Metabolic Fate of 3,4-Dichloropropionanilide in Plants: The Metabolism of the Propionic Acid Moiety

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Abstract. 3,4-Dichloropropionanilide-14C (propanil) labeled in either the C-1 or C-3 carbon atoms of the propionic acid moiety was applied to the roots of pea (Pisum sativum L.) and rice (Oryza sativa L.) plants in nutrient solution (0.1 mM-0.28 mM). Radioactivity was detected throughout the treated plants, but the greatest labeling was found in the roots. None of the products that contained aniline were radioactive, suggesting that the plants split the propionic acid moiety from propanil. The fate of the propionate moiety of propanil was determined by recovery of 14CO2 from plants exposed to propanil-14C. The time-course of the 14CO2 production demonstrated that the intact propionic acid was cleaved from the propanil and subsequently catabolized by the β-oxidation catabolic sequence. The appearance of radioactivity in the shoots was attributed to the incorporation of products of propionate metabolism. Both the susceptible pea plants and the tolerant rice plants converted a high percentage of the administered propanil-14C to 14CO2.

For the last 6 years propanil (3,4-dichloropropionanilide) has been used for selective control of barnyard grass (Echinochloa spp.) in rice (Oryza sativa L.). When rice is treated simultaneously with propanil and certain carbamate or phosphoric insecticides, the rice loses its tolerance for propanil. Preliminary reports by Unger et al. (8) and McRae et al. (5) state that resistant rice plants readily cleave propanil to 3,4-dichloroaniline, while susceptible barnyard grass plants yield a metabolite in which the amide bond of propanil remains intact. The authors proposed that this difference in the metabolism of propanil between susceptible and resistant species was the basis for herbicidal selectivity of substituted aniline herbicides.

If this basis for selectivity is correct, the liberation of propionic acid from propanil and its catabolism to CO2 would lend itself to a comparative study of propanil metabolism. Time-course studies following the 14CO2 recovery from propanil-14C, labeled in the C-1 and C-3 of the propionic acid moiety of propanil, indicated that propanil was readily cleaved to 3,4-dichloroaniline and propionate. The propionic acid was then further catabolized to CO2 by the β-oxidation catabolic sequence. There was no clear-cut difference in the ability of tolerant and susceptible species to cleave the amide bond of propanil.

Materials and Methods

Plant Materials and Treatments. Rice (Oryza sativa L. var. Nato), and pea (Pisum sativum L. var. Little Marvel) seeds were germinated between moist paper towels at 25° in the dark. The 7-day-old seedlings were transferred to pint jars containing Hoagland’s solution. Metal jar lids, perforated with holes large enough to accommodate mature plant stems, were used to support the seedlings. Aeration was provided through a 0.06 in. o.d. polyethylene aeration tube. These jars were then placed either in controlled-environment rooms or a greenhouse. Light was totally excluded from the root zone. A respirometer chamber was constructed to accept the plant with its jar-lid collar. The specially designed plant respirometer chamber completely isolated the root from the shoot.

Propionic acid-1,14C and propionic acid-3,14C and 3,4-dichloroaniline were used to synthesize specifically labeled propanil using the procedures

1 Use of trade names is for the purpose of identification of equipment employed and does not constitute endorsement by the United States Department of Agriculture.
described by Brown (2). The specific activity of the resulting products, propanil-1-14C and propanil-3-14C, were 20.0 μc per mmole and 23.1 μc per mmole, respectively. Labeled propanil was dissolved to give 0.1 mM to 0.28 mM concentrations in full-strength Hoagland’s solution.

Plant Respirometer. The respirometer consisted of 2 glass resin reaction kettles and a specially machined plastic center member. The center member was 23 cm in diameter and 5.5 cm thick at the outside edge. The center well of the shoot chamber (upper chamber) was designed with a raised ring with the same dimensions as the pint jar neck. Thus a gas-tight seal was made between the center member and the metal jar lid. Stud bolts were placed in the plastic center member so that the 3 retaining rings would secure and seal the jar lid and keep the 2 resin reaction kettles in place. Access ports were drilled through the center member to accommodate tubes connecting the manometers, air pumps, and traps to the chamber. Funnels of various sizes were modified to form a container to hold the root media. A tube through the root zone access port passing to the bottom of the funnel-shaped root container allowed the root media to be changed at any time during the experiment without opening the respirometer system. The volume of the chamber may be changed by placing various-sized resin reaction kettles in position. The assembled plant chamber was placed in a controlled-environment room, thus controlling the temperature and light conditions within the respirometer chamber.

The flow system used in conjunction with the respirometer chamber was designed so the pressure in the shoot and root zone chambers is maintained at or near atmospheric pressure by adjusting bleed valves in the pump reservoir tanks. The effluent gas from the shoot zone chamber was freed of CO2 in one of two CO2 traps. The traps were similar to those described by Wang et al. (9). By adjusting the two 3-way stopcocks, CO2 sampling may be changed from one trap to the other thus permitting continuous sample collections.

A positive pressure pump fitted with a membrane filter, to remove airborne contaminants, was used to aerate the root media. The effluent gas from the root zone chamber was either trapped or vented to waste.

Quantitation. 14CO2 was trapped in 10 ml of 2:1 absolute ethanol:ethanolamine. The trapping solution was replaced at 3-hour intervals, diluted to 15 ml with absolute ethanol, and a 5 ml portion mixed with 10 ml of toluene containing 2,5-diphenyloxazole (5 g/l) and 1,4-bis-2'-(4-methyl-5-phenyl-oxazolyl) benzene (300 mg/l) in a 20-ml glass counting vial. The scintillation mixture was counted with a liquid scintillation counter.

Qualitative Analysis and Thin-layer Chromatography. The plant material was lyophilized, ground to pass through 80-mesh screen, and extracted with 100% methanol. The concentrated methanol extract was spotted on 20-cm square glass plates coated with silica gel HF. The chromatograms were developed ascendingly to 10 cm in butanol:ethanol:water (2:1:0.5, v/v/v). The air-dried plates were sprayed at 10-minute intervals with 6 n HCl in methanol, 1% NaNO2 in 1 n HCl, and 1% N-1-naphthylethylene-diamine dihydrochloride in 2 n HCl (1) for color development of primary aromatic amines.

Results and Discussion

When intact pea or rice plants were root-treated with propanil-14C (carboxyl, C-1; or methyl, C-3 carbon atoms 14C labeled) the 14C activity was detected throughout the entire plant. Most of the radioactivity was in the roots. Thin-layer chromatography of plant extracts and radioautography of chromatograms showed that no 14C activity was

![Fig. 1. Calculated cumulative percent interval recovery of 14CO2 from pea plants treated with 0.1 mM to 0.28 mM propanil-1-14C or propanil-3-14C (0.3 μc-1.0 μc) in Hoagland's solution. A photoperiod regime of 12 hours of light and 12 hours of dark was used. The dark hatched area represents the dark respiration, the light area represents photosynthetic period.](image-url)
present in the aniline metabolites. These results strongly suggest that the plant hydrolyzed the aniline bond to liberate 3,4-dichloroaniline (6,7) and propionic acid. To determine the metabolic fate of the propionic acid moiety of propanil, young, rapidly-growing pea and rice plants were treated with 0.1 mm to 0.28 mm propanil-1-14C (0.3 μc) or propanil-3-14C (1.0 μc) in Hoagland's solution. These experiments were conducted in the plant respirometer and the 14CO2 evolution was continuously assayed from 0 time until termination of the experiment. At the termination of the experiment, the roots were washed and the concentration of propanil remaining in the solution was determined. In all cases the 14C activity remaining in the nutrient solution was predominantly unchanged propanil-14C. The percent 14C activity recovered as 14CO2 is based on the total propanil-14C absorbed by the plants.

Figure 1 is a calculated cumulative percent interval recovery of 14CO2 as a function of time from pea plants treated with propanil-14C. The recovery of 14CO2 from propanil-1-14C was 82.2 % and from propanil-3-14C was 17.7 %. During the photosynthetic period little or no 14CO2 was evolved from the labeled propionic acid moiety, but in the dark there was major evolution of C-1 as 14CO2. The 14C activity remaining in the plant tissues was 17.8 % in the case of propanil-1-14C and 82.3 % in the case of propanil-3-14C.

Similar studies with intact rice plants yielded the data presented in figure 2. The curves are similar to those in figure 1. There was little evolution of 14CO2 during photosynthesis with a major evolution of 14CO2 during the dark period. The rate of 14CO2 evolution was much greater when the plants were treated with propanil-1-14C as compared to propanil-3-14C. 14CO2 recovery from propanil-1-14C was 62.3 % of the total propanil absorbed by the plants. In the case of propanil-3-14C, 44.2 % of the absorbed propanil-3-14C was recovered as 14CO2.

Table I is a summary of the data collected from peas and rice. These data are consistent with the proposed mechanism for propionate metabolism in plants (3,4). Hatch and Stumpf (4) reported data collected from pea cotyledons, safflower root, and wheat epicotyl treated with propionate-1-14C, propionate-2-14C, and propionate-3-14C. Their findings are in close agreement with the 14CO2 recoveries from C-1 and C-3 carbons of propionate liberated from propanil. Stumpf reported a C-1/C-3 14CO2 recovery ratio of 4.8 from pea cotyledons treated with propionate-1-14C and propionate-3-14C. Our results with intact pea plants root-treated with propanil-1-14C and propanil-3-14C gave a C-1/C-3 14CO2 recovery ratio of 4.6.

The data presented indicate that propanil is rapidly absorbed by the roots of growing rice and pea plants and the propanil is cleaved to an unknown aromatic moiety and propionic acid. The resulting propionic acid is further catabolized to CO2 via the β-oxidation sequence. There is no evidence that the alternate metabolic sequence for propionate found in mammals and bacteria exists in these plant tissues.

The high 14CO2 recovery from the carboxyl carbon of propanil-1-14C is indicative of rapid cleavage of the amide bond by these plants. There seems to be little correlation between the hydrolysis of propanil and plant resistance. Peas, which are not tolerant to propanil, yielded more CO2 from C-1 of propanil than did the tolerant rice plants. This fact is not in agreement with the hypothesis that propanil is detoxified by cleavage of the amide bond (5,8).

<table>
<thead>
<tr>
<th>Specific labeled carbon</th>
<th>14CO2</th>
<th>Plant incorporation</th>
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<tbody>
<tr>
<td>Pea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>C-3</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td>Rice</td>
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</tr>
<tr>
<td>C-1</td>
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<td>38</td>
</tr>
<tr>
<td>C-3</td>
<td>44</td>
<td>56</td>
</tr>
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![Fig. 2. Calculated cumulative percent interval recovery of 14CO2 from rice plants treated with 0.1 mm to 0.28 mm propanil-1-14C or propanil-3-14C (0.3 μc-1.0 μc) in Hoagland's solution. The experiments were of 65 hours to 96 hours duration and employed a dark period of 14 hours with a light period of 10 hours. The dark hatched area represents the dark respiration, the light area represents the photosynthetic period.](image)
Acknowledgments

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Literature Cited