolan, Stockholm, Sweden; the free acid was prepared by their method (2). Samples of 1-methyl-IAA, 2-methyl-IAA, and 2-carboxy-IAA were obtained from Dr. Sidney Fox of the University of Florida. Indole-3-carboxylic acid was supplied by Dr. Norman E. Good of the Science Service Laboratory, London, Ontario, Canada. Dr. K. Mann of the Upjohn Laboratories, Kalamazoo, Michigan, provided the aldehydes.

### Table I

<table>
<thead>
<tr>
<th>IAA-oxidase Preparation</th>
<th>Cytochrome C</th>
<th>Oxygen Uptake*</th>
<th>IAA Used</th>
<th>Product # 4**</th>
<th>Indole-3-carboxaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>moles</td>
<td>micro-moles</td>
<td>micro-moles</td>
<td>micro-moles</td>
<td>micro-moles</td>
</tr>
<tr>
<td>0.0</td>
<td>1 x 10\textsuperscript{-4}</td>
<td>-0.49</td>
<td>0.9</td>
<td>0.02</td>
<td>0.21</td>
</tr>
<tr>
<td>0.5</td>
<td>1 x 10\textsuperscript{-4}</td>
<td>0.22</td>
<td>0.9</td>
<td>0.1</td>
<td>0.62</td>
</tr>
<tr>
<td>&quot;</td>
<td>5 x 10\textsuperscript{-5}</td>
<td>2.9</td>
<td>6.35</td>
<td>0.34</td>
<td>2.69</td>
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<tr>
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<td>14.7</td>
<td>0.79</td>
<td>4.0</td>
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<tr>
<td>&quot;</td>
<td>5 x 10\textsuperscript{-5}</td>
<td>11.2</td>
<td>15.8</td>
<td>1.03</td>
<td>3.52</td>
</tr>
<tr>
<td>&quot;</td>
<td>1 x 10\textsuperscript{-4}</td>
<td>14.3</td>
<td>18.9</td>
<td>1.1</td>
<td>2.28</td>
</tr>
<tr>
<td>&quot;</td>
<td>0 x 0</td>
<td>13.9</td>
<td>16.6</td>
<td>1.05</td>
<td>0.83</td>
</tr>
<tr>
<td>1.0</td>
<td>1 x 10\textsuperscript{-4}</td>
<td>-0.31</td>
<td>1.43</td>
<td>0.07</td>
<td>0.62</td>
</tr>
<tr>
<td>&quot;</td>
<td>5 x 10\textsuperscript{-5}</td>
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<td>6.0</td>
<td>0.34</td>
<td>2.28</td>
</tr>
<tr>
<td>&quot;</td>
<td>1 x 10\textsuperscript{-4}</td>
<td>14.5</td>
<td>20.0</td>
<td>1.35</td>
<td>4.35</td>
</tr>
<tr>
<td>&quot;</td>
<td>5 x 10\textsuperscript{-5}</td>
<td>17.5</td>
<td>21.7</td>
<td>1.7</td>
<td>4.14</td>
</tr>
<tr>
<td>&quot;</td>
<td>1 x 10\textsuperscript{-4}</td>
<td>18.0</td>
<td>22.1</td>
<td>1.66</td>
<td>3.9</td>
</tr>
<tr>
<td>&quot;</td>
<td>0 x 0</td>
<td>17.3</td>
<td>21.1</td>
<td>1.58</td>
<td>1.79</td>
</tr>
</tbody>
</table>

* These values were obtained after 90 min at 30 °C.

The amounts of reaction products listed were obtained from 3.0 ml reaction mixture. Flasks containing cytochrome c also contained 0.2 ml of 5 % rat liver homogenate.

** The estimation of reaction product # 4 is described under Materials and Methods.
the 5-hydroxy-IAA. The indole-3-carboxaldehyde was obtained from Dow Chemical Company, Midland, Michigan.

**RESULTS AND DISCUSSION**

Indole-3-carboxaldehyde has for a long time figured prominently as a possible reaction product of IAA oxidation. The data on substrate utilization (14) and the respiratory quotients observed (15) were suggestive in this respect. Only recently (9) has this product been detected in small amounts. Earlier studies of the author (13) showed that with the enzyme from *Lupinus albus*, regardless of its state of purity, significant amounts of the aldehyde were not accumulated. Therefore, in order to maintain the role of the aldehyde as an intermediate prod-

**FIG. 1.** Effect of cytochrome system on oxygen uptake with a dialyzed oxidase preparation, Mn++, and 2,4-dichlorophenol. O, reaction mixture without added ingredients; ×, cytochrome oxidase; +, Al+++; Δ, cytochrome c; ■, cytochrome c + cytochrome oxidase; ●, cytochrome c + cytochrome oxidase + Al+++; ▲, cytochrome system minus IAA oxidase, or IAA oxidase plus the cytochrome system minus 2,4-dichlorophenol.

**FIG. 2.** The ultraviolet absorption spectrum of synthetic indole-3-carboxaldehyde and of material isolated from enzyme reaction mixture, in ethanol.  

uct of IAA-oxidation, one must assume either its rapid further oxidation in the reaction sequence or that it is not formed in quantity under the experimental conditions. However, the first hypothesis cannot be substantiated since the oxidase obtained from lupine is incapable of utilizing indole-3-carboxaldehyde as a substrate.

In the present experiments it was found that, if the right concentration ratios of oxidase and cytochrome c are selected, a 50% depression in the oxygen uptake is observed as is nearly the case in figure 1. Before the concentration response data in table I were established, this restriction of the oxygen uptake was thought to indicate a complete shift in the metabolic pattern. However, the relationship between oxygen uptake and cytochrome c (table I) may be conservatively interpreted to suggest that cytochrome c is inhibitory up to a concentration of $10^{-6}$ M, at which level it is slightly stimulatory. The aldehyde response is parallel to the oxygen uptake in part, but the highest level of aldehyde is reached at $10^{-5}$ M cytochrome c, and the most efficient conversion of IAA to aldehyde is at $5 \times 10^{-5}$ M cytochrome c. At this level, with 0.5 ml of enzyme, approximately 6 moles of IAA were oxidized by three moles of oxygen to give three moles of aldehyde. This molar ratio would be significant if the Salkowski assay could be assumed to be reliable; however, the 1-ml enzyme experiment (table I) and the C14 data (fig 4 and 5) suggest that this is not the case. It is attractive to suggest that the cytochrome system is competing as the terminal oxidase by diverting hydrogen from the peroxidase portion of the oxidase-peroxidase system so that a reducible intermediate is not regenerated.

The relation of the formation of the #4 product
to the aldehyde and the cytochrome system is more clearly shown in the methylene-C14 labeled IAA experiments than the data in table I indicate.

In figure 2 the ultraviolet absorption spectrum of synthetic indole-3-carboxaldehyde is compared with that of the material obtained from the enzyme experiment, and the corresponding infrared absorption spectra are shown in figure 3. The present identification and isolation in quantity of indole-3-carboxaldehyde after action of IAA oxidase in presence of cytochrome c and cytochrome oxidase shows that the usual reaction sequence can be diverted from its main course. The relative significance of this part in the metabolism of IAA by plants remains to be established (5).

Two possible intermediate precursors of the aldehyde were tested with crude and purified enzyme, in the absence and in the presence of the cytochrome c system. Indole-3-glyoxylic acid was totally inert even with the crude enzyme, since there was no oxygen uptake or product formation as shown by the chromatograms, while indole-3-glycolic acid (table II) gave the aldehyde in substantial amounts independent of the cytochrome system. Neither acid shows any oxygen uptake with the cytochrome system in the absence of the oxidase, although a trace of the aldehyde could be detected on the chromatograms from the reaction of indole-3-glycolic acid and the cytochrome system. Recently Greenburg and Gals ton (4) have observed the instability of indole-3-glycolic acid and have suggested a role for it in the formation of auxin. Indole-3-glyoxylic acid should presumably be the intermediate between indole-3-glycolic acid and the aldehyde; the fact that it was not utilized by IAA oxidase suggests that decarboxylation occurs simultaneously with dehydrogenation.

![Infrared spectra of synthetic and enzymatic indole-3-carboxaldehyde; the KBr disc technique was used. In the region not shown in the figure, the spectra of the two compounds were essentially identical.](image)

To shed light on the primary site of oxidation, three substituted IAAs were studied with enzyme preparations of different degrees of purity. The results with crude and with highly purified IAA oxidase are given in table II. Despite the substitution in the side chain, indole-3-isobutyric acid was found to be a better substrate than IAA in that it supported a higher oxygen uptake, and, in the case of the electrophoretically homogeneous enzyme, the chromatograms showed no residual substrate. Chromatograms of the reaction mixture from the crude enzyme showed residual substrate. Similar oxygen uptake data have been reported by Ray and Thimann (10) with the enzyme from *Omphalia flavida*.

### Table II

**Response of Substituted Indole-3-acetic Acid to the Oxidase from L. albus**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cytochrome system (2 x 10^-4 M cytochrome c)</th>
<th>Crude oxidase from etiolated Lupine hypocotyls</th>
<th>Electrophoretically homogenous oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>uptake *&lt;br&gt;IAA used **&lt;br&gt;Product #4 †&lt;br&gt;Aldehyde ‡</td>
<td>Oxygen uptake *&lt;br&gt;IAA used **&lt;br&gt;Product #4 †&lt;br&gt;Aldehyde ‡</td>
<td></td>
</tr>
<tr>
<td>IAA</td>
<td>-</td>
<td>10.6&lt;br&gt;16&lt;br&gt;0.69&lt;br&gt;0.21</td>
<td>15.2&lt;br&gt;22&lt;br&gt;1.06&lt;br&gt;1.03</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>11.4&lt;br&gt;16&lt;br&gt;0.99&lt;br&gt;3.93</td>
<td>11&lt;br&gt;16.3&lt;br&gt;0.67&lt;br&gt;5.17</td>
</tr>
<tr>
<td>Indole-3-glycolic</td>
<td>-</td>
<td>6.5&lt;br&gt;...&lt;br&gt;0.085&lt;br&gt;16.1</td>
<td>9.25&lt;br&gt;...&lt;br&gt;0.17&lt;br&gt;24.9</td>
</tr>
<tr>
<td>acid</td>
<td>+</td>
<td>7.2&lt;br&gt;...&lt;br&gt;0.12&lt;br&gt;18.2</td>
<td>8.84&lt;br&gt;...&lt;br&gt;0.17&lt;br&gt;25.9</td>
</tr>
<tr>
<td>Indole-3-isobutyric acid</td>
<td>-</td>
<td>16.1&lt;br&gt;...&lt;br&gt;1.08&lt;br&gt;0.21</td>
<td>20.9&lt;br&gt;...&lt;br&gt;1.89&lt;br&gt;1.03</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>19&lt;br&gt;...&lt;br&gt;1.73&lt;br&gt;0.21</td>
<td>20.3&lt;br&gt;...&lt;br&gt;2.91&lt;br&gt;1.45</td>
</tr>
<tr>
<td>1-Methy1indole-3-acetic acid</td>
<td>-</td>
<td>11.4&lt;br&gt;...&lt;br&gt;0.65&lt;br&gt;0.41</td>
<td>6.83&lt;br&gt;...&lt;br&gt;0.64&lt;br&gt;0.85</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>12&lt;br&gt;...&lt;br&gt;0.67&lt;br&gt;3.86</td>
<td>8.0&lt;br&gt;...&lt;br&gt;0.89&lt;br&gt;5.59</td>
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<tr>
<td>2-Carboxyindole-3-acetic acid</td>
<td>-</td>
<td>10&lt;br&gt;...&lt;br&gt;0.1&lt;br&gt;0.0</td>
<td>0.0&lt;br&gt;...&lt;br&gt;0.0&lt;br&gt;0.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>4.3&lt;br&gt;...&lt;br&gt;0.0&lt;br&gt;0.0</td>
<td>0.0&lt;br&gt;...&lt;br&gt;0.0&lt;br&gt;0.0</td>
</tr>
</tbody>
</table>

All values are expressed in micromoles.

* These values were obtained after 90 min at 30° C. The initial substrate concentration was 30 micromoles.

** Salkowski test with IAA as the reference standard.

† The analysis of product #4 and of indole-3-carboxaldehyde is described in the text.

‡ In the colorimetric determination, indole-3-carboxaldehyde was used as the reference standard. This restricts the accuracy of the results obtained with the IAA derivatives.
Considering the level of substrate utilized and the oxygen uptake, the amount of substance reacting with 2,4-dinitrophenylhydrazine was low; it is possible that an oxindole (11) causes the response. However, these data suggest that the alpha carbon of the side chain is not the primary site of oxidation. It appears rather that the ring system is the site of attack and that ring substitution should block the oxidation. Two compounds of this type were tested: 1-methylindole-3-acetic acid and 2-carboxyindole-3-acetic acid.

With the purified IAA oxidase, no oxygen uptake was observed with 2-carboxyindole-3-acetic acid as a substrate. The ability of the frozen crude enzyme preparations from 10-day-old etiolated hypocotyls, in contrast to the electrophoretically purified material, to metabolize the 2-carboxy-IAA constitutes the first evidence of a restriction in range introduced by purification of the system. This suggests that part of the changes in activity observed on aging and purification of the crude enzyme from both etiolated and non-etiolated tissue may be caused by the loss of some essential material. The dialyzed enzyme gave results similar to the electrophoretically purified enzyme preparation.

In further experiments, none of the lupine enzyme preparations has been found capable of oxidizing 5-hydroxy-IAA, tryptophan, indole-3-acetonitrile, indole-3-carboxaldehyde, or indole-3-carboxylic acid.

Attempts were made to amplify and corroborate the data on the reaction by experiments with C14-labeled IAA, with the label either in the carboxyl group (IAA-1-C14) or in the methylene group (IAA-2-C14) of the side chain (12, 13).

The concentration of IAA-2-C14 declined during the course of the reaction, as measured by the decrease in its C14 counts, and increasing activity was recovered in the products. This correlation was observed with samples taken after 30-, 60-, and 90-minute reaction periods (see figs 4 and 5, in which the 60-minute values are omitted). Fig 4 shows the activity distribution found on the chromatograms when the oxidase used IAA-2-C14 as a substrate, in the presence and absence of the cytochrome system, in TRIS buffer at pH 7.2. Similarly, figure 5 shows the response to the same enzymes in phosphate buffer at pH 6.3. pH 6.3 is the optimum for the partially purified oxidase used, while the pH 7.2 TRIS buffer represents compromise with the optimum for cytochrome oxidase.

The effect of the cytochrome system (minus the

![Fig. 4. Paper chromatography of the products from IAA-2-C14 obtained by enzymatic reaction in TRIS buffer at pH 7.2. The locations of residual IAA, products #2, #4, indole-3-carboxaldehyde, and of the solvent front are indicated. The reaction time was 30 minutes (●), or 90 minutes (+). The solid lines show the response to the IAA oxidase system alone, the broken lines the results with addition of the cytochrome c-cytochrome oxidase system (2 x 10^-4 M cytochrome c).](image)

![Fig. 5. Paper chromatography of the reaction products from IAA-2-C14 obtained in phosphate buffer, pH 6.3. The solid lines show the effect of the oxidase system, the broken lines indicate the results in presence of IAA oxidase and cytochrome system (2 x 10^-4 cytochrome c). The reaction times were 30 minutes (●) and 90 minutes (+). Shaking of the IAA for 90 minutes (○) without enzyme proved that little spontaneous decomposition had taken place.](image)
IAA oxidase) after 90 minutes is also shown in figure 5 by the presence of activity in the aldehyde and product #5 areas of the chromatogram. The pre-
dominant shift in activity from the #4 product to the aldehyde, which is found on the addition of the
cytochrome system in both figures 4 and 5, is evidence of
a major change in the reaction pathway.

The unstable and apparently complex reaction
product #4 (fig 4 and 5) may contain the oxindole
described by Ray and Thimann (10). However, when
this fraction was removed from the chromatograms
and examined, the ultraviolet and infrared spectra
were complex, and on rechromatography, three or
four components were found, including one which ap-
ppears to be an anthranilate. It is assumed that oxida-
tion or decomposition, or both, occurred during
manipulation and rechromatography.

The immobile fraction found below IAA on the
chromatogram (fig 5) could be detected only by the
tracer technique; it is neither fluorescent nor does it
show indolyl reactions. Phosphate buffer at pH 6.3
favors its formation. The presence of the cyto-
chrome-cytochrome oxidase system had little influence
on its formation. Infrared spectra of this material
indicated the absence of phenyl or indolyl groups;
the only discernible functional groups were methylene
and carboxyl groups. The aliphatic nature of this
reaction product is thus indicated, and because of the
immobility on chromatograms, a strongly acid char-
acter is suggested.

Experiments with carboxy-C14-IAA yielded pre-
dominantly non-radioactive products; the C14 was
recovered as BaC14O3 from the center well. An ex-
ception to this was the non-indolyl product #2
formed in TRIS buffer (fig 4), which still carried C14,
whether carboxyl- or methylene-labeled IAA was used
as a substrate. It is weakly acidic, shows green fluo-
rescence, and is assumed to be aromatic. The buffer
used for its formation is immaterial.

The present data show that catabolism of IAA
may occur by more than one route. With respect to
the primary site of action of IAA oxidase, the obser-
vations are in harmony with the results of other lab-
oratories (7, 10, 11); the ring system is altered by
the primary attack. Further work will be required
to unravel the reaction sequence and identity of the
products.

The author wishes to acknowledge the assistance
of Mrs. Sara Berliner in carrying out many of these
experiments.

SUMMARY

Indole-3-carboxaldehyde is a major reaction prod-
cut of the action of IAA oxidase provided the system
is coupled to the cytochrome-cytochrome oxidase sys-
tem. Indole-3-glycolic acid yields indole-3-carboxa-
aldehyde with equal efficiency both in the absence and
presence of cytochrome c. Experiments with substi-
tuted indole-3-acetic acids as substrate indicate that
the ring system, not the side chain, is the primary
site of oxidation. The two position of the indole ring
is probably the primary site of dehydrogenation. The
use of IAA-2-C14 as substrate made possible the de-
tection of a reaction product which apparently is an
aliphatic acid. The formation of this product is un-
affected by the cytochrome system suggesting that it is
the terminal oxidation product.

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