Metabolism of 2-Chloro-*N*-Isopropylacetanilide (Propachlor) in the Leaves of Corn, Sorghum, Sugarcane, and Barley

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The metabolism of propachlor in corn seedlings and in excised leaves of corn, sorghum, sugarcane, and barley is similar during the first 6 to 24 hr following treatment. At least three water-soluble metabolites are produced in each species during this period. Two of these metabolites, compounds I and II, were isolated. Compound I was identified as the glutathione conjugate of propachlor. Compound II appears to be the γ-glutamylcysteine conjugate of propachlor. In corn seedlings compounds I and II were shown to be transitory metabolites, and they were not detected in significant concentrations 72 hr following treatment. The possibility that *N,N*-diallyl-2-chloroacetamide (CDAA) and 4-chloro-2-butynyl-*m*-chlorocarbanilate (barban) might form similar conjugates was considered.

Glutathione has been the subject of many biochemical investigations, but its role in biological systems is still not completely understood. Glutathione is a substrate or coenzyme in several enzymatic reactions and is believed to protect thiol groups of proteins. In addition, it plays an important role in the detoxication of exogenous chemicals by formation of glutathione conjugates (Boyland and Chasseaud, 1969). Mercapturic acids are believed to be formed from the corresponding glutathione conjugates by successive removal of the glutamyl and glycine residues, followed by *N*-acetylation of the cysteine residue (Boyland and Chasseaud, 1969). The metabolism of organic halides and related compounds to glutathione conjugates or mercapturic acids occurs in mammals (Booth *et al.*, 1961), birds (Wit and Leeuwangh, 1969), and insects (Cohen and Smith, 1964). A similar mode of metabolism was established in plants when 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) was found to be metabolized to a glutathione conjugate and a γ-glutamylcysteine conjugate in sorghum (Lamoureux *et al.*, 1970); the process was shown to be enzymatic (Frear and Swanson, 1970). Glutathione transferase activity has been demonstrated in corn, sorghum, sugarcane, Sudan grass, and Johnson grass. The enzyme is capable of catalyzing the formation of glutathione conjugates with a number of herbicidal 2-chloro-*s*-triazines (Frear and Swanson, 1970). The report that 2-chloro-*N*-isopropylacetanilide (propachlor) reacts nonenzymatically *in vitro* with glutathione (Frear and Swanson, 1970) indicated that glutathione conjugation may be a pathway for metabolism of the α-chloroacetamide herbicides in higher plants. This paper reports on the metabolism of propachlor by this route in several species of higher plants.

EXPERIMENTAL

General Methods. Quantitative amino acid analyses were performed on a Technicon Amino Acid Analyzer using a 140-cm column of Chromobeads Type B and the standard citrate buffer gradient (Technicon Chromatography Corp., 1962). The ^{14}C content of insoluble plant residues was determined by liquid scintillation techniques after the samples were combusted in an oxygen atmosphere (Shimabukuro, 1967). The general methods used for monitoring ^{14}C, preparing silica gel thin-layer plates, and concentrating plant extracts have been described previously (Lamoureux *et al.*, 1970). Thin-layer chromatograms were developed with 1-butanol-glacial acetic acid-water (12:3:5) unless otherwise stated.

Plant Material. Corn (*Zea mays* L., var. N.D. KE 47101) seedlings were grown in vermiculite in greenhouses with supplementary fluorescent light to give at least a 12-hr photoperiod. Half-strength Hoagland's solution was used intermittently for subirrigation. Leaves were excised 14 to 28 days after germination. Barley (*Hordeum vulgare* L., var. Larker), sorghum (*Sorghum vulgare* Pers., var. N.D. 104) and sugarcane (*Saccharum officianarum* hybrid C.P. 61-37 × C.P. 56-59) were grown under similar conditions. Excised leaves were obtained from 47-day-old sorghum and 5-month-old sugarcane. The shoots of 28-day-old barley plants were excised above the crown. In whole plant studies, corn seedlings were grown in continuously aerated one-third strength Hoagland's solution. These plants were treated 10 to 15 days after germination.

Treatment of Plant Material. Duplicate 2-g samples, containing two to six excised leaves of sorghum, sugarcane, and corn, and shoots of barley were treated by immersing the cut ends in 4 ml of 0.9 to 1.5 × $10^{-5}M$ ^{14}C carbonyl-labeled propachlor (specific activity of 2.0 μCi per μmole). Another duplicate set of excised corn leaves was treated similarly with uniformly ^{14}C ring-labeled propachlor (specific activity 4.0 μCi per μmole). The leaves were incubated in a growth chamber with a 12-hr photoperiod, 1600 ft-candles light intensity, at 26° C and a 12-hr dark period at 21° C. The relative humidity was maintained at 40% throughout the cycle. The excised leaves were rinsed with water and blotted dry after a 6- or 24-hr treatment period. Each sample was homogenized in a water-cooled blender with 50 ml of cold 80% methanol. During homogenization, the temperature increased from 5° C to a final temperature of 17° C. The homogenates were filtered and the residues were reextracted with two 50-ml portions of cold 80% methanol. The combined filtrates from each sample were concentrated to near dryness, brought to a volume of 25 ml with water, and partitioned three times with 25-ml volumes of chloroform.

Intact corn seedlings were treated by injecting 20 μl of a 2% ethanol solution (0.0585 μmole of carbonyl-^{14}C-propachlor) into the stem of each seedling. Corn seedlings were also treated by immersing the roots in 50 ml of one-third strength Hoagland's solution containing 0.318 μmole of carbonyl-^{14}C-propachlor or ring-^{14}C-propachlor. The seedlings were placed in the growth chamber and harvested after 18 and 72 hr. The roots and shoots were extracted separately as described for excised leaves and shoots.

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Purification of Metabolites. Nineteen sets of excised corn shoots (*ca.* 40 shoots per set) were used for the isolation and identification of propachlor metabolites. Each set was treated with 150 ml of 1×10^{-4} *M* carbonyl-^{14}C-propachlor (specific activity of 0.107 μCi per μmole). The excised corn shoots (528 g fresh wt) were harvested after a 24-hr treatment period, extracted, and partitioned with chloroform as described previously (Lamoureux *et al.*, 1970). The aqueous phase from the extraction was concentrated, placed in pH 2.1 pyridine-acetate buffer and chromatographed on a 2.4×100 cm water-jacketed column (15° C) of AG 50W-X2 resin by stepwise elution (Lamoureux *et al.*, 1970). The first radioactive fraction (fraction IA) was eluted with pH 3.1 buffer and contained 1.6×10^6 dpm. A second fraction containing 9.2×10^6 dpm (fraction IIA) and a third fraction containing 3.7×10^6 dpm (fraction IIIA) were eluted with pH 3.7 buffer (a mixture of 80% pH 3.1 buffer and 20% pH 5.7 buffer). Total recovery of radioactivity from this column was 88%.

Fraction IIA was concentrated and chromatographed on a 2.4×110 cm water-jacketed column (15° C) of AG 1-X2 (200–400-mesh) resin by stepwise elution with 400 ml of 0.75 *N* acetic acid, 350 ml of 1.5 *N* acetic acid, 790 ml of 2.5 *N* acetic acid, and with 3.0 *N* acetic acid until radioactivity was no longer eluted. Two radioactive fractions, fraction IB and fraction IIB were eluted from this column. Fraction IB (5.6×10^6 dpm) was eluted with 2.5 *N* acetic acid and fraction IIB (2.9×10^6 dpm) was eluted with 3.0 *N* acetic acid. Recovery of radioactivity from this column was 92%. Aliquots of fractions IB and IIB (600,000 dpm each) were individually chromatographed with methanol on a 1×100 cm water-jacketed column (15° C) of Sephadex LH-20. Each fraction chromatographed as a single peak on this column. In each case about 85% of the radioactivity was recovered. The products were designated as fractions IC and IIC, respectively. Fractions IC and IIC were further purified by preparative thin-layer chromatography. The radioactive zones of gel (R_f 0.28 to 0.34) were scraped from the plates and eluted with 50% methanol. These products were designated as compounds I and II, respectively.

Analysis of Hydrolytic Products of Compounds I and II. Compounds I and II were hydrolyzed under an inert atmosphere with 6 *N* HCl at 110° C for 20 hr. An aliquot of each hydrolyzate was analyzed on the amino acid analyzer. The qualitative results of these analyses were verified by thin-layer chromatography using ethanol-water (70 to 30) as the developing solvent. An aliquot of each hydrolyzate was treated with dilute sodium hydroxide until slightly basic, and then partitioned with hexane. The hexane phases were analyzed for aniline bases by the gas chromatographic procedure of Henkel (1965). Aniline, *N*-ethylaniline, and *N*-isopropylaniline were used as chromatographic standards.

Chromatographic Comparison of the Metabolites Derived from Excised Tissues and Corn Seedlings. The aqueous extracts were compared by thin-layer chromatography and column chromatography. For column chromatography, a 1×110 cm water-jacketed column (15° C) of AG 1-X2 (200–400 mesh) resin in the acetate form was eluted with an acetic acid gradient at a flow rate of 0.30 ml per min. The column was standardized with compounds I and II extracted from corn leaves. Retention volumes were reproducible to within ±8 ml. The acetic acid gradient used to elute the column was produced from a nine-chamber gradient device (Technicon Chromatography Corp., 1962). Initially, each chamber of the gradient device contained 60 ml of solvent filled in the following manner: chambers 1, 2, 4, 6, and 8 contained water, chamber 3 contained 1.38 *N* acetic acid, chambers 5 and 9 contained 6.88 *N* acetic acid, and chamber 7 contained 5.30 *N* acetic acid. After each chromatographic analysis, the column was washed with water until the pH of the effluent was between 4 and 5.

Table I. Percent Distribution of Radioactivity into Solubility Classes in Excised Leaves and Shoots Treated with ^{14}C-Propachlor

Tissue	Water Soluble	Chloroform Soluble	80% Methanol Insoluble	% Recovery[a] of ^{14}C
Corn leaves[b]	93.5	5.9	0.6	72
Corn leaves[c]	96.9	2.4	0.7	76
Sugarcane leaves[d]	91.5	5.0	3.5	86
Sorghum leaves[b]	96.5	1.5	2.0	88
Barley shoots[b]	95.7	3.4	0.9	74

[a] These values represent the total % recovery of radioactivity used in the experiment. [b] Treated with carbonyl-^{14}C-propachlor for 24 hr. [c] Treated with ring-^{14}C-propachlor for 24 hr. [d] Treated with carbonyl-^{14}C-propachlor for 6 hr.

Synthesis. *S*-Carboxymethylcysteine was prepared in excellent yield by reacting 12.1 mg of cysteine with 10 mg of α-chloroacetic acid in 5 ml of 0.1 *N* methanolic sodium hydroxide for 6 hr at 77° C. The resulting product was used without further purification.

The glutathione conjugate of ^{14}C-propachlor (Figure 4, Compound I) was prepared by reacting 0.045 μmole of carbonyl-^{14}C-propachlor for 3 hr with 10 μmoles of reduced glutathione in 1 ml of 0.1 *M* pH 6.8 phosphate buffer. The resulting solution was lyophilized, dissolved in 0.5 ml of methanol, and centrifuged. The radioactive glutathione conjugate was obtained from the supernatant in almost quantitative yield.

Propachlor and *N*,*N*-diallyl-2-chloroacetamide (CDAA) were prepared with ^{14}C-labeling at the carbonyl position. Chloroacetic acid-1-^{14}C (1 mCi, 7.87 μCi per μmole) was purchased from New England Nuclear Corp. Chloroacetyl chloride was prepared from chloroacetic acid based on the method of Cox and Turner (1950). A small reaction flask (5-ml capacity) with a side arm and a cold finger was used to scale down the reaction to the 0.5 mmole level. Phthaloyl chloride (2 mmoles, 0.3 ml) was added to the chloroacetic acid-^{14}C (1 mCi, 0.5 mmole, 47.3 mg) in the reaction flask. The reaction temperature was held at 125° C in an oil bath. A cold trap, cooled with dry ice-2-propanol, was initially vented to the atmosphere while generated acid chloride was allowed to reflux for 30 min. The system was slowly evacuated to a pressure of 0.1 mm and the reaction mixture was refluxed for 1 hr. The generated chloroacetyl chloride was pumped from the reaction vessel into the cold trap as refluxing occurred. After completion of reaction, the cold trap was removed from the vacuum line and cold anhydrous chloroform was added to the acid chloride while the acid chloride was held in the dry ice-2-propanol bath. The chloroacetyl chloride solution was divided into two equal volumes and added to 2 mmoles each of diallylamine and *N*-isopropylaniline which were dissolved in dry chloroform. The reactions were completed at room temperature, and the products were purified by preparative thin-layer chromatography with benzene-acetone (90 to 10) as the developing solvent (combined yield—78.6%).

RESULTS AND DISCUSSION

Propachlor is rapidly metabolized to water-soluble compounds by all of the tissues examined (Table I and Figure 1).

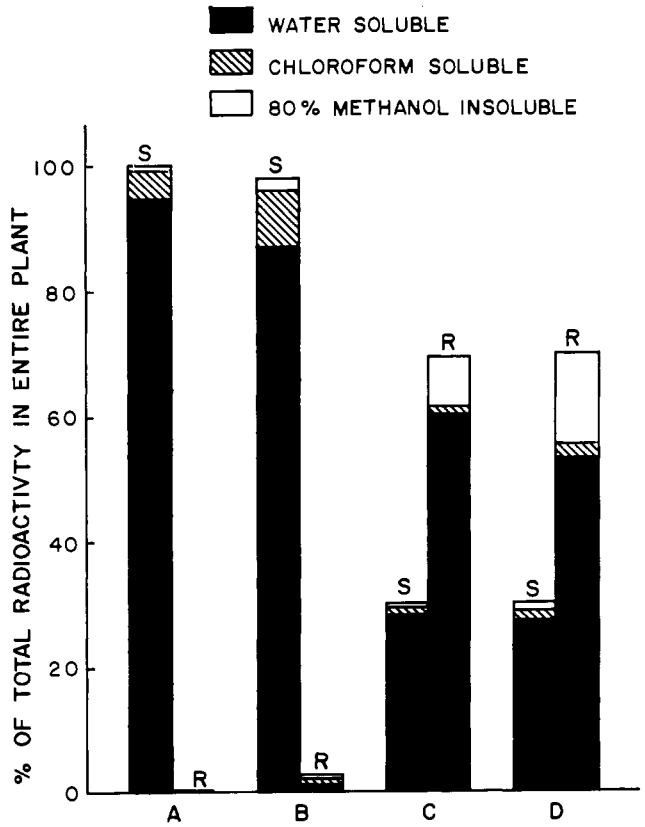

Figure 1. Percent distribution of radioactivity into solubility classes in roots and shoots of corn seedlings treated with ^{14}C-propachlor. Seedlings A and B were treated by stem-injection with carbonyl-^{14}C-propachlor and harvested 18 and 72 hr later. Seedlings C and D, respectively, were root-treated with carbonyl-^{14}C- and ring-^{14}C-propachlor and harvested 72 hr later. Shoots = S, roots = R. The average recovery of radioactivity was 88 ± 6%

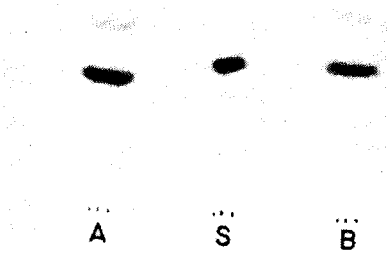

Figure 2. Authentic carbonyl-^{14}C-glutathione conjugate of propachlor (S) prepared by *in vitro* reaction was compared by thin-layer chromatography to the radioactive products present in the aqueous extracts from excised corn leaves treated with ring-^{14}C-propachlor (A) and carbonyl-^{14}C-propachlor (B)

In the foliar tissues the distribution of radioactivity into water-soluble, chloroform-soluble, and 80% methanol insoluble fractions is similar (Table I). However, the percent radioactivity in the 80% methanol insoluble residue from the roots of root-treated corn seedlings was noticeably higher than in the leaves (Figure 1). This could be due to a basic difference in the metabolism of propachlor between the roots and shoots. The slight increase in chloroform-soluble radioactivity observed in corn leaves between 18 and 72 hr following treatment (A and B, Figure 1) is probably not significant. Results in Figure 1 indicate that propachlor and/or its metabolites are absorbed through the roots and translocated to the shoots. Propachlor and/or its metabolites are not readily translocated downward, as indicated by the absence of significant amounts of radioactivity in the roots of corn seedlings treated by injection of radioactive herbicide into the stems.

The water-soluble fractions of extracts from different plant species (Table I) were analyzed by thin-layer chromatography. Each extract showed a characteristic intense radioactive zone at R_f 0.29 and a weak radioactive zone at R_f 0.43. Thin-layer chromatograms of extracts of excised corn leaves treated with ^{14}C-propachlor are shown in Figure 2. Chromatographic results from corn seedlings harvested 18 hr after stem-injection of carbonyl-^{14}C-propachlor were similar to those from the excised leaf tissues shown in Figure 2; however, corn seedlings incubated for 72 hr following stem-injection or root-feeding of ^{14}C-propachlor gave different results from those described above. These extracts (72 hr incubation) produced radioactive zones of nearly equal intensity at R_f 0.36, 0.44, and 0.52. With the possible exception of the product at R_f 0.44, these metabolites appeared to be different from those obtained from excised leaf tissues and from corn seedlings harvested 18 hr after stem-injection.

The water-soluble plant extracts were chromatographed on a column of AG 1-X2 with an acetic acid gradient to further characterize the metabolites. The chromatographic results are shown in Figure 3. Metabolites with elution volumes of 118, 338, and 405 ml were detected in extracts from excised leaves of the different species and from stem-injected corn seedlings incubated for 18 hr. The product with an elution volume of 118 ml corresponds to the radioactive zone at R_f 0.43 by thin-layer chromatography. The products with elution volumes of 338 ml and 405 ml have R_f values of 0.29 and 0.31, respectively, and correspond to the major radioactive zone observed at R_f 0.29 upon initial analysis by thin-layer chromatography. The principal metabolites with elution volumes of 338 ml and 450 ml were either not detected in plant material incubated for 72 hr, or the concentrations of these metabolites were very low. Recovery of radioactivity from the column during chromatography of these extracts was greatly reduced from that observed with the other extracts. The results in Figure 3 indicate that the principal metabolites in corn seedlings with elution volumes of 338 ml and 405 ml (designated as compounds I and II, respectively) are transitory.

Compounds I and II were isolated from excised corn shoots treated with carbonyl-^{14}C-propachlor. During the first isolation procedure, 37% of the radioactivity eluted from the column of AG 50W-X2 was in the form of metabolites other than compounds I and II. These radioactive products were not examined in this study. Compounds I and II were the only major radioactive products detected in the other isolation steps, and the recovery of radioactivity from each ranged from 75 to 92%. The chemical and radiochemical homogeneity of I and II was confirmed by thin-layer and column chromatography.

Compounds I and II were hydrolyzed in *6 N* HCl. Gas chromatographic analysis indicated that *N*-isopropylaniline was liberated by both compounds in a yield of approximately 80 mole%. The results obtained from amino acid analyses of these hydrolyzates are shown in Table II. Glutamic acid and glycine were detected in a 1 to 1 stoichiometric relationship for

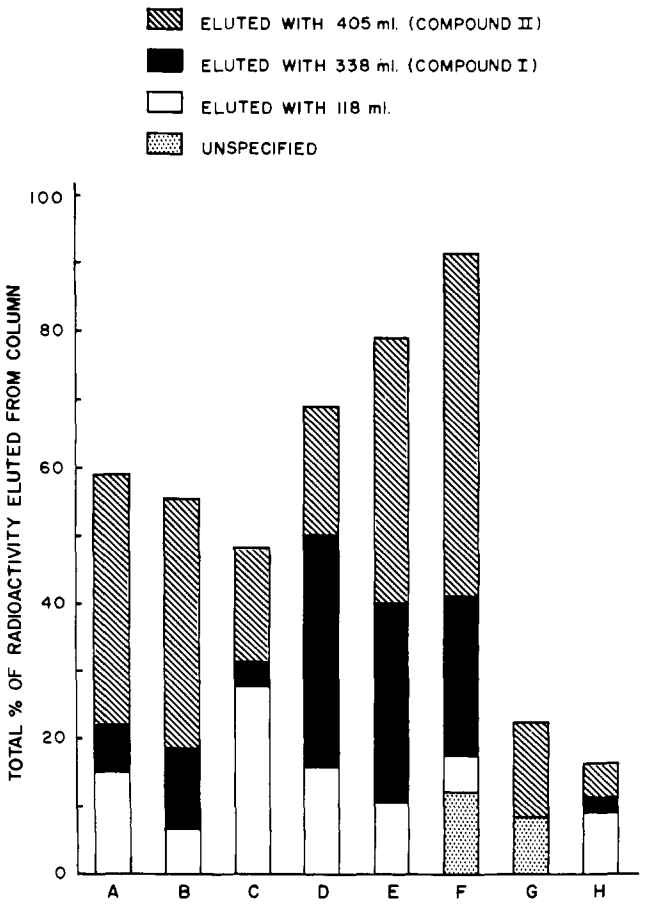

Figure 3. A column-chromatographic comparison of the water-soluble metabolites produced from ^{14}C-propachlor by: A, sugarcane leaves, 6 hr; B, barley shoots, 24 hr; C, sorghum leaves, 24 hr; D, corn leaves, 24 hr; E, corn leaves, ring-^{14}C-propachlor, 24 hr; F, foliar tissue from stem-injected corn seedlings, 18 hr; G, foliar tissue from stem-injected corn seedlings, 72 hr; H, root tissue from root-treated corn seedlings, ring-^{14}C-propachlor, 72 hr. Unless otherwise stated, carbonyl-^{14}C-propachlor was used in all treatments

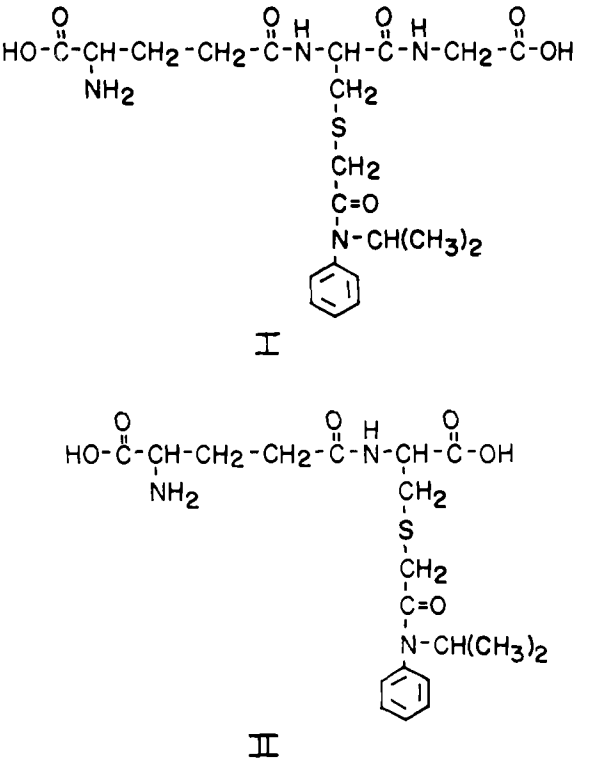

Figure 4. Compound I, the glutathione conjugate of propachlor; Compound II, the γ-glutamylcysteine conjugate of propachlor

Table II. Amino Acid Analysis of Hydrolyzates of Compounds I and II

Amino Acid	Residues per Molecule of Compound Hydrolyzed	
	Compound I	Compound II
^{14}C-*S*-carboxymethylcysteine[a]	0.996	0.570
Glutamic acid	1.14	1.10
Glycine	1.11	0.080
1/2 Cystine	0.174	0.498

[a] The color value of *S*-carboxymethylcysteine was estimated from an impure reaction mixture and the values for this compound are only approximate.

Table III. Chromatographic Comparison of the Glutathione Conjugate of Propachlor and Compounds I and II

Chromatographic System	Glutathione Conjugate	Compound I	Compound II
Technicon amino acid analyzer	0.751[a]	0.751[a]	0.833[a]
Gradient elution from AG 1-X2 Resin	338 ml	338 ml	405 ml
Tlc (1-butanol-glacial acetic acid-water)	R_f 0.29	R_f 0.29	R_f 0.31
Tlc (ethanol-water)	R_f 0.61	R_f 0.60	...

[a] Value is retention volume relative to norleucine.

the amount of I hydrolyzed. In addition, cystine and ^{14}C-*S*-carboxymethylcysteine were detected. The sum of 1/2 cystine plus ^{14}C-*S*-carboxymethylcysteine was equivalent, on a molar basis, to the amount of I hydrolyzed. Similar results were observed with II, except only a trace of glycine was detected. The cystine detected in these hydrolyzates was probably formed from ^{14}C-*S*-carboxymethylcysteine during hydrolysis. The presence of ^{14}C-*S*-carboxymethylcysteine in these hydrolyzates suggests that I and II are conjugates of propachlor in which a cysteine or cysteinyl residue is bonded to the α-carbon of the acetyl moiety of propachlor by a sulfide bond.

The results in Table II indicate that I is the glutathione conjugate of propachlor as shown in Figure 4. This structure was confirmed by synthesis and comparison of the authentic product with I by several different chromatographic methods (Table III). The chromatographic properties of II are quite similar to those of I (Table III). Based upon the chromatographic character and the results of hydrolysis, the most likely structure of II was concluded to be the γ-glutamylcysteine conjugate of propachlor, as shown in Figure 4.

The results of this study show that the metabolism of propachlor in the leaves of barley, sorghum, corn, and sugarcane is similar during the first 6 to 24 hr. The primary mode of metabolism is a nucleophilic displacement of the α-chloro group of propachlor by the sulfhydryl group of a peptide. The principal products resulting from this displacement are the glutathione conjugate (I) and the γ-glutamylcysteine conjugate (II). A similar mode of metabolism has been established for atrazine in sorghum. The reaction of atrazine with glutathione has been shown to be catalyzed by an enzyme which has a high degree of specificity with regards to the sulfhydryl reactant. However, in the case of propachlor, the reaction

proceeds nonenzymatically *in vitro* and the possibility exists that the *in vivo* reaction may be enzymatic and/or nonenzymatic. The high percent of propachlor converted to compounds I and II in corn seedlings during the first 18 hr following treatment indicates that the reaction of propachlor with glutathione and/or γ-glutamylcysteine is quite specific. This would be expected if the reaction is enzymatic.

Evidence has been presented that the glutathione conjugate and the γ-glutamylcysteine conjugate of atrazine are transitory (Lamoureux *et al.*, 1970). The results of this paper indicate that similar metabolites of propachlor, I and II, are transitory in corn. This is in agreement with the work of Porter and Jaworski (1965) and Jaworski and Porter (1965), who reported the presence of transitory metabolites of propachlor in corn. These workers did not characterize the transitory metabolites, but partial identification of the apparent terminal metabolite was carried out. Evidence indicated that the α-chloro group in the terminal metabolite was replaced by an unidentified moiety of an acidic nature. Possibly, the terminal metabolite observed by Porter and Jaworksi is produced from I and/or II by modification or nucleophilic displacement of the peptide side chain.

The metabolism of other α-chloroacetamides and related compounds by glutathione conjugation was considered in this study. A fast *in vitro* reaction of 4-chloro-2-butynyl-*m*-chlorocarbanilate (barban) with reduced glutathione was reported by Frear and Swanson (1970), and an interaction of glutathione with *N,N*-diallyl-2-chloroacetamide (CDAA) was noted in the respiration of ryegrass by Jaworski (1956). Therefore, barban and CDAA were examined in metabolism experiments similar to those described for propachlor. Both barban and CDAA produced products in excised leaves which had thin-layer chromatographic R_f values comparable to the glutathione conjugates of the 2-chloro-*s*-triazines and propachlor. The *in vitro* reaction of CDAA with glutathione frequently resulted in more than one product, and the correspondence of these products with those present in extracts of excised leaves was not conclusive. However, the *in vitro* reaction product of barban with glutathione and the principal metabolite of barban from excised leaves have the same thin-layer chromatographic R_f value. Attempts to purify this metabolite by ion-exchange chromatography were not successful; consequently the structure was not verified.

A limited ability of higher plants to detoxify certain halogenated compounds by conjugation with glutathione is indicated by this paper and by others (Frear and Swanson, 1970; Lamoureux *et al.*, 1970), but additional studies are needed to determine whether higher plants have the capacity, comparable to mammals, to detoxify a wide variety of organic halides and related compounds by this mechanism. Although mercapturic acids appear to be the most common end-products of metabolism *via* glutathione conjugation in mammals, the final products of metabolism *via* glutathione conjugation in higher plants have not been determined. Some glutathione conjugates and γ-glutamylcysteine conjugates in plants may be end-products, but in the case of propachlor and atrazine, these metabolites are transient intermediates and further studies are needed to establish the final steps in their metabolism.

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LITERATURE CITED

Booth, J., Boyland, E., Sims, P., *Biochem. J.* **79,** 516 (1961).
Boyland, E., Chasseaud, L. F., "Advances in Enzymology," Vol. 32, pp. 173–219, Interscience Publishers, New York, 1969.
Cohen, A. J., Smith, J. N., *Biochem. J.* **90,** 449 (1964).
Cox, J. D., Turner, H. S., *J. Chem. Soc.*, 3176 (1950).
Frear, D. S., Swanson, H. R., *Phytochemistry* **9,** 2123 (1970).
Henkel, H. G., *J. Gas Chromatogr.* **3,** 320 (1965).
Jaworski, E. G., *Science* **123,** 847 (1956).
Jaworski, E. G., Porter, C. A., American Chemical Society, 148th Meeting, Detroit, 1965.
Lamoureux, G. L., Shimabukuro, R. H., Swanson, H. R., Frear, D. S., J. AGR. FOOD CHEM. **18,** 81 (1970).
Porter, C. A., Jaworski, E. G., American Society of Plant Physiologists, Urbana, Ill., 1965.
Shimabukuro, R. H., J. AGR. FOOD CHEM. **15,** 557 (1967).
Technicon Chromatography Corp., Chauncey, N.Y., "Buffers for Micro-Column Amino Acid Analysis," *Res. Bull.* **10** (1962).
Wit, J. G., Leeuwangh, P., *Biochim. Biophys. Acta* **177,** 329 (1969).

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