

ON THE MECHANISM OF ACTION OF 2, 4-DICHLOROPHENOXYACETIC ACID

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Summary

The rate of destruction of indole-3-acetic acid by a crude enzyme preparation from etiolated pea epicotyls is increased by 2, 4-dichlorophenoxyacetic acid.

A natural inhibitor present in boiled onion juice opposes this increase.

A mechanism is suggested for the action of 2, 4-D on the growth of plants.

I. INTRODUCTION

The use of synthetic plant growth-regulating compounds is of increasing economic importance in agriculture but little is known of their fundamental mechanism of action. The hormone-like character and general structural similarity of certain substituted aryl-acetates and -oxyacetates or their potential precursors (Zimmerman and Hitchcock 1941-42) to that of indole-3-acetic acid (I.A.A.) suggest that these compounds may act indirectly by altering the activity of this natural plant hormone. Tang and Bonner (1947) have partially characterized an enzyme prepared from pea epicotyls (and referred to here as I.A.A. oxidase) which oxidizes and inactivates I.A.A. On adding 11 auxin analogues to a crude enzyme preparation they found no change in the rate of I.A.A. inactivation. However, as these workers used a *substrate* (I.A.A.) *concentration* that was rate-limiting, the only rate-change necessarily to be detected would have been a *decrease* due to competition with the substrate.

II. EXPERIMENTAL AND RESULTS

In this laboratory a crude preparation containing I.A.A. oxidase (the "whole cytoplasm" of Tang and Bonner) was prepared from etiolated epicotyls of peas grown in the dark at 24°C. for seven to ten days. With an initial substrate concentration of 2×10^{-4} M the I.A.A.-destroying activity was found to be directly proportional to the enzyme concentration up to an activity of approximately 95 μ M/l./hr. at 27.5°C. This condition holds for subsequent batches of enzyme irrespective of the absolute activity per unit volume of the enzyme suspension, provided the initial substrate concentration and incubation temperature are the same. In experiments reported here the enzyme concentrations were always rate-limiting. On the addition of 2, 4-dichlorophenoxyacetic acid (2, 4-D), a

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marked stimulation in the rate of inactivation was obtained, increasing with increasing concentration of 2, 4-D (Fig. 1).

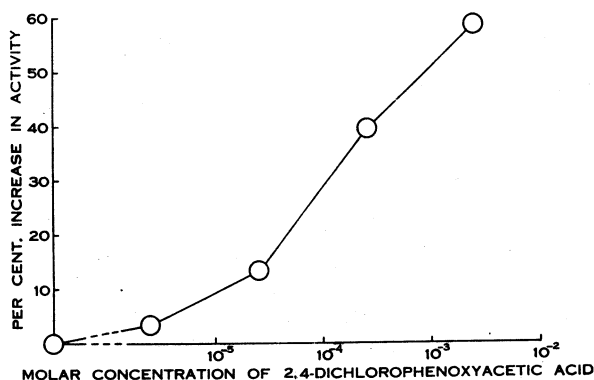


Fig. 1.—The effects of 2, 4-dichlorophenoxyacetic acid on the activity of a crude preparation of indole-3-acetic acid oxidase. Each tube contains 0.5 ml. of 10^{-3} M indole-3-acetic acid, 0.5 ml. of M/15 phosphate buffer, pH = 6.64, 0.5 ml. of crude enzyme, the appropriate amount of 2, 4-D, and sufficient distilled water to make the total volume 2.5 ml. Incubated at 27.5°C. for 60 min.

Rates of inactivation of I.A.A. were measured by determining the residual concentration of I.A.A. at time intervals, using the $\text{FeCl}_3\text{-H}_2\text{SO}_4$ reagent of Tang and Bonner (1947). 2, 4-D alone or with boiled extract caused no destruction of I.A.A., nor did 2, 4-D interfere with the determination of I.A.A.

TABLE 1

EFFECT OF 2, 4-D AND BOILED ONION JUICE ON THE ACTIVITY OF CRUDE I.A.A. OXIDASE

Tube	Substance Added*		I.A.A. destroyed in 60 min. ($\mu\text{M}/\text{l.}$)†	Inhibition (%)
	10^{-2} M 2, 4-D (ml.)	Boiled, Filtered Onion Juice (ml.)		
A	0	0	53.7	0
B	0.5	0	92.7	- 73
C	0	0.5	14.3	+ 73
D	0.5	0.5	31.3	+ 42

* Each tube contains 0.5 ml. of 10^{-3} M indole-3-acetic acid, 0.5 ml. of M/15 phosphate buffer, pH = 6.64, 0.5 ml. of crude enzyme preparation, and sufficient distilled water to make the total volume 2.5 ml. Incubated at 27.5°C. for 60 min.

† Arithmetic mean from three batches of crude enzyme. Statistical analysis made on square root transformed data which substantially equalizes the variance due to different relative activities of enzyme and inhibitor present in each batch. Effect of boiled extract on I.A.A. destruction significant at < 1 per cent. Effect of 2, 4-D on I.A.A. destruction significant at 6 per cent.

Tang and Bonner (1948) have reported that boiled extracts of several plants strongly inhibit the I.A.A. oxidase. In this laboratory it has been shown that such inhibition by boiled onion juice can be partly or wholly reversed by 2, 4-D. A set of data for a series of experiments is given in Table 1.

Tang and Bonner (1948) have been able to demonstrate the presence of a heat-stable inhibitor of I.A.A. oxidase in several plant tissues and in the crude enzyme preparation from such tissues. Since 2, 4-D can oppose the inhibition caused by added boiled plant extract, it is likely that the rate-stimulation observed on adding 2, 4-D alone to the crude pea enzyme is due to its reversal of the effect of the natural inhibitor present there.

It is here suggested that the enzyme *in situ* is normally functioning at suboptimal rates which are controlled by a heat-stable inhibitor present in the tissues. Applied 2, 4-D counteracts this inhibition, permitting a greater rate of I.A.A. destruction. As there appears to be a dynamic equilibrium between production of I.A.A. from tryptophane (Wildman, Ferri, and Bonner 1947) and its destruction by I.A.A. oxidase, the characteristic effects on plant growth produced by relatively large amounts of 2, 4-D might well arise through disturbing this equilibrium.

III. ACKNOWLEDGMENT

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IV. REFERENCES

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