Absorption and Translocation of 2-I^{131}-2,3,5-Triiodobenzoic Acid

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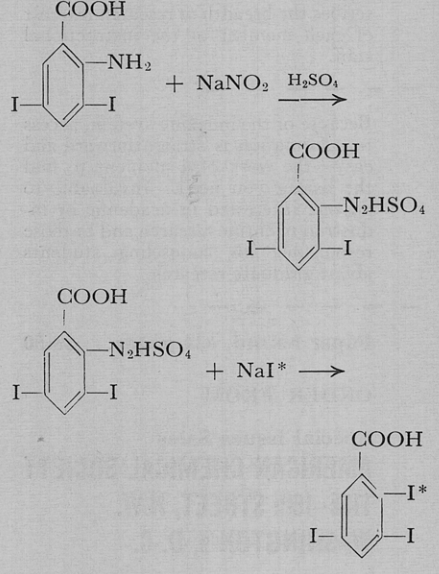
2,3,5-Triiodobenzoic acid (TIBA) is known to be a plant growth substance. It was synthesized and labeled with iodine-131 at the 2 position, to trace its absorption and translocation. Foliar as well as root applications were tried and traced by autoradiography. Extraction methods were also used to identify as well as verify the absorption and translocation of the TIBA. Dicots (tomatoes) absorbed it readily. In the case of root application both monocot and dicot readily absorbed and translocated the compound throughout the plant within 12 to 24 hours. The compound, absorbed through the root, was easily extracted and identified both by chromatography and autoradiography.

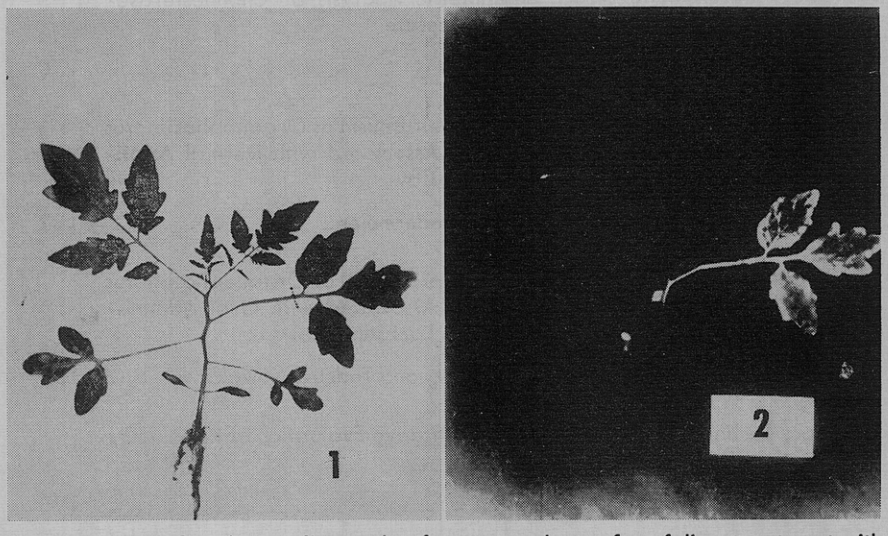
ALTHOUGH there are reports in the literature on the action of 2,3,5-triiodobenzoic acid (TIBA) such as alteration of the flowering of tomatoes, loss of apical dominance (*1*), inhibition of auxin (*2*, *3*), and abscission inducing activity (*5*), there is no information on absorption and translocation. The relatively minute quantities used in physiological studies make it difficult to detect TIBA by the standard chemical method, which is not sensitive enough to allow a critical evaluation of its action. The use of the isotopic technique with iodine-131 labeled TIBA permits the study of absorption and translocation.

The present report concerns the synthesis of iodine-131 labeled TIBA and the physiology of its absorption and translocation in plants.

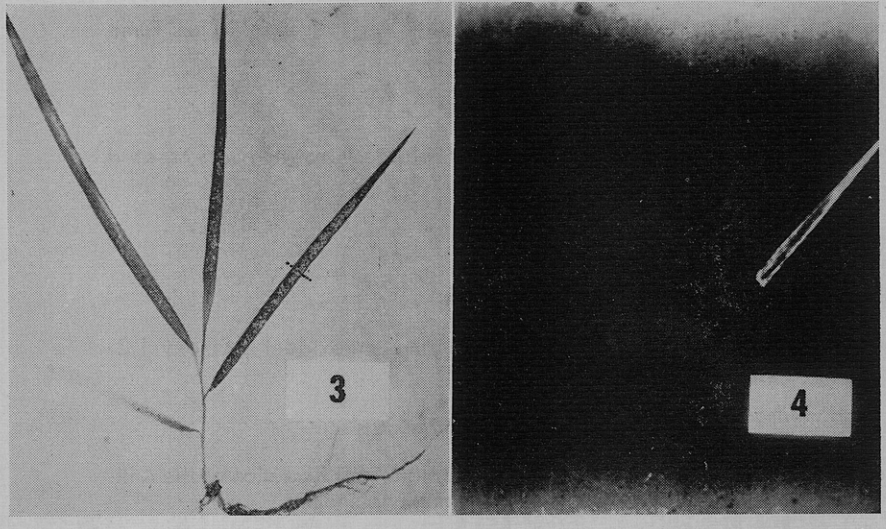
Materials and Methods

2-I^{131}-2,3,5-triiodobenzoic acid was synthesized using iodine-131 labeled sodium iodide according to the following reactions (*1*).





Figures 1 and 2. Autoradiograph of tomato plant after foliar treatment with TIBA



Figures 3 and 4. Autoradiograph of barley plant after foliar treatment with TIBA

2-Amino-3,5-diiodobenzoic acid (2.5 grams) was dissolved in 8 ml. of concentrated sulfuric acid. After cooling to 0° C. with an ice-salt mixture, 0.6 gram of powdered sodium nitrite was added gradually. The mixture was allowed to stand for 1 hour, after which it was mixed with 50 grams of crushed ice.

The solution was filtered to remove any insoluble substances. The filtrate was then mixed with 2.5 grams of sodium iodide, containing approximately 1 mc. of sodium iodide containing iodine-131 in 10 ml. of water. The halide was obtained from the Radioisotopic Chemical Center of Japan.

Ninety-two per cent of the calculated yield or 2.7 grams was obtained as an orange precipitate. To remove the free iodine, the precipitate was heated on a steam bath and treated with sodium hyposulfite. After two recrystallizations from hot ethyl alcohol, colorless crystals which melted at 225° C. were obtained. The specific radioactivity was 5.48 × 10^3 counts per minute per milligram which corresponds to 29.2 mμc. per mmole of TIBA.

A solution of 0.1 gram per liter was made with the labeled TIBA. This solution was then used in both the foliar application and in the root absorption experiment. The plants used for the absorption experiments were tomato (Aichi tomato strain No. 2) to represent a dicot and barley (Golden Mellon variety) to represent a monocot as shown in Figures 1 to 8.

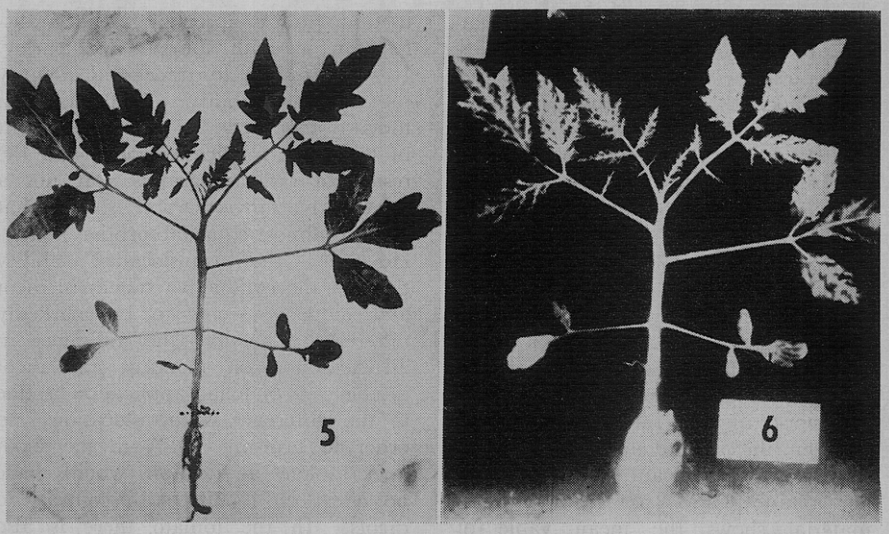
To observe the absorption and translocation of labeled TIBA a concentration of 0.1 gram per liter was applied to the leaves and the roots. An art brush was used to paint the solution on the leaf. It was allowed to be absorbed for 24 hours. In another experiment the roots were immersed in a similar TIBA solution for 6, 12, and 24 hours. All experiments were conducted at 25° C.

When the plants were harvested they were rinsed twice with distilled water, to remove any unabsorbed TIBA adhering to the external surfaces. The plants were then air dried, pressed, mounted on drawing paper, and finally covered with a thin sheet of cellophane. For autoradiography, Fuji medical x-ray films (Fuji Photo Film Co., Ltd., Tokyo) were allowed to come in contact with the pressed plants over the cellophane. For a thorough exposure, 7 days in the dark were required. The films were then developed by the usual x-ray film developing method.

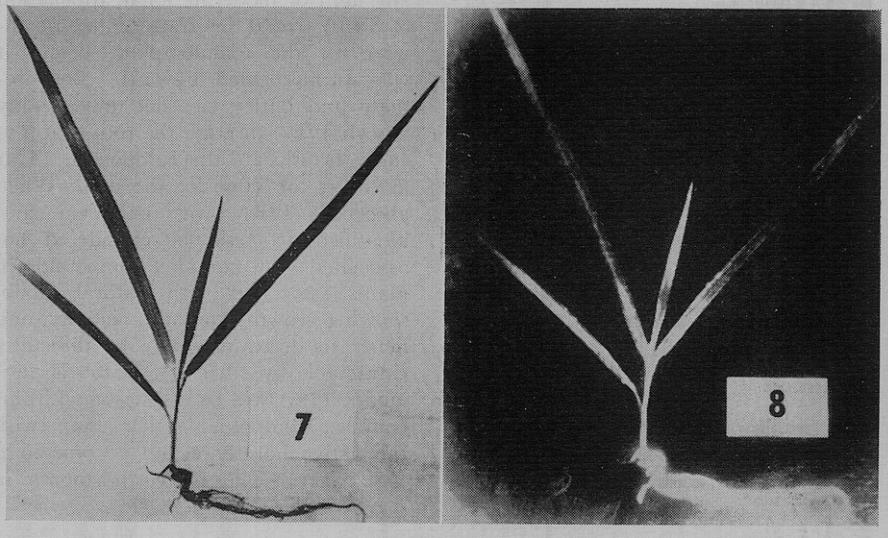
Results

Autoradiographs show that foliar absorption and translocation of TIBA occurred in tomato, whereas in barley absorption took place only at the site of application and no translocation was observed. Figures 1 and 3 correspond to Figures 2 and 4, respectively. The dark line across the petiole in the case of the tomato and the blade in the case of barley indicates the extent of foliar application. In the tomato the TIBA was readily absorbed and translocated into the rest of the petiole. It was also translocated to a lower primary leaf on the left, as shown by the bright spot on the autoradiograph. In the case of barley only absorption occurred. Any TIBA adhering externally was washed off. In this case no translocation took place; this is shown by the sharp demarcation between the applied and non-applied portions of the blade.

In the root absorption experiment, labeled TIBA was not only readily absorbed, but easily translocated in both tomato and in barley, as shown in Figures 6 and 8. Here again dark lines on the corresponding Figures 5 and 7 indicate the extent to which the roots were exposed to the TIBA solution.



Figures 5 and 6. Autoradiograph of tomato plant after a root absorption experiment with TIBA



Figures 7 and 8. Autoradiograph of barley plant after a root absorption experiment with TIBA

Table I. Radioactivity of Tomato and Barley in the Root Absorption Test of Iodine-131-2,3,5-Triiodobenzoic Acid

Plants	Tomato			Barley		
Hours of absorption	3	6	12	3	6	12
Average c.p.m. per gram from several experiments	1766	2723	3930	1201	2675	3565

Table II. R_f Values of I^{131}-2,3,5-Triiodobenzoic Acid Extracted from Plants

Solvent and Ratio	R_f Phenol-water (75:25)	R_f Isopropyl alcohol–ammonia-water (10:1:1)
TIBA[a]	0.92	0.78
TIBA[b]	0.91	0.74
TIBA[c]	0.89	0.74

[a] Pure triiodobenzoic acid.
[b] Known I^{131}-2,3,5-triiodobenzoic acid, identified by autoradiography.
[c] Substance extracted from tomato plant and identified by autoradiography.

Although there were no doubts that the absorbed radioactive material was TIBA, tests were conducted to verify the autoradiograph. The plants from the root absorption experiments were cut just above the line of immersion after 3, 6, and 12 hours, dried at room temperature under reduced pressure, and powdered to pass a 60-mesh screen. The powdered samples were then tested for radioactivity by the usual radioassay method. The results of the radioassay show that the amount of TIBA absorption and translocation increases steadily with time, and that the slight difference in the rate of absorption between the tomato and barley is not statistically significant. The counts per minute (CPM) per gram of plant material shows the mean value of three experiments subtracted from the background count ranging from 25 to 28 (Table I).

For a chemical assay the absorbed TIBA was extracted with acidified ether. Powdered tomato plants from the 12-hour root absorption experiment were used and the TIBA was identified by paper chromatographic technique, followed by autoradiography of the paper chromatogram. These results are shown in Table II, which indicates that the labeled TIBA was the radioactive compound absorbed. However, this was not determined quantitatively.

Discussion

A simple procedure was used here to synthesize 2-iodine-131–labeled 2,3,5-triiodobenzoic acid, which was then used to trace absorption and translocation in both tomato and barley. It was possible to trace and identify the absorptive ability of TIBA in both tomato and barley. The experiment in the foliar application only with the results of radioautographs, but not a quantitative investigation shows that there is almost equal absorptive ability. However, the translocative ability through the conductive system in dicot and monocot seems to be markedly different. Therefore the difference in TIBA action on monocot and dicot in the case of foliar application is due to the difference in translocation. In order to transmit TIBA to the shoot apex, foliar application would have no effect on monocots in contrast to dicots. In the tomato there is also indication that TIBA is first translocated downward. Hence it would seem that any light or moderate foliar application of TIBA would be translocated downward in the tomato plant before it was retranslocated upward. Both tomato and barley can not only readily absorb TIBA through the root, but also easily translocate the compound. This ability is the same for the two. When absorbed TIBA was extracted and identified, it could be considered not associated with complex compounds in plants associated with natural auxin, showing growth-promoting activity, and hence its derivatives may be difficultly extractable by ether. If TIBA is anti-auxin, TIBA will be associated with this complex compound rather than with the native hormones and become hard to extract. The native hormones in plants on the contrary become more soluble and extractable. In this paper the authors could not report on the quantitative investigation of the native hormones affected by the TIBA treatment. As a result of extraction and radioautography experiments with TIBA, it is supposed that TIBA is not antagonistic to native auxins, but that it inhibits growth in some manner other than that of an antagonist of native auxins.

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INSECTICIDE MEASUREMENT

Direct Colorimetric Analysis of Cholinesterase-Inhibiting Insecticides with Indophenyl Acetate

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Anticholinesterase activity due to the hydrolysis of indophenyl acetate is measured colorimetrically in the presence of the following insecticide residues: Sevin, Thimet, Guthion, and Trithion. The variables affecting the reaction such as enzyme concentration, temperature, preincubation time, and pH have been studied.

A PRECISE AND REPRODUCIBLE PROCEDURE for the measurement of anticholinesterase insecticides is based on the direct colorimetric measurement at 625 mμ of the hydrolysis product of indophenyl acetate at a constant pH of 8.0. Standard curves and residue data are presented using crystalline bovine erythrocyte acetylcholinesterase as the enzyme. A preincubation time of 30 minutes and a reaction time of 30 minutes at 30° C. are recommended. Honey bee brain as a source of esteratic enzymes is also discussed.

Enzymatic methods for the analysis of cholinesterase-inhibiting insecticides have been recently reviewed by Schechter and Hornstein (*13*). Several of these procedures have been adapted for insecticide residue analyses in agricultural crops (*4, 14*). Recently, Kramer and Gamson (*7*) published a method for the colorimetric determination of acetylcholinesterase activity utilizing indophenyl acetate as the chromogenic substrate. The method reported here utilizes the color reaction described by Kramer and Gamson for the quantitative measurement of cholinesterase-inhibiting insecticides.