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

Abstract. *Studies to elucidate the fate of 3,4-dichloropropionanilide (propanil) in rice (*Oryza sativa *L. var. Nato) plants have shown that the propanil molecule is cleaved and the propionic acid moiety metabolized. To ascertain the fate of the 3,4-dichloroaniline moiety of propanil, rice plants were exposed to propanil in liquid culture. The roots and shoots of treated rice plants were extracted and quantitatively assayed for four aniline-containing metabolites. One of the four metabolites proved to be 3,4-dichloroaniline, while the remaining three metabolites contained complexed 3,4-dichloroaniline.* N-*(3,4-dichlorophenyl)-glucosylamine was identified as one of the complexes. A time-course study of the four metabolites indicated the appearance of the 3,4-dichloroaniline-containing metabolites within 6 hours. After 14 days of treatment, the complexed aniline metabolites amounted to only 10 percent of the total propanil administered to the rice plants.*

3,4-Dichloropropionanilide (propanil), a selective herbicide used as a postemergence treatment, has found wide use both nationally and internationally in the cultivation of rice (*Oryza sativa* L. var. Nato). Propanil is very effective in controlling both monocotyledonous and dicotyledonous weeds in rice (*1*). Investigations on propanil metabolism in intact rice plants have shown that roots readily absorb propanil and translocate and metabolize the propionic acid moiety of propanil (*2*). However, the metabolic fate of the 3,4-dichloroaniline (3,4-DCA) moiety of propanil is still unknown. This report will describe investigations which showed that the free 3,4-DCA was first complexed as *N*-(3,4-dichlorophenyl)-glucosylamine. Two other metabolites were isolated that yielded 3,4-DCA after hydrolysis. A precursor-product relation was shown to exist between propanil and its metabolites.

The roots of 1-month-old rice plants were treated with $8.3 \times 10^{-5}M$ to $9.2 \times 10^{-5}M$ propanil in full-strength Hoagland's solution. The plants were harvested after 5 days of exposure. The root and shoot portions were separated and lyophilized. The methanol extract of the dried tissue was chromatographed on silica gel in butanol : ethanol : water (2 : 1 : 0.5, by volume). The metabolites containing aniline were identified on the chromatogram by use of the sensitive Bratton-Marshall diazotization reaction with 1-*N*-naphthylethylenediamine dihydrochloride (*3*). The chromatograms were sprayed with 6*N* HCl and allowed to stand at room temperature for 10 minutes to hydrolyze the aniline complexes before color development. Under these conditions, propanil was not hydrolyzed. Four aniline-positive spots were detected by this procedure. For means of identification the metabolites were designated as M-1, M-2, M-3, and M-4 with increasing R_F values. Metabolite M-4 was identified as 3,4-DCA as indicated by thin-layer chromatography and gas-liquid chromatography (*4*). Isolation of metabolites M-1, M-2, and M-3 from paper or thin-layer chromatograms followed by hydrolysis and gas-liquid chromatography proved that the only isomer of aniline present was 3,4-DCA.

Metabolite M-1 was readily hydrolyzed to an unknown derivative and metabolite M-3. Metabolite M-2 was present in small quantities and seemed to be stable. A survey of the hydrolysis products with various indicating reagents showed that the predominant component of all three metabolites was reducing sugars. Gas-liquid chromatography of the trimethyl silyl ether derivatives (*5*) of the hydrolysis products of M-1, M-2, and M-3 showed that these sugars were hexoses and predominantly glucose in the case of metabolite M-3.

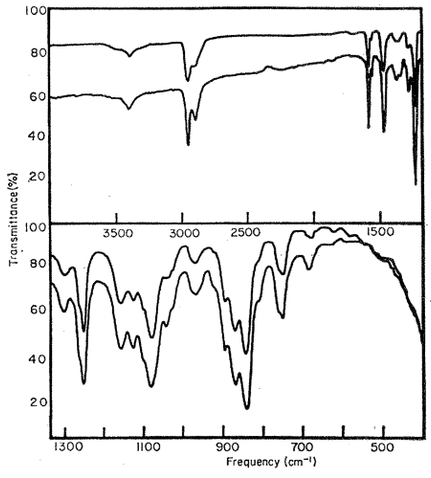

Fig. 1. Infrared spectrum of the trimethylsilyl ether derivatives of synthetic *N*-(3,4-dichlorophenyl)-glucosylamine (lower) and the metabolite (upper) isolated from intact rice plants.

The data indicated that *N*-(3,4-dichlorophenyl)-glucosylamine was a possible structure for metabolite M-3. This compound was chemically synthesized as described by Kadunce (*5*). The isolated metabolite M-3 and the synthetic glucoside were compared by thin-layer and paper chromatographic analysis. The trimethyl silyl ether derivative of the *N*-(3,4-dichlorophenyl)-glucosylamine had a retention time of 20.4 minutes on a Carbowax (5 percent) gas-liquid chromatograph column at a temperature of 195°C and coincided with the retention time of the 3,4-DCA complex (M-3) isolated from rice. The trimethyl silyl ether derivatives were collected directly on KBr crystals, by use of a modification of the method of Giuffrida (*6*) to collect the effluent from the gas-liquid chromatographic column. Figure 1 shows that the infrared spectra of the trimethyl silyl ether derivatives of synthetic *N*-(3,4-dichlorophenyl)-glucosylamine (lower) and the isolated M-3 metabolite (upper) were superimposable. This confirmed that metabolite M-3 isolated from treated intact rice was *N*-(3,4-dichlorophenyl)-glucosylamine. Efforts to characterize metabolites M-1 and M-2 have failed because of the instability of M-1 and our failure to separate and purify M-1 and M-2 sufficiently to carry out structural analysis.

A series of time-course studies were conducted to determine the precursor-product relationship between the three metabolites containing 3,4-DCA. Roots of 1-month-old rice plants were treated with $8.3 \times 10^{-5}M$ to $9.2 \times 10^{-5}M$ propanil in full-strength Hoagland's solution. Plant samples were harvested at 6- and 12-hour and at 1-, 2-, 4-, 5-, 7-, 8-, 10-, and 14-day intervals. A sample of the treating solution was taken at each harvest. The roots were washed and the plant was severed at the crown, and the shoots and roots were analyzed separately. All tissues were frozen and lyophilized. The ground, dry tissues were extracted with anhydrous methanol, and unchanged propanil and its metabolites were separated by paper and thin-layer chromatography. Methanolic eluates from the chromatograms were quantitatively analyzed by the Bratton-Marshall colorimetric procedure. Propanil was positive to the Bratton-Marshall colorimetric procedure

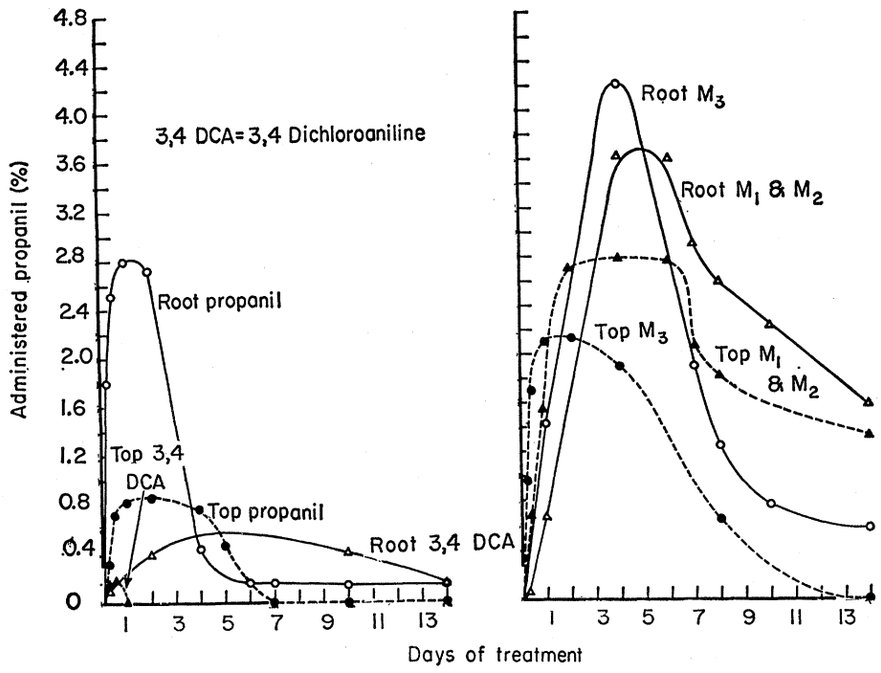

Fig. 2. A time-course study of propanil metabolism in rice.

only after a 6*N* HCl hydrolysis for 30 minutes at 100°C. Therefore, we were able to determine propanil and 3,4-DCA separately in a chromatographic system in which they were not widely separated.

Figure 2 shows the time-course metabolism of propanil in rice. The propanil concentration in the roots reached a maximum at 1 to 2 days and fell to a constant level at 6 days. This was contrasted to the simultaneous increase of propanil concentration in the shoots which fell to zero concentration at 7 days after treatment.

3,4-Dichloroaniline, the first hydrolysis product of propanil, was detected only within the first 24 hours in the shoots. The roots showed a slow increase in 3,4-DCA concentration followed by a decrease approaching zero at 14 days of treatment. After 24 hours of exposure to propanil, the shoots contained no free 3,4-DCA. However, these same tissues contained a high concentration of propanil after 24 hours of treatment. These observations suggest that the foliar tissues have the enzymatic capability to rapidly complex free 3,4-DCA. The persistence of free 3,4-DCA in the roots suggests that root tissues may not have the same enzymatic capabilities as the shoots.

The concentration of metabolite M-3 increased abruptly in the shoots and decreased in concentration after 3 days. Simultaneously, M-3 concentration increased in the roots, reaching a maximum at 4 days. There was a steady decrease in concentration of metabolite M-3 in the roots thereafter, until a constant concentration was established at 10 to 13 days.

The same relationship was observed with metabolites M-1 and M-2, which are represented in these curves as the sum of the two metabolites. These metabolites increased in the foliar material, and shifted in time with relation to metabolite M-3, reaching a plateau between 2 and 6 days. They reach a maximum concentration at 5 days, and subsequently decrease in concentration to the termination of the experiment.

The results of these studies show that a precursor-product relationship does exist between propanil and its metabolites. The first stable catabolic intermediate of propanil metabolism, M-3, is found in both the shoot and root in about equal concentrations (435 μmole per total treated root, 371 μmole per total shoot treated). Propanil and free 3,4-DCA are found in much lower concentrations in the shoot (propanil, 343 μmole per total treated root and 121 μmole per total treated shoot; 3,4-DCA, 95 μmole per total treated root and 10 μmole per total treated shoot). This difference in propanil and 3,4-DCA concentration suggests that the foliar tissues more readily hydrolyze propanil and complex the liberated 3,4-DCA to M-3. Both root and shoot appear to convert M-3 to metabolites M-1 and M-2.

When rice plants are grown in the presence of 3,4-DCA in Hoagland's solution, the metabolites M-1, M-2 and M-3 are all found in the foliar and root tissues of these treated plants, in concentrations similar to that found in the propanil-treated rice. An extensive time-course study using free 3,4-DCA as substrate has not been performed, but the observed translocation and enzymatic glucoside formation yielded a precursor-product relationship identical to that seen with the herbicide propanil.

Bartha (*7*) demonstrated that soil microflora readily hydrolyzed propanil to 3,4-DCA. Subsequent metabolism of the free aniline resulted in the accumulation of 3,3′,4,4′,-tetrachloro-azobenzene in soil cultures. We have no evidence that azobenzene is one of the metabolites of propanil in rice.

At 6 days of treatment an assay of the total aniline content in the treating solution and root and shoot tissues gave the following percentage distribution: 12.5 percent of the total administered propanil remained unchanged in the treating solution; the roots contained 7.1 percent and the shoots 4.9 percent of the total administered propanil aniline equivalents. Thus at 6 days 75.5 percent of the total aniline equivalent from propanil had not been accounted for as propanil, 3,4-DCA, or as a metabolite intermediate containing 3,4-DCA. A similar assay at 14 days of treatment accounted for only 10 percent of the total aniline equivalent administered to the system. Thus, there is a rapid rate of turnover of the parent propanil molecule and its catabolic intermediates to some unknown intermediate or intermediates.

GERALD G. STILL
Agricultural Research Service,
U.S. Department of Agriculture,
Fargo, North Dakota 58103

References and Notes

1. P. de Sarjas, *Comptes Rendus des Journées d'Etudes sur les Herbicides* (European Weed Research Council–Columa, Paris, 1961); N. Yamada and H. Nakamura, *Nippon Sakumotsu Gakkai Kiji* **32**, 69 (1963).
2. G. G. Still, *Abstracts, 1967 Meeting of the Weed Society of America* (Weed Society of America, Urbana, Ill., 1967), p. 64; *Plant Physiol.*, in press.
3. C. A. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.* **128**, 537 (1939).
4. K. J. Bombaugh, *Anal. Chem.* **37**, 72 (1965).
5. R. E. Kadunce, *J. Chromatog.* **30**, 204 (1967).
6. L. Giuffrida, *J. Assoc. Offic. Agr. Chemists* **48**, 354 (1965).
7. R. Bartha and D. Pramer, *Science* **156**, 1617 (1967).
8. I gratefully acknowledge the highly competent assistance of E. R. Mansager.

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