Metabolism of Isopropyl-3-chlorocarbanilate by Cucumber Plants

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Root-treated cucumber plants absorb, translocate, and metabolize ^{14}C-labeled isopropyl-3-chlorocarbanilate (chlorpropham-^{14}C). Polar products and solid residues are found in the roots, stems, and leaves after a 3-day treatment period. Pulse time-course experiments demonstrate a precursor-product relationship between chlorpropham, soluble polar products, and solid residual materials. The polar metabolites are not translocated once they are formed in either the roots or shoots. The radiocarbon distribution patterns of phenyl-^{14}C and isopropyl-^{14}C-labeled chlorpropham were similar in all comparative experiments. Polar metabolites were isolated and partly purified, and the chlorpropham oxidized moiety was characterized. Cucumber tissues rapidly convert chlorpropham to isopropyl-4-hydroxy-3-chlorocarbanilate, which is conjugated with unknown plant components. These chlorpropham metabolites were found in both root and shoot tissues.

The metabolism of isopropyl-3-chlorocarbanilate-^{14}C (chlorpropham-^{14}C) in the resistant crop plant, soybean, has been established and shown to have a precursor-product relationship between the parent herbicide, its polar metabolites, and the solid residual materials (Still and Mansager, 1971, 1972, 1973). In soybean, the polar metabolites were shown to be the *O*-glucosyl derivatives of isopropyl-2-hydroxy-5-chlorocarbanilate (2-hydroxychlorpropham) and isopropyl-4-hydroxy-3-chlorocarbanilate (4-hydroxychlorpropham). During the soybean investigation (Still and Mansager, 1972), there was an apparent need for an experimental system that would yield large quantities of leaf tissue polar metabolites so that new methods of extraction, purification, and characterization could be developed. Cucumber plants met this criterion. This paper reports data elucidating the metabolic fate of chlorpropham in root-treated cucumber plants and the characterization of the chlorpropham moiety of the polar metabolites.

EXPERIMENTAL SECTION

Pulse Time-Course Experiments, Plant Material, and Treatment. Cucumber (*Cucumis sativus* L. 'national pickling') seeds were germinated, transplanted, and grown in an environmental chamber. Methods and equipment previously described by Still and Mansager (1971) were used. The 4-day-old seedlings were transferred to pint jars (two seedlings/jar) containing one-third strength Hoagland's solution and allowed to grow until the cotyledons, three fully expanded leaves, and the fourth leaf had emerged. This was usually 10 to 11 days after the plants were placed in the environmental chamber. When the plants reached that point of development, the time-course experiments were initiated by placing the plants in 400 g of one-half strength Hoagland's solution containing the radiolabeled substrate. After 3 days, the ^{14}C treating solution was replaced with one-half strength Hoagland's nutrient solution (1-qt jars). Tissue samples were taken 0, 1, 3, 5, and 9 days after removal of radiolabel from the nutrient solution.

Plants were removed from the nutrient solution at each sampling time and the roots blotted. Each plant was then sectioned into different tissue groups: root, leaf group 1 (consisting of the cotyledon, the primary leaves, and the fully expanded leaves 1 through 4), leaf group 2 (consisting of all the newly expanded leaves), stem section 1 (the section of stem from the root to the junction of the petiole of leaf 4), and stem section 2 (the section of stem above leaf 4). All the tissue sections were freeze-dried and stored dry at freezer temperatures until analysis. Four plants (two jars) were sampled at each harvest. The radiochemical content of each tissue section group was assayed (Oliveria *et al.*, 1962).

All radiochemical assays and extraction procedures used in this study were previously described (Still and Mansager, 1971).

Radiochemical Substrates. Chlorpropham-phenyl-^{14}C and chlorpropham-2-isopropyl-^{14}C were synthesized by New England Nuclear Corp. and were shown to be both chemically and radiochemically pure using the procedures described by Still and Mansager (1971). Nutrient solutions containing chlorpropham-^{14}C were prepared as previously described (Still and Mansager, 1971). The specific activity of chlorpropham-phenyl-^{14}C was 1.27 mCi/mmol and that of chlorpropham-2-isopropyl-^{14}C was 1.13 mCi/mmol. The concentration of chlorpropham-phenyl-^{14}C was 0.97 μCi (0.77 μmol)/800 ml of Hoagland's solution ($0.96 \times 10^{-6}\ M$) and that of chlorpropham-2-isopropyl-^{14}C was 0.91 μCi (0.81 μmol)/800 ml of Hoagland's solution ($1.01 \times 10^{-6}\ M$).

Chlorpropham-^{14}C Incorporation Experiments. Cucumber seeds were germinated, grown, and root treated in stainless steel trays with one-half strength Hoagland's solution containing either chlorpropham-phenyl-^{14}C (2.90 μCi/tray) or chlorpropham-2-isopropyl-^{14}C (2.83 μCi/tray), at $4.0 \times 10^{-6}\ M$, as described by Still and Mansager (1971, 1972). Twenty trays of each radiolabeled substrate with 24 14-day-old cucumber plants per tray were treated for 7 days. The experiment was terminated and the roots and shoots were separated at the point where the first lateral root hairs appeared on the stem. The chlorpropham-phenyl-^{14}C-treated plant tissues yielded 4110 g of shoot and 1150 g of root (fresh weight), whereas the chlorpropham-2-isopropyl-^{14}C-treated plants yielded 4040 g of shoot and 1040 g of root tissue.

At the end of the experiment, the nutrient treating solution was subjected to liquid-liquid extraction (Still and Mansager, 1971) and the polar and nonpolar phases were assayed for radiocarbon. Chlorpropham-^{14}C was the only ^{14}C-labeled compound found in the nutrient treating solution, which was characterized by thin-layer chromatography (tlc) and gas-liquid chromatography (glc) retention with relation to standards. These procedures were also used to identify the chlorpropham present in the nonpolar phase after Bligh-Dyer extraction.

Extraction and Purification of Polar Metabolites. A modified Bligh-Dyer procedure was used to extract shoot and root tissues after treatment with either chlorpropham-phenyl-^{14}C or chlorpropham-2-isopropyl-^{14}C (Still and Mansager, 1972, 1973).

Extracted polar metabolites were purified by methods described previously (Still and Mansager, 1973). Chroma-

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Table I. Percent Distribution of Radiocarbon Incorporated into Cucumber Plants Pretreated for 3 Days with Chlorpropham-phenyl-^{14}C or Chlorpropham-2-isopropyl-^{14}C

	Days posttreatment									
	0		1		3		5		9	
Tissue	Phenyl	Isopropyl	Phenyl	Isopropyl	Phenyl	Isopropyl	Phenyl	Isopropyl	Phenyl	Isopropyl
Root	35	31	34	26	34	29	35	30	36	27
Stem 1	9	8	8	5	6	2	7	6	7	6
Stem 2	N[a]	N	N	N	N	N	N	N	N	N
Leaf group 1	56	61	58	69	60	66	58	64	58	66
Leaf group 2	N	N	N	N	N	N	N	N	N	N

[a] N = tissue did not contain enough radiocarbon for assay.

tography on Sephadex G-25 or G-10 columns, equilibrated with water and eluted with water or 0.5 *M* potassium acetate, was used for preliminary purification. Shoot polar metabolite samples were chromatographed on DEAE-cellulose (DE-52 in the acetate form) and eluted with either water or 0.1 *M* of sodium chloride. The DE-52 purified shoot metabolites and the root metabolites were adsorbed on XAD-2 (Amberlite), which was washed with water and then eluted with 100% methanol. Tlc with either 250 μ or 2-mm thick silica gel HF plates developed with chloroform–methanol–water (60:40:5) was used to purify the polar metabolites. Recoveries of polar metabolites from the Sephadex G-25, Sephadex G-10, and XAD-2 columns were greater than 80%. Recoveries from the DEAE-cellulose columns or tlc were between 60 and 70%.

Hydrolysis and Derivatization. Hesperidinase and β-glucosidase reactions were conducted in pH 4.8 sodium acetate buffer (0.1 *M*) at 37° for 4 hr. Both enzymes were purchased from Sigma Chemical Co. Solvolysis with anhydrous methanol–HCl (2 *N*) was conducted in sealed conical glass vials at 60° for 2 to 18 hr. All hydrolyses were performed under dry nitrogen.

Acetylation reactions were carried out as described by Paulson and Portnoy (1970). Trimethylsilyl derivatives (TMS) were prepared by dissolving the samples in Regisil (Regis Chemical Co.) and heating in a sealed vial for 2 hr at 60°. Reaction mixtures were injected directly into the gas–liquid chromatograph.

Halogen Acid Hydrolysis of Cucumber Metabolites and Hydroxychlorpropham Analogs. Purified cucumber metabolites were hydrolyzed with 1 ml of 6.0 *N* HCl or HBr at 100° for 18 to 22 hr. Standard hydroxychlorpropham analogs (10 mg) were also hydrolyzed with 1 ml of 6.0 *N* halogen acid. Each tube was purged with nitrogen before the sample was sealed in a glass ampoule. At the end of hydrolysis, the tubes were opened and extracted with 1,2-dimethoxyethane. The solvent was removed under a stream of nitrogen and the residue acetylated (Paulson and Portnoy, 1970). The acetylated mixture was separated by glc and the individual radiolabeled components were subjected to mass spectral analysis. The ^{14}C recovery from 6.0 *N* HCl hydrolysis of cucumber metabolites was estimated to be from 40 to 60%.

Instrumentation. Gas chromatography was conducted with 1.8 m × 0.6 cm glass columns packed with either 3% OV-1 or 3% Dexsil-300 on 80–100 mesh Gas Chrom Q. Both columns were used with a glass inlet. Chromatography was carried out under the following conditions: helium carrier gas, 60 ml/min; column inlet temperature, 185°; and column oven programmed from 100 to 165° at 1°/min with a 10- or 15-min hold on the initial temperature. The column effluent was split 10:1; the smallest part was passed through a flame ionization detector. Components from the larger fraction were trapped. Trapped components were assayed for ^{14}C by liquid scintillation or analyzed by mass spectroscopy.

The mass spectra were measured with a Varian M-66 mass spectrometer equipped with a V-5500 console. The spectra were obtained at 70 eV at a source temperature of 180° and a probe temperature of 20–50°.

Nuclear magnetic resonance (nmr) spectra were measured with a Varian A-60A spectrometer equipped with a Digilab NMR-3 Fourier transform system. All nmr data were obtained as carbon tetrachloride solutions using TMS as an internal standard.

RESULTS

Pulse Time–Course. Chlorpropham-phenyl-^{14}C and chlorpropham-2-isopropyl-^{14}C were supplied to cucumber plants for a 72-hr pretreatment period, after which the ^{14}C-labeled treating solution was replaced with fresh nutrient solution. The movement of the radiocarbon throughout the plant was followed by sampling the plants 0, 1, 3, 5, and 9 days posttreatment. At harvest, the plants were sectioned as described in the Experimental Section. Each tissue section was assayed separately.

The distribution of radiocarbon incorporated into cucumber plants is shown in Table I. A comparison of distribution of the radiocarbon in root and shoot tissues shows some difference between the phenyl-^{14}C and 2-isopropyl-^{14}C-labeled herbicide. Root tissues showed a higher incorporation of chlorpropham-phenyl-^{14}C than chlorpropham-2-isopropyl-^{14}C. This could be interpreted to indicate that the carbanilate bond was cleaved to a limited extent in roots. The general trend, however, as indicated by the similarity between the isopropyl alcohol-^{14}C and the phenyl-^{14}C-labeled substrates, suggests that the carbamate bond of chlorpropham-^{14}C was not cleaved by cucumber metabolism. The very low percentage of incorporation of radiolabel from either substrate into stem section 2 or leaf group 2 indicates that there was little movement of chlorpropham-^{14}C or chlorpropham-^{14}C metabolites from the older tissues into the newly emerging stem and leaf tissues.

To determine the chemical character of the radiolabeled components found in each tissue group at each sampling period, we extracted the treated tissues with a modified Bligh–Dyer procedure (Still and Mansager, 1971). Stem section 2 and leaf group 2 were omitted from this extraction because of their low radiocarbon content. The ^{14}C distribution in nonpolar, polar, and solid residual fractions and between tissue sections were independent of the specifically labeled ^{14}C substrates. This suggested that the two specifically labeled substrates were absorbed, translocated, and metabolized similarly. Because of these observations and because both substrates had the same specific activity, the radiocarbon distribution data from

Table II. Percent Distribution in Bligh–Dyer Extraction of Cucumber Tissues, Pretreated for 3 Days with either Chlorpropham-phenyl-^{14}C or Chlorpropham-2-isopropyl-^{14}C (Data Normalized to 100%)

Tissue	Nonpolar extract, days posttreatment					Polar extract, days posttreatment					Solid residue, days posttreatment				
	0	1	3	5	9	0	1	3	5	9	0	1	3	5	9
Root	2.6	1.4	1.5	1.2	1.4	2.1	1.7	1.8	1.8	1.5	1.7	2.4	2.8	2.8	2.8
Stem section 1	0.7	0.2	0.1	N[a]	N	0.9	0.6	0.6	0.6	0.7	0.4	0.4	0.4	0.3	0.5
Leaf group 1	2.7	0.9	0.2	N	N	7.9	8.2	9.8	9.3	8.7	2.3	3.3	3.4	3.5	3.9

[a] N = tissue did not contain enough radiocarbon to carry out the Bligh–Dyer extraction and assay.

the Bligh–Dyer extraction were combined and normalized (Table II).

The concentration of chlorpropham-^{14}C (nonpolar extract) decreases with time in both stem and leaf (Table II). In root tissue, chlorpropham-^{14}C decreased rapidly during the first time period (0 to 1 days posttreatment) and thereafter the concentration did not change. Soybean roots (Still and Mansager, 1971) nearly completely metabolize chlorpropham within 3 days. The presence of unaltered chlorpropham in cucumber roots may indicate compartmentalization or adsorption of the parent compound. The rapid and extensive conversion of chlorpropham-^{14}C to polar metabolites and solid residues in cucumber leaf tissue suggests that these tissues have the capacity to rapidly conjugate or metabolize chlorpropham.

Root and stem tissues contained low concentrations of polar chlorpropham metabolites. From 1 to 9 days after posttreatment, the concentration of the polar metabolites in root and stem tissues did not change. The decrease in polar metabolites between 0 and 1 day posttreatment was reflected in roots by the increase in the solid residual materials. The highest concentration of ^{14}C in the polar metabolites and the solid residue was found in leaf tissues. Apparently cucumber plants absorb and translocate large quantities of chlorpropham to the expanded and newly emerging leaf tissues where chlorpropham is rapidly converted to polar metabolites. The concentration of polar leaf metabolites decreased between 3 and 9 days posttreatment with the concomitant increase in solid residues.

In contrast to the metabolism of chlorpropham by soybean leaves (Still and Mansager, 1971), where there was only a small conversion to solid residues, cucumber leaves converted a major percentage of the herbicide to insoluble residual materials. At the termination of the experiment (9 days posttreatment), the distribution in all plant parts (root, stem sections, and leaf sections) was 7.2% chlorpropham-^{14}C, 55.9% polar metabolites, and 36.9% solid residual materials. The distribution of solid residual materials was nearly equally divided between leaf tissues and the stem and root tissues.

Characterization of the Chlorpropham-^{14}C Cucumber Metabolites. The radiochemical concentration of cucumber polar metabolites in root and shoot tissues is reported in Table III. Shoot tissues from chlorpropham-phenyl-^{14}C and chlorpropham-2-isopropyl-^{14}C-treated cucumber plants contain five times as much radiocarbon as the root tissue. However, the specific activity of the roots was nearly twice that of the shoots.

After the unknown cucumber polar metabolites (either root or shoot) were passed through various chromatographic procedures, the polar metabolites remained impure and exhibited the characteristics of macromolecules. Further purification was impractical with the techniques available; consequently, hydrolysis procedures were investigated to release the chlorpropham metabolite moiety from its conjugate.

Incubation of partly purified polar metabolites from either cucumber root or shoot tissue with β-glucosidase yielded no nonpolar products. Previous investigations with soybean (Still and Mansager, 1972, 1973) showed that soybean polar metabolites could be hydrolyzed with β-glucosidase to yield either 2-hydroxychlorpropham (isopropyl-2-hydroxy-5-chlorocarbanilate) or 4-hydroxychlorpropham (isopropyl-4-hydroxy-3-chlorocarbanilate). Incubation of the polar cucumber root or shoot metabolites with hesperidinase yielded only 4% of the radiolabel as hexane-soluble material. To recover the unreacted partly purified polar metabolites, the hesperidinase enzyme mixture was adsorbed on XAD-2 and eluted with water that yielded no radiolabeled eluate. Subsequent elution with methanol, however, removed greater than 90% of the ^{14}C-labeled polar metabolites. This sample was again subjected to hesperidinase hydrolysis and again only 4% of the total radiolabel appeared as a nonpolar soluble material.

Table III. Radiochemical[a] Concentration of Cucumber Polar Metabolites

	Shoot	Root
Chlorpropham-phenyl-^{14}C		
Specific activity, dpm/mg	178	349
Total activity, dpm	19.7×10^6	4.09×10^6
Chlorpropham-2-isopropyl-^{14}C		
Specific activity, dpm/mg	160	289
Total activity, dpm	14.6×10^6	2.84×10^6

[a] Radiochemical analysis by oxygen combustion (Oliveria *et al.*, 1962).

No hydroxychlorpropham was recovered when polar cucumber metabolites were hydrolyzed with 20% aqueous HCl. Instead, benzoquinone derivatives were recovered. These derivatives will be discussed elsewhere in this paper. When polar metabolites were treated with anhydrous methanol–HCl, more than 60% of the radiolabel was converted to a hexane-soluble product.

Additional partly purified polar metabolites from cucumber root or shoot were subjected to anhydrous methanolic-HCl solvolysis. The product of this solvolysis was derivatized either as the acetate or as the TMS derivatives and separated by glc. Glc retention times and parent ions of the acetate and TMS derivatives from the unknown and from standard 4-hydroxychlorpropham were identical. These data agreed with findings previously reported for the hydroxychlorpropham analogs found in soybean root and shoot tissues (Still and Mansager, 1973).

The mass spectral fragmentation of acetylated 4-hydroxychlorpropham has been reported (Still, 1971). In the present study significant differences were observed in the mass spectral fragmentation of the mono- and di-TMS derivatives of 4-hydroxychlorpropham. Table IV reports the major fragment ions from the mono-TMS derivative (glc peak 2) and the di-TMS derivative (glc peak 1) of 4-hydroxychlorpropham. Both derivatives lost 42 from their parent ions (*m/e* 373 or 301), which was interpreted to be the loss of C_3H_6 resulting from a McLafferty rearrangement (M_a^+). This was followed by the loss of 44 (CO_2) (M_b^+). The TMS derivative, whether it was bonded to a

Table IV. Major Ion Fragments from Trimethylsilyl Derivatives of Hydroxychlorpropham[a]

	m/e	Fragment		RI
		Ion	Lost	
Glc peak 1				
	373	M^+		29
	331	M_a^+	C_3H_6 (−42)	20
	287	M_b^+	$C_4H_6O_2$ (−86)	19
Glc peak 2				
	301	M^+		74
	259	M_a^+	C_3H_6 (−42)	55
	215	M_b^+	$C_4H_6O_2$ (−86)	17

[a] The conditions of the ms experiment were as described in the Experimental Section. The base peak was *m/e* 42. Each of the ions M^+, M_a^+, and M_b^+ also shows fragment ions of $-CH_3$ (−15).

nitrogen or an oxygen atom, appeared to be remarkably stable to electron bombardment. This was not the case with the acetate derivatives of 4-hydroxychlorpropham. Fragment ions *m/e* 287 and 215 (M_a^+) from the di- and mono-TMS derivatives represent *N*-TMS-3-chloro-4-*O*-TMS aniline and 3-chloro-4-*O*-TMS aniline, respectively. The observed and calculated ratios between the *m/e* 301, 302, and 303 ions or the *m/e* 373, 374, and 375 ions agreed for a molecule with one chlorine and one or two silicon atoms for each fragment. These mass spectral fragmentation data supported the conclusion that the parent ion was either the mono- or di-TMS derivative of isopropyl-3-chlorohydroxycarbanilate (hydroxychlorpropham).

Some samples of polar cucumber metabolites yielded large amounts of TMS-glucose, which glc chromatographed near the TMS-hydroxychlorpropham fractions. The TMS-glucose derivatives were characterized by mass spectral analysis (DeJongh *et al.*, 1969). We do not know, however, if the hydroxychlorpropham was conjugated with glucose.

To determine the structure of the hydroxychlorpropham metabolite fraction, the di-TMS derivative was collected from glc and subjected to Fourier transform nmr (FT nmr). The protons of the TMS-methyls were observed at 0.14 and 0.25 ppm as a singlet (9 H each) for the O and N substituted derivatives. The isopropyl methyl protons were at 1.14 and 1.23 ppm (singlets, 3 H each), the isopropyl methine proton was a septet at 4.62 to 5.06 ppm (1 H), and the aromatic protons were 6.56 to 4.35 ppm, a multiplet (3 H). The splitting pattern of the aromatic protons was an exact duplicate of the pattern reported for 3,4-dichloronitrobenzene (Zanger, 1972). The chemical shift and coupling found for the hydroxychlorpropham aromatic protons were: H_5, 6.56 and 6.52 ppm (ortho coupled); H_6, 6.95, 7.00, 7.15, and 7.18 (ortho–meta coupled); and H_2, 7.29 and 7.35 (meta coupled). The coupling constants were found to be 9.5 and 2.4 Hz ortho and meta coupling, respectively. These data are in agreement with the structure isopropyl-3-chloro-4-hydroxycarbanilate (4-hydroxychlorpropham). The chemical shift of the TMS-methyl protons was similar to shifts reported for TMS-flavanoids (Rodriguez *et al.*, 1972).

Synthetic standards of 4-hydroxychlorpropham were silonized and the mono- and di-TMS ethers were separated by glc and subjected to FT nmr. The data from the synthetic standards and the data from the cucumber metabolite were identical. A soybean shoot metabolite was shown to be 4-hydroxychlorpropham (Still and Mansager, 1973). This metabolite was characterized by FT nmr of the diacetate derivative. To compare the cucumber metabolite and the soybean metabolite, the TMS derivatives of the cucumber metabolite were acetylated (Paulson and Portnoy, 1970) and the diacetate was purified by glc. Data from the cucumber FT nmr experiment were identical to the soybean 4-hydroxychlorpropham data previously reported (Still and Mansager, 1973).

On the basis of the mass and the FT nmr we concluded that cucumber root and shoot tissue hydroxylated the chlorpropham molecule in the 4-position to yield 4-hydroxychlorpropham, which was conjugated with an unknown plant component.

Halogen Acid Hydrolysis of the Chlorpropham Metabolite. In an effort to characterize the cucumber chlorpropham polar metabolite conjugates, partly purified metabolites were subjected to hydrolysis in 6 *N* aqueous HCl. At the end of the hydrolysis, the solvent was removed and the residue was subjected to glc analysis. No radiolabeled elution peaks were detected. The residue was acetylated (Paulson and Portnoy, 1970) and yielded two radiolabeled glc peaks. The mass spectral data from these two acetylated derivatives showed a major fragment ion group at *m/e* 212 through *m/e* 218 from glc peak 1, and a major fragment ion group from glc peak 2 was shown to be *m/e* 246 through *m/e* 252. The ratio of the M, M + 2, M + 4, and M + 6 ion fragments agreed with the literature for a molecule containing three and four chlorine atoms (Beynon, 1960). The parent ion for glc peak 1 ($C_8H_5O_3Cl_3$, *m/e* 254) agreed with the proposed structure of monoacetoxy-trichlorohydroquinone. The parent ion of glc peak 2 ($C_{10}H_6O_4Cl_4$, *m/e* 330) agreed with the speculated structure of diacetoxy-tetrachlorohydroquinone.

To further test this hypothesis, a comparative hydrolysis with 6 *N* HCl or HBr was conducted. Recoveries from the hydrolysis were 57% for HCl and 29% for HBr. Recoveries from acetylation of the hydrolytic products were 95–96%. Glc followed by mass spectral analysis indicated that the HCl hydrolysis again yielded the tri- and tetrachlorohydroquinone derivatives, whereas the HBr hydrolysis yielded a mixture of the possible hydroquinone isomers.

Chlorpropham and the four different hydroxy analogs of chlorpropham were subjected to 6 *N* HCl hydrolysis. The recovery of chlorpropham from the chlorpropham hydrolysis mixture was quantitative. Analysis of the acetylated derivatives of the hydroxy analogs of chlorpropham showed that only the 4-hydroxy-3-chlorocarbanilate analog yielded the tri- or tetrachlorohydroquinone derivatives. These data indicate that the para hydroxy analog of chlorpropham was probably required for the formation of the polychlorinated hydroquinones found from the cucumber metabolite.

Literature reviewed by Cason (1948) suggested a free radical mechanism to explain the oxidation of a halogenated phenol or amine to a quinone containing more than the expected number of halogen atoms. In the presence of 6 *N* aqueous HCl, 4-hydroxychlorpropham must be hydrolyzed to 2-chloro-4-aminophenol, which is subsequently oxidized and hydrohalogenated to yield the observed tri- and tetrachlorinated hydroquinone derivatives. The results of these experiments suggest that the conjugated chlorpropham moiety of the cucumber metabolite was 4-hydroxychlorpropham. Analysts must be cautioned to avoid acid hydrolysis and possible hydroquinone formation during quantitative estimations of 4-hydroxychlorpropham.

DISCUSSION

Interest in the metabolism of chlorpropham by cucumber plants was based on the fact that cucumber was susceptible to the herbicide and that preliminary experiments showed that cucumber produced large quantities of polar metabolites. It was our desire to investigate the nature of the polar metabolites in a susceptible chlorpropham crop plant.

The results of the pulse time-course experiments indicated that the major fraction of chlorpropham was not

cleaved at the carbanilate bond by the cucumber plant. Similar results have been reported for soybean plants (Still and Mansager, 1971, 1972, 1973). A precursor–product relationship between chlorpropham and the polar metabolites was established (Table II). Only minor concentrations of the parent compound were present in the plant after 3 days treatment. In all tissue sections, the greatest rate of metabolic activity appeared between 0 and 1 day after herbicide treatment. A slow conversion of polar metabolites to solid residues was observed in leaf tissues (group 1) between 1 and 9 days after treatment. Unchanged chlorpropham (nonpolar extract) was observed in root tissue (Table II) and appeared to be either adsorbed to the surface of the root or compartmentalized and bound in the cell structure. Because the concentration of unchanged chlorpropham did not decrease between the 1 and 9 day posttreatment, it was apparently metabolically inert and was not translocated during the experiment. Further, if this chlorpropham were mobile, it should have appeared in the newly emerging tissues (stem section 2 and leaf group 2, Table I) where only negligible radiolabeled concentrations from chlorpropham-^{14}C were found. Cucumber plants did not acropetally translocate the polar metabolites formed in the leaf tissues during the course of plant growth. Similar results have been reported for soybean (Still and Mansager, 1971). The immobilization of polar metabolites was indicated by the low concentration of radiolabel in the newly emerging leaves (leaf group 2) throughout the later stages of the pulse time-course study. The significance of the higher concentration of solid residues found in the leaf tissues and stems of cucumber as compared with that in soybean is not understood.

The isolation and characterization of the methanol–HCl solvolysis product from cucumber polar metabolites indicated that the cucumber plants hydroxylated chlorpropham to yield 4-hydroxychlorpropham, which was subsequently conjugated to form unknown polar metabolites. The nature of these conjugates was not determined. Evidence indicates that the conjugate was not a β-glucoside or a common glucoside because it was not hydrolyzed by either β-glucosidase or the broad spectrum enzyme hesperidinase. Both the β-glucosidase and hesperidinase enzyme preparations were demonstrated to be active in the presence of the cucumber metabolites, indicating that these negative results were not due to endogenous inhibitors. Acid hydrolysis yielded an artifact that was characterized as a polychlorinated hydroquinone. Analysis of the aqueous fraction after hydrolysis yielded no information to indicate that the conjugate was an amino acid, a peptide, or a small protein.

It is interesting to compare the metabolism of the resistant soybean with that of the susceptible cucumber and to correlate the data from the pulse time-course studies and the nature of the polar metabolites with susceptibility and resistance. Note that susceptible cucumber yielded only the 4-hydroxychlorpropham polar conjugate in root and shoot tissues, whereas soybean yielded the 2-hydroxychlorpropham glucoside in root and the 2-hydroxychlorpropham and 4-hydroxychlorpropham glucosides in shoot tissue. However, the existence of the 4-hydroxychlorpropham moiety in the resistant soybean would seem to negate this isomer as a phytotoxic factor in cucumber. The apparent absence of glucosides in cucumber and the formation of the unknown conjugates could be the key to the sensitivity of cucumber to chlorpropham. However, it appears that other factors must be involved in the mechanism of action and the phenomenon of susceptibility and resistance. With the information that we have, the correlation of the mechanism of action of chlorpropham in resistant and susceptible plants does not seem possible.

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