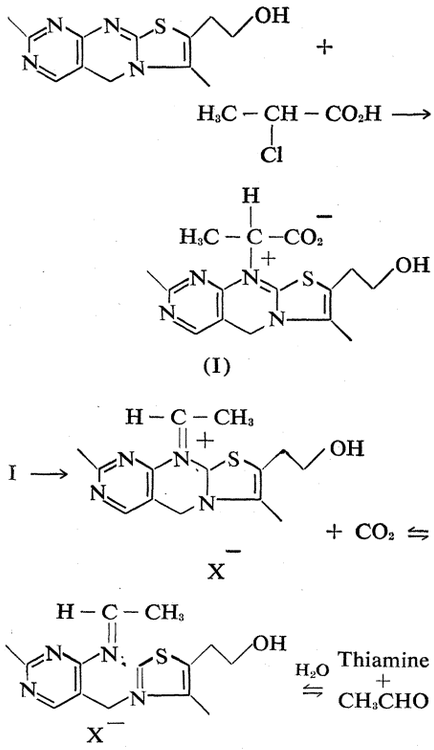
water) was added to the solution, and the mixture was stirred and heated in an oil bath at 50°C for 5 hours. Then a portion was acidified to *p*H 2 with dilute hydrochloric acid and analyzed by paper chromatography. The chromatogram showed the presence of acetaldehyde, thiamine hydrochloride, thiochrome, and thiochrome hydrochloride. The relative frequencies, R_F, of these compounds in a mixture of alcohol, acetic acid, and water (1:1:1) were 1.0, 0.57, 0.92, and 0.78, respectively; in a mixture of isobutyl alcohol, acetic acid, and water (4:5:1) R_F's were 1.0, 0.26, 0.84, and 0.49. The reaction sequence can be formulated in these terms:



At present the biological significance of this reaction to the thiamine-catalyzed decarboxylation of pyruvic acid is not known.

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Indoleacetamide as an Intermediate in the Synthesis of Indoleacetic Acid in Pseudomonas savastanoi

Abstract. *When* DL-*tryptophan-2-C*^{14} *was incubated with washed cells or cell-free preparations of* Pseudomonas savastanoi, *two radioactive metabolites were formed. One was identified as indoleacetamide and the second, indoleacetic acid. The amount of indoleacetamide in the reaction mixture increased rapidly during the early stages of incubation; it reached a peak after 15 minutes and declined steadily thereafter. Indoleacetic acid, on the other hand, accumulated slowly throughout the incubation period. Cell-free preparations preferentially utilize the* L-*isomer of tryptophan for the synthesis of indoleacetamide and indoleacetic acid. The results of these experiments suggest, therefore, that* P. savastanoi *synthesizes indoleacetic acid by the following reactions:* L-*tryptophan→ indoleacetamide→indoleacetic acid.*

The bacterium, *Pseudomonas savastanoi*, incites the production of tumorous outgrowths on plants. Indoleacetic acid accumulates in the tumors and, presumably, plays some part in their formation (*1*). Relatively large quantities of indoleacetic acid accumulate in culture media in which the bacteria are growing. Since tryptophan is known to be a precursor of indoleacetic acid in the metabolism of a number of organisms (*2*), studies were made on its metabolism in *P. savastanoi*.

When DL-tryptophan-C^{14} was incubated with extracts of cells of *P. savastanoi* that had been broken up by high frequency sound, indoleacetic acid and an unknown compound with properties resembling those of indoleacetamide were produced. We are reporting the identification of the unknown compound as indoleacetamide and its role as an intermediate in the conversion of tryptophan to indoleacetic acid by this bacterium.

Cells of *P. savastanoi* were washed from a solid proteose peptone-glycerol medium with sterile, distilled water. Cell-free suspensions were prepared by disrupting the bacterial cells, at 2°C under hydrogen, in a Raytheon sonic oscillator. The suspension was then centrifuged at 15,000*g* for 10 minutes at 0°C and passed through a Millipore filter (pore size 0.45 μ) to remove cellular debris. The reaction mixtures consisted of an enzyme preparation containing 26 μg of protein, 0.1 μc of DL-tryptophan-2-C^{14} (1.1 mc/mmole), 2.5 ml of 0.01*M* tris buffer (*p*H 7.4), and water with a final volume of 3.1 ml. The reactions were carried out at 27°C for specified periods of time and were terminated by heating in boiling water for 5 minutes. The denatured protein in the heated reaction mixtures was removed by centrifugation.

The formation of the products of the reactions was followed by separating the components on chromatographic paper by a mixture of isopropanol, concentrated NH_4OH, and water (10:1:1, v/v). Radioactive components on the chromatograms were detected by a chromatographic-strip counter and then radioactivity was determined with a Packard tri-carb liquid scintillation counter (*3*). In addition, radioactivity was determined with a Tracerlab gas flow counter.

Chromatograms thus prepared and treated bore three radioactive areas with R_F values of 0.23, 0.36, and 0.73. These values corresponded with the R_F values of tryptophan, indoleactic acid (IAA), and indoleacetamide (IAM), respectively. The unknown radioactive compounds at R_F 0.36 (unknown A) and at R_F 0.73 (unknown B) were eluted from chromatograms. Unknown A was cochromatographed with unlabeled, authentic IAA and unknown B with unlabeled, authentic IAM. The chromatograms were then developed in the first dimension with 70-percent ethanol and in the second dimension with the mixture of isopropanol, ammonia, and water. Radioautograms were prepared to determine the positions of the radioactive compounds and the chromatograms were then sprayed with Salkowski's reagent (*4*). In both cases radioactive areas corresponded exactly in size and position with the Salkowski-positive areas.

In another experiment, radioactive unknown A and unknown B were eluted from chromatograms with 95-percent ethanol and the eluates diluted with water to give a final solution of 30-percent ethanol. Then 17 mg of authentic IAA were dissolved in the solution of unknown A and 40 mg of authentic IAM were dissolved in the solution of unknown B. After acidification to *p*H 3.0, the solutions were shaken with ether and the ether fractions were separated. The ether was evaporated and the residues were crystallized from water. Weighed samples of the crystalline compounds were measured for radioactivity and their specific activities were calculated. The

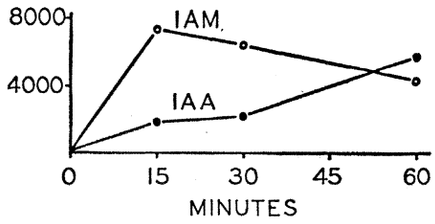

Fig. 1. Rate of formation of IAA and IAM in reaction mixture. The ordinate is count/min.

specific activity of both samples remained constant through three crystallizations.

These experiments indicated that unknown A was indoleacetic acid and unknown B was indoleacetamide.

Because it is possible that IAM was formed as an artifact by ammonolysis of an ester of IAA (*5*) when the chromatogram was developed in the isopropanol, ammonia, and water mixture, other chromatograms of the reaction products were developed in 70-percent ethanol or in deionized water. Only IAM and IAA were detected. These were in amounts similar to those on chromatograms developed in the mixture of isopropanol, ammonia, and water. Apparently, therefore, ammonolysis was not involved in IAM production. Because good separation of IAA and IAM is obtained with this mixture as solvent, it was used to develop all subsequent chromatograms of the two compounds.

Figure 1 illustrates the rate at which IAM and IAA appeared in the reaction mixture. IAM increased rapidly during the first few minutes of incubation, reached a maximum within 15 minutes, and decreased slowly thereafter. On the other hand, IAA increased slowly throughout the incubation period and appeared to accumulate as the end product. The amount of tryptophan in the reaction mixture decreased to approximately 50 percent within 15 minutes and remained relatively constant thereafter. When $10^{-3}M$ D-tryptophan was used as the substrate, neither IAM nor IAA was detected in the reaction mixture. Both products were detected, however, when either $10^{-3}M$ DL- or L-tryptophan was used as the substrate. Therefore, it appears that the L-isomer is utilized preferentially.

Enzyme preparations aged by storage at 4°C retain the capacity to convert tryptophan to IAM for several weeks, but rapidly lose the capacity to convert IAM to IAA, suggesting that at least two enzymes are involved in the reactions.

A number of compounds ($7 \times 10^{-4}M$ NaN_3, $7 \times 10^{-4}M$ KCN, $7 \times 10^{-5}M$ chlorogenic acid, and catalase) inhibited the conversion of tryptophan to IAM. Potassium cyanide at $7 \times 10^{-4}M$ was the most effective, inhibiting the reaction 73 percent. Incubation of the reaction mixture under an atmosphere of nitrogen reduced the conversion of tryptophan to IAM by 77 percent and indicated that oxygen is required for the reaction.

Several pathways have been suggested for the conversion of tryptophan to IAA (*2*, *6*). These are represented by the following reactions or their modifications.

1. tryptophan→tryptamine→ indoleacetaldehyde→IAA.
2. tryptophan→indolepyruvate→ indoleacetaldehyde→IAA.
3. tryptophan→indoleacetonitrile→IAM→IAA.

Only the last scheme includes IAM as an intermediate. This scheme suggests that IAM arises from indoleacetonitrile (*6*). There is very little evidence from studies on enzymes to confirm the occurrence of the last pathway. Earlier reports of the natural occurrence of IAM were open to question when it was shown that IAM may be formed by ammonolysis if NH_4OH were used in the developing solvent (*5*).

The evidence presented in our studies indicates that IAM is an intermediate in the synthesis of IAA from tryptophan in *P. savastanoi* and that the sequence of reactions is: tryptophan→IAM→IAA. Recently, Riddle and Mazelis (*7*) showed that preparations from cabbage convert tryptophan to IAM, thus providing evidence of a similar pathway in higher plants.

Because no radioactive products other than IAM and IAA could be detected in the reaction mixtures in the present studies, it appears that the conversion of tryptophan to IAM might be catalyzed by a single enzyme. A reaction of this type has been discovered and studied by Mazelis (*8*) who showed that methionine is converted to 3-methylthiopropionamide by peroxidase from horseradish. The aforementioned compounds which inhibited the conversion of tryptophan to IAM according to Mazelis also inhibited the conversion of methionine to 3-methylthiopropionamide. Moreover, enzyme preparations used in our studies showed high peroxidase activity when assayed by the method of Willstätter and Stoll (*9*). The second step, the hydrolysis of indoleacetamide to indoleacetic acid, could be catalyzed by an amidase. Reactions of this type are well known (*10; 11*).

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Experimental Differentiation between Phototaxis and Motility in Chlamydomonas snowiae

Abstract. *A reversible inhibition of phototaxis by acetic acid is demonstrated in* Chlamydomonas snowiae. *The inhibition is dependent on the* pH *of the medium and on the concentration of the inhibitor and does not act by affecting motility. Some substances closely related to fatty acids are also shown to be inhibitors of phototaxis, while certain metabolites have no effect. The possible mechanism by which the phototactic response is prevented is discussed.*

The phototactic response of *Chlamydomonas snowiae* is extremely variable (*1–3*). Sachs and Mayer (*2*) found that the cells in a culture might be fully motile yet would not respond to a light stimulus. Although a number of compounds that inhibit phototaxis are known, all of them appear