Metabolism of Chlorpropham (Isopropyl *m*-Chlorocarbanilate) in Various Plant Species

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Chlorpropham is extensively converted to water-soluble metabolites in the susceptible species, smartweed, pigweed, and tomato, but only slightly in the tolerant species, parsnip. The metabolites from smartweed, tomato, and parsnip consist mainly of β-glycosides with a modified chlorpropham molecule as the aglycone entity; that from pigweed also contained the modified chlorpropham molecule, but was not glycosidic in nature. Glycoside formation did not account for the various susceptibilities of these species to chlorpropham. The modification of the chlorpropham molecule did not occur in the ring and did not involve hydroxylation at the bridging nitrogen atom.

Although carbamate herbicides have found considerable use in agriculture over the past 20 years, relatively little work has been reported on their metabolism within plant tissues.

Riden and Hopkins (1962) found that barban (4-chloro-2-butynyl *m*-chlorocarbanilate) was converted to a water-soluble, 3-chloroaniline-containing, substance by a number of plant species. They also showed that a similar substance was formed by various structural analogs of barban, including chlorpropham (isopropyl *m*-chlorocarbanilate). These authors concluded that the metabolite involved more than one component, was dialyzable, nonproteinaceous, contained acidic groups neutralized by sodium hydroxide, but not by sodium bicarbonate, might contain sugars, and was associated in solubility with the flavonoids.

Baskakov and Zemskaya (1959) earlier postulated that herbicidal carbamates owed their activity to conversion to their *N*-hydroxy derivatives. They showed that the *N*-hydroxy derivative of propham (isopropyl carbanilate) had greater water solubility than propham itself, and that in distinction from propham, *N*-hydroxy propham deposited on oat leaves was capable of penetrating into the plant and exerting a herbicidal effect.

Riden and Hopkins (1962) suggested, however, that their water-soluble metabolite was not the *N*-hydroxy derivative of barban, since its water solubility and its partition coefficient between water and ethylene dichloride was only slightly increased over that of barban.

Another carbamate herbicide, swep (methyl 3,4-dichlorocarbanilate) was reported to complex with the relatively inert plant constituent lignin in the straw, hull, and bran layers of rice (Chin *et al.*, 1964). The formation of this stable complex was suggested as involving the trapping of the swep molecule within the lignin molecule and was considered as an example of a common detoxication mechanism by plants through immobilization.

Prendeville *et al.*, (1968) reported that similar water-soluble metabolites were isolated from C^{14}-chlorpropham treated pale smartweed (*Polygonum lapathifolium* L.), redroot pigweed (*Amaranthus retroflexus* L.), and parsnip (*Pastinaca sativa* L.), regardless of whether the herbicide was ring- or chain-labeled.

This paper reports further work on the elucidation of the nature of these metabolites.

MATERIALS

Plants used were pale smartweed (*Polygonum lapathifolium* L.), redroot pigweed (*Amaranthus retroflexus* L.), parsnip (*Pastinaca sativa* L.), and tomato (*Lycopersicum esculentum* Mill). They were grown in a greenhouse kept at 24° C. day temperature, 18° C. night temperature and 16-hour photoperiod.

The chlorpropham used was either an emulsifiable concentrate in water or C^{14}-chlorpropham labeled either uniformly in the ring or at carbon atom 2 of the isopropyl group. β-Glucosidase (B grade) and β-glucuronidase were obtained from Calbiochem, and β-galactosidase from Sigma Chemical Co.

Ascending thin-layer chromatography was carried out using silica gel G of thickness 0.25 mm. containing a fluorescein indicator (SilicAR TLC-7F, Mallinckrodt) on 200- × 200-mm. plates. The solvent systems used were chloroform to *n*-hexane (3 to 1) and acetone–hexane–benzene (1:2:5) (Finocchiaro and Benson, 1967). Anilines were detected with Ehrlich's spray reagent (1% solution of *p*-dimethylaminobenzaldehyde in 5*N* hydrochloric acid). Other nonradioactive substances were detected visually under an ultraviolet lamp. Positions of radioactive substances were detected by allowing the solvent to ascend 9.5 cm., drying, and dividing the plate into 20 sections of 0.5 cm. each. The silica gel in each section was evenly spread into planchets by the addition of 0.5 ml. of acetone and then counted in a thin-window gas flow counter.

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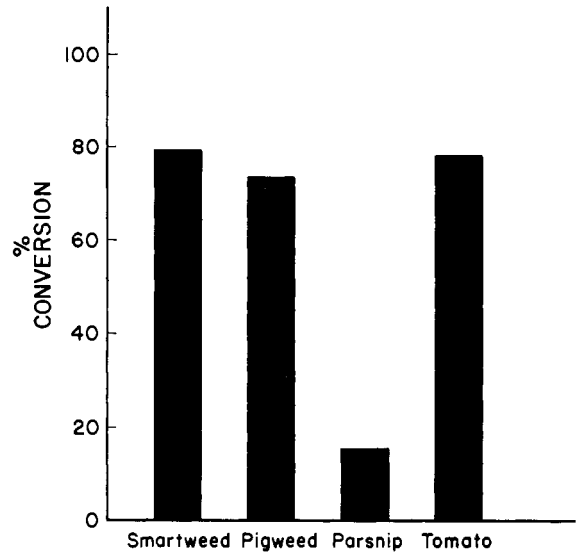

Figure 1. Percentage conversion after 10 days of chain labeled ^{14}C-chlorpropham to water-soluble metabolites in treated leaves of smartweed, pigweed, parsnip, and tomato

Conversion expressed as percentage of radioactivity in aqueous layer relative to aqueous plus chloroform layers

SYNTHESIS OF ANALOGS

3-Chlorophenylhydroxylamine (by a modification of the method of Utzinger, 1944). 1-Chloro-3-nitro benzene was suspended in 600 ml. of warm 50% ethanol. Zinc powder (65 grams) was added and the mixture stirred vigorously. A solution of 53.5 grams ammonium chloride in 150 ml. water was then added gradually with external cooling to prevent the reaction from proceeding too vigorously. After 20 to 25 minutes, the mixture was filtered and washed with 200 ml. of warm water. After cooling, the filtrate was extracted with 2 × 250 ml. of chloroform. The chloroform extract was dried over magnesium sulfate and the chloroform removed under reduced pressure. The product was recrystallized from benzene/petroleum ether to give 26 grams (37%) 3-chlorophenylhydroxylamine, m.p. 46–7° C.

Isopropyl *N*-Hydroxy *m*-chlorocarbanilate. A solution of 9.3 grams of 3-chlorophenylhydroxylamine and 5.1 grams of pyridine in 125 ml. of dry benzene was stirred at 10–15° C. and a solution of 8.0 grams of isopropyl chloroformate in 25 ml. of dry benzene added dropwise over 30 minutes. The slurry formed was stirred at room temperature for a further 3 hours and then extracted with 3 × 30 ml. of water. The benzene layer was dried over magnesium sulfate and the benzene then removed under reduced pressure. Purification of the resulting oil was achieved by elution through a Florisil column with the solvent system acetone–hexane–benzene (1:2:5). After removal of the solvent under reduced pressure, remaining traces of benzene were removed by allowing the oil to stand over lubricating grease for 3 days. This resulted in crystallization of the oil as a pale brown solid m.p. 54–5° C., its identity as isopropyl *N*-hydroxy *m*-chlorocarbanilate being confirmed by elemental analysis.

Hydroxy Anilines. The various nuclear hydroxylated 3-chloroanilines were either purchased or synthesized by sodium dithionite reduction of the corresponding chlorhydroxy nitro benzenes (Christiansen, 1923).

EXPERIMENTAL PROCEDURE

For investigations with nonradioactive chlorpropham, 60 plants were sprayed with an emulsifiable concentrate in water at a rate of 2.4 kilograms per hectare when the plants were in the 3- to 4-leaf stage. This rate was noninjurious to the plant.

With radioactive chlorpropham, solutions of the C^{14}-labeled herbicide were applied evenly to the leaves by means of a 1-ml. tuberculin syringe. Approximately 0.25 ml. of an 80% ethanolic solution at sublethal concentration was applied to each plant. There were 30 plants per treatment.

Since chlorpropham has been shown to be immobile following foliar application (Prendeville *et al.*, 1968), only treated leaves were extracted in the present study. After 10-day treatment, the leaves were homogenized with twice their weight of 80% ethanol, the homogenate was filtered and taken to dryness on a rotary film evaporator. The residue was partitioned between chloroform and water and the former removed and kept. The aqueous layer was further extracted with chloroform, so as to remove any remaining chlorpropham, and both chloroform extracts were combined. In the case of radioactive treatments, aliquots were taken from both chloroform and aqueous layers and counted in a thin-window gas flow counter.

ENZYMATIC AND CHEMICAL HYDROLYSIS

Cleavage of the water-soluble metabolites was accomplished by adopting the method of Kuhr and Casida (1967). The aqueous layer obtained after partitioning was divided into five equal fractions and each fraction taken to dryness on a rotary film evaporator. The residues were treated with 4 ml. of citrate-phosphate buffer, pH 4.4 (Kuhr and Casida, 1967), and individually with one of the following: no addition, 3 mg. of β-glucosidase, 3 mg. of β-glucuronidase, 3 mg. of β-galactosidase, or hydrochloric acid adjusted to a final pH of 1.0. After shaking on a water-bath at 37° C. for 5 hours, the contents of each flask were extracted with 3 × 8-ml. portions of chloroform, the three chloroform portions combined, and the radioactivity of both aqueous and chloroform fractions was measured. The chloroform fractions were then concentrated and chromatographed.

In addition to enzymatic and acidic hydrolysis, the initial aqueous fraction was also subjected to alkaline hydrolysis. The entire fraction was refluxed for 1 hour with 35 ml. of 2*N* sodium hydroxide solution, cooled, and extracted with 3 × 25-ml. portions of chloroform. The chloroform extracts were combined, concentrated to 0.5 ml., and chromatographed.

RESULTS AND DISCUSSION

The percentage conversion of chlorpropham to water-soluble metabolites after 10 days in the four plant species studied is shown in Figure 1. Although these data represent values obtained for chain-labeled chlorpropham, similar results also were obtained with ring-labeled chlorpropham. Previously Prendeville *et al.*, (1968) showed that these metabolites could be resolved into various components by the use of different chromatographic solvents. Thus, the water-soluble metabolites obtained from parsnip were resolved into three components and that from pigweed into two. The metabolite obtained from smartweed, on the other hand, had one component only. The water-soluble metabolite obtained from chlorpropham-treated tomato leaves in the present study also had only one component but its R_f values were different from the smartweed metabolite.

Figure 1 shows that smartweed, pigweed, and tomato, all species susceptible to either or both of the slow or fast actions of chlorpropham (Eshel and Warren, 1967), convert chlorpropham into approximately equal amounts of water-soluble

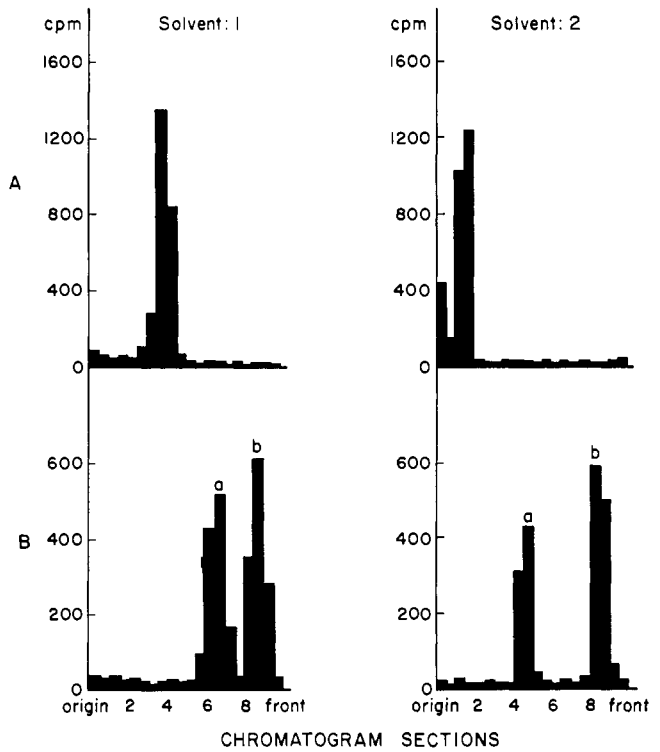

Figure 2. Radioactivity of thin-layer chromatograms

A. Chloroform extract after incubation with either β-glucosidase or hydrochloric acid at pH 1.0
B. Authentic ring-labeled 3-chloroaniline (*a*), and chlorpropham (*b*), added to chloroform extract of control solution after incubation.
Solvent 1. Acetone–*n*-hexane–benzene (1:2:5)
Solvent 2. Chloroform:*n*-hexane (3:1)
Silica gel was scraped off plates and counted by thin-window gas flow counter

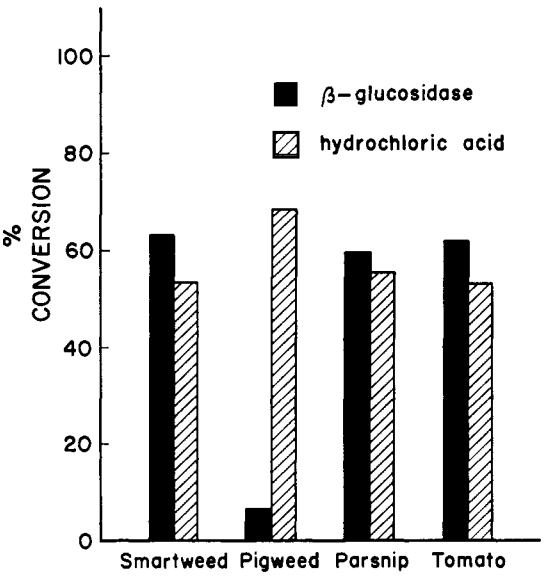

Figure 3. Relative cleavage of water-soluble metabolites by β-glucosidase and hydrochloric acid

Cleavage expressed as percentage of radioactivity in chloroform layer relative to chloroform plus aqueous layers after incubation

metabolites after 10 days. Parsnip, on the other hand, a tolerant species, gives a much lower percentage conversion.

Alkaline hydrolysis by refluxing with 2*N* sodium hydroxide resulted in the formation of 3-chloroaniline from the water-soluble metabolites obtained from all plant species. The 3-chloroaniline was detected by its intense yellow coloration with Ehrlich's reagent and was characterized by cochromatography with authentic material. No evidence was obtained to indicate the presence of any ring-hydroxylated products, the R_f values and color characteristics of the various hydroxy 3-chloroanilines being distinctly different from those of 3-chloroaniline itself. The water-soluble metabolites must therefore contain at least the unchanged 3-chloroaniline moiety.

Figure 2 shows the results of chromatography of the chloroform extracts obtained from solutions of the water-soluble metabolites after incubation with either hydrochloric acid or β-glucosidase. The chloroform-soluble compounds resulting from both these two treatments ran to a lower R_f than either chlorpropham or 3-chloroaniline in both solvent systems. Additionally, this same compound was obtained irrespective of whether the chlorpropham was ring- or chain-labeled. This indicated that the metabolite probably contained the basic chlorpropham structure modified somewhat, thus resulting in different R_f values from chlorpropham itself. The water-soluble metabolites formed from chlorpropham in treated leaves must therefore contain not only the 3-chloroaniline moiety but also a modified chlorpropham entity.

Since β-glucosidase exhibits a complex of hydrolase activities (Pridham, 1965), it was investigated whether the cleavage of the chlorpropham water-soluble metabolites by β-glucosidase was due to other enzymes present as impurities. Thus, cleavage of the metabolites was attempted with both β-glucuronidase and β-galactosidase. The cleavage obtained was not significantly different from the control, thus indicating that the hydrolysis was due entirely to β-glucosidase.

The extent of this hydrolysis by both β-glucosidase and hydrochloric acid is shown in Figure 3. Hydrochloric acid hydrolysis resulted in approximately equal conversion of all metabolites to the modified chlorpropham molecule. β-glucosidase hydrolysis, on the other hand, resulted in substantial conversion of the metabolites obtained from smartweed, parsnip, and tomato, but not of the pigweed metabolite. However, this β-glucoside formation is not sufficient, in itself, to account for the various susceptibilities of these species to chlorpropham. It does, however, indicate that the water-soluble metabolites consist, at least in part, of glycosides with a modified chlorpropham molecule as the aglycone portion.

To investigate the nature of the modified chlorpropham entity, one must consider the possible sites of degradation in the molecule. The formation of 3-chloroaniline by alkaline hydrolysis indicates that degradation or modification of the aromatic ring had not occurred. Furthermore, the use of chlorpropham labeled at carbon atom 2 of the isopropyl group showed that this portion of the molecule was still present in the final metabolite. The two remaining sites for degradation are the carbamate linkage and isopropyl ester grouping. Baskakov and Zemskaya (1959) suggested that herbicidal carbamates could possibly be activated by conversion to their *N*-hydroxy derivatives within plant tissues. To examine this possibility, the *N*-hydroxy derivative of chlorpropham was synthesized and its chromatographic characteristics were compared with those of the product obtained after acid or enzyme hydrolysis of the water-soluble metabolites. The R_f values of the *N*-hydroxy compound proved to be distinctly different from those of the unknown product after hydrolysis, thus indicating that chlorpropham is not converted to its *N*-hydroxy derivative within plant tissues.

The isopropyl portion of the molecule must be modified prior to conjugate formation. This might involve oxidation of the $-CH_3$ group(s) to $-CH_2OH$. The glycoside formation could then be explained on this basis since formation of glycosides in plants occurs readily with hydroxyl groups (Kosuge and Conn, 1961; Pridham, 1965). This process can be visualized as a detoxication mechanism for chlorpropham within plant tissue. This hypothesis is supported by the fact that the water-soluble metabolite obtained from barban by Riden and Hopkins (1962) was nontoxic. Our preliminary studies on the metabolic fate of barban coupled with the results obtained by Riden and Hopkins (1962) on a large number of related carbamate analogs indicate that this modification of the carbamate molecule prior to complex formation could be a common process for all carbamate herbicides. Any correlation between the formation of this modified carbamate molecule and toxicity will, however, necessitate the identification and chemical synthesis of the metabolite.

ACKNOWLEDGMENT

The authors thank L. V. Phillips and J. R. Riden, Gulf Research and Development Co., Merriam, Kansas, for helpful advice, and PPG industries, Pittsburgh, Pa., for supplying the chlorpropham and isopropyl chloroformate. Thanks are also due to G. F. Warren and M. M. Schreiber for helpful advice and to Joseph Deli and Roy Nishimoto for helpful criticism of this article.

LITERATURE CITED

Baskakov, Yu. A., Zemskaya, V. A., *Fiziol. Rast.* **6,** 67 (1959).
Chin, W. T., Stanovick, R. P., Cullen, T. E., Holsing, G. C., *Weeds* **12,** 201 (1964).
Christiansen, W. G., *J. Am. Chem. Soc.* **45,** 2192 (1923).
Eshel, Y., Warren, G. F., *Weeds* **15,** 237 (1967).
Finocchiaro, J. M., Benson, W. R., *J. Assoc. Offic. Agr. Chemists* **50,** 881 (1967).
Kosuge, T., Conn, E. E., *J. Biol. Chem.* **236,** 1617 (1961).
Kuhr, R. J., Casida, J. E., J. AGR. FOOD CHEM. **15,** 814 (1967).
Prendeville, G. N., Eshel, Y., James, C. S., Warren, G. F., Schreiber, M. M., *Weed Sci.* **16,** 432 (1968).
Pridham, J. B., *Advan. Carbohydrate Chem.* **20,** 371 (1965).
Riden, J. R., Hopkins, T. R., J. AGR. FOOD CHEM. **10,** 455 (1962).
Utzinger, G. E., *Ann.* **556,** 50 (1944).

Received for review March 10, 1969. Accepted August 11, 1969. Cooperative investigation between Purdue University, Agricultural Experiment Station, and the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, under Cooperative Agreement No. 12-14-100-8462(34). Approved as Journal Paper No. 3603, Purdue University Agricultural Experiment Station, Lafayette, Ind.