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Herbicide-Derived Chloroazobenzene Residues: Pathway of Formation

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In soil, the chloroaniline moieties of certain phenylamide herbicides are liberated by microbial acylamidases, and are subsequently transformed by peroxidases to stable chloroazobenzene residues. The intermediate steps of this transformation were studied by allowing 4-chloroaniline or 3,4-dichloroaniline to react under steady state conditions with peroxidase and H_2O_2. The results indicated that the initial attack of peroxidase produced a free chloroanilino radical. Formation of another labile intermediate, chlorophenylhydroxylamine, was detected by the spectrum of its trisodium pentacyanoaminoferroate complex. The obtained results are consistent with a proposed pathway involving the transformation of the chloroanilines by peroxidases to chlorophenylhydroxylamines, with or without involvement of free chloroanilino radicals. The chlorophenylhydroxylamines spontaneously condense with excess chloroanilines and form chloroazobenzenes. The last step may be indirect and may involve the rapid autoxidation of the respective chlorohydrazobenzene intermediates.

The fate of certain phenylamide herbicides in soil is unusual in the respect that the initial step of their biodegradation is followed by a combination of enzymatic and chemical reactions which are synthetic rather than degradative in nature. They give rise to azobenzenes and to polyaromatic products of higher complexity. This type of transformation affects primarily the acylanilides (Bartha and Pramer, 1970), but the fate of methyl *N*-(3,4-dichlorophenyl)-carbamate (swep) is similar (Bartha and Pramer, 1969). Recent studies on ^{14}C ring-labeled compounds (Chisaka and Kearney, 1970; Bartha, 1971) demonstrated that both polymerized and nonpolymerized chloroaniline moieties have extended residual lives in soil, the latter ones apparently persisting in the form of humic complexes. Sprott and Corke (1971) observed the disappearance of low 3,3′,4,4′-tetrachloroazobenzene (3,3′,4,4′-TCAB) concentrations from some Ontario soils within a few weeks, but Kearney *et al.* (1970) detected 3,3′,4,4′-TCAB residues in rice field soils that were treated with 3′,4′-dichloropropionanilide (propanil) 2 and 3 yr prior to sampling. The weight of the evidence indicates that chloroazobenzenes and other chloroaniline transformation products (Rosen and Siewierski, 1971) are relatively persistent environmental pollutants. An understanding of their formation mechanism is basic to any attempt to prevent or reduce their production.

Peroxidases have a wide distribution in nature and occur in soil (Galstyan, 1958, 1959; Kozlov, 1964; Bartha and Bordeleau, 1969). They are specific in respect to their primary substrate (H_2O_2 or some alkylperoxides) but are capable of using a wide range of electron donors, including substituted anilines (Saunders *et al.*, 1964). The mode of action of peroxidases was studied by Chance (1949a,b, 1952) and numerous other workers (George, 1953a,b; Yamazaki *et al.*, 1960; Yamazaki and Piette, 1961). From these studies the currently accepted mechanism of peroxidase action (Figure 1) has emerged. In the presence of H_2O_2 complex I is formed very rapidly (less than 1 sec), whereas its conversion to complex II requires a longer time (Saunders *et al.*, 1964; Cormier and Prichard, 1968).

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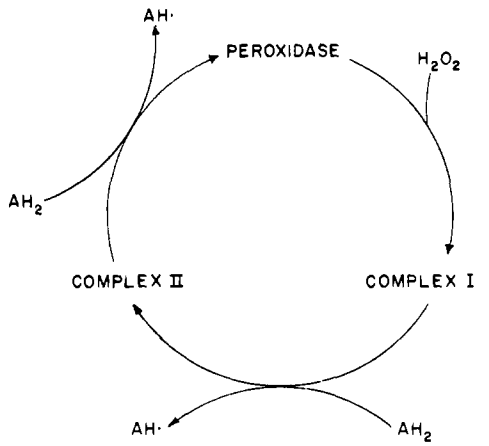

Figure 1. Currently accepted mechanism of peroxidase reaction. AH_2 is the hydrogen donor, *e.g.*, an aniline; AH· is the free radical formed

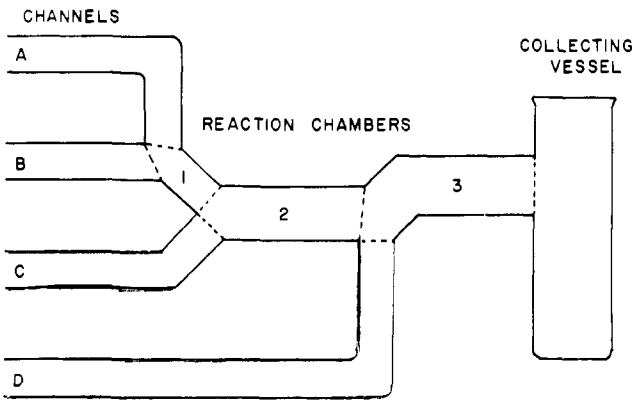

Figure 2. Diagram of a continuous flow apparatus designed for the study of steady state peroxidase reactions. For explanation see text

Studies conducted both in soil and *in vitro* implicated peroxidases as the biological agents that mediate the transformation of pesticide-derived chloroanilines to chloroazobenzenes (Bartha *et al.*, 1968; Bartha and Bordeleau, 1969). Furthermore, the involvement of labile intermediates has been established (Bordeleau and Bartha, 1970). The aims of the present study were to identify the nature of these labile intermediates and to propose a chloroazobenzene formation pathway that is consistent with experimental evidence.

EXPERIMENTAL

Chloroanilines (Aldrich Chem. Co.) were purified by distillation or by recrystallization from light petroleum ether. Symmetric azobenzenes were synthesized as reported by Linke *et al.* (1969). Asymmetric azobenzene standards were obtained by letting appropriately substituted anilines and nitrosobenzenes react overnight in glacial acetic acid (Bray *et al.*, 1957). The products of the reaction were separated by column chromatography. 4-Chlorophenylhydroxylamine and 3,4-dichlorophenylhydroxylamine were synthesized from their nitrobenzene analogs by reduction with zinc powder in the presence of ammonium chloride (Vogel, 1948) using ethanol:water (1:1 v/v) as solvent. 4-Chloronitrosobenzene and 3,4-dichloronitrosobenzene were prepared by sodium dichromate oxidation of their hydroxylamine analogs in acid medium (Fieser and Fieser, 1961). The products were purified by distillation and recrystallized from an acetone–water mixture (1:1). The homogeneity of all compounds was established by gas–liquid and thin-layer chromatography. Melting points (uncorrected): 4-chloronitrosobenzene, 91°C; 4-chlorophenylhydroxylamine, 88°C; 3,4-dichloronitrosobenzene, 46°C; 3,4-dichlorophenylhydroxylamine, 38°C. The melting points of the 4-chloro compounds matched the ones reported in the literature (Ingold, 1925; Farrow and Ingold, 1924). The authenticity of the 3,4-dichloro analogs, which are new compounds, was established by the comparison of their ir spectra with those of the respective 4-chloro analogs. Further proof of authenticity was obtained by reacting the synthesized 3,4-dichloronitrosobenzene with 3-chloro-4-methylaniline in glacial acetic acid (Bray *et al.*, 1957), and by the mass spectrometric characterization of the resulting 3,3′,4-trichloro-4′-methylazobenzene (Bartha, 1969). In the enzyme experiments, horseradish peroxidase Type II, 135 purpurogallin units/mg (Sigma Chemical Co., St. Louis, Mo.) was used.

Metabolites from the peroxidase reaction mixture were partitioned into benzene and were subjected to chromatographic and mass spectrometric analysis. Thin-layer chromatography was performed on 20 × 20 cm sheets coated with a 250-nm layer of silica gel with fluorescent indicator (Chromagram 6060, Eastman, Rochester, N.Y.). Sheets were activated 1 hr at 100°C before use. Separation of substituted anilines was achieved using benzene, and azo compounds were separated from other reaction products by hexane:benzene:acetone (7:3:1, v/v). Individual azobenzenes were resolved by ligroin (60–70°C). R_f values are listed in Table I.

Preparatory separations were performed on 30 × 2 cm columns packed with neutral aluminum oxide (Woelm, Activity II). Nonpolar solvents (ligroin, 60–70°C, or benzene) were used for development.

Gas–liquid chromatography was performed on an F&M Model 700 gas chromatograph equipped with dual flame ionization detector, temperature programmer, and an F&M Model 7127-A recorder with a disc integrator. The gas chromatographic columns were stainless steel, 1.8 m long, 3 mm o.d., packed with 10% UC-W 98 on Chromosorb W. The operational parameters for the gas chromatograph were as follows: injector temperature, 275°C; column temperature, 175°C isothermal for chloroanilines, and 250°C for chloroazobenzenes; detector temperature, 275°C; carrier gas (helium), 40 cm^3/min; hydrogen, 40 cm^3/min; oxygen, 250 cm^3/min. Before use, the columns were aged at 270°C for 48 hr with a carrier gas flow of 30 cm^3/min. Under the listed conditions the lower limits of detection for chloroanilines and chloroazobenzenes were 0.01 and 0.05 μg, respectively. Mass spectra were obtained using a Hitachi Perkin-Elmer Model RMU-7 combination gas chromatograph–mass spectrometer. High-resolution mass spectrometry was performed in the laboratories of the Morgan & Schaffer Corp., Montreal, Canada. The purpose of these experiments was to detect and identify the formation of intermediates and azo compounds as a means of defining their formation pathway, with no attempt to measure either the rate or the extent of the transformations.

For detection of short-lived labile intermediates of enzymatic chloroazobenzene production, a continuous flow system was constructed in which stoichiometric concentrations of peroxidase, H_2O_2, and substituted aniline were permitted to react at steady state. The system was modeled after those of Roughton (1953), Bray (1961), and Gibson and Milnes (1964). It is illustrated schematically in Figure 2. Channels A, B, C, and D carried to the reaction chambers solutions of

peroxidase, hydrogen peroxide, hydrogen donor, and reaction terminator, respectively. The channels consisted of 0.073 in. i.d. polyethylene manifold tubing (Technicon Instruments Corp., Tarrytown, N.Y.). Chambers 1, 2, and 3 were borosilicate glass mixing cells (Technicon) with a volume of 0.2, 0.6, and 0.8 ml, respectively. The channels were connected to the entrance arms of the chambers by transmission tubing of 0.073 in. i.d. (Technicon). The mixing cells were joined together by outer tubing joints (Technicon). Continuous flow through each chamber was obtained by using peristaltic pumps (Buchler Instruments, Fort Lee, N.J.). The flow was adjusted to the same rate in each channel. This mode of operation rendered the system capable of continuous flow operation with a constant relationship of 1:2:2 between reaction times in chambers 1, 2, and 3, respectively. In this apparatus, the steps of peroxidase reaction were induced in a sequential manner. For greater clarity the operational mode of the apparatus is summarized in Table II.

Equimolar concentrations of peroxidase and hydrogen peroxide were passed at equal flow rates through channels A and B into chamber 1, and were permitted to form Complex I. The substituted aniline was passed through channel C to the entrance of chamber 2 in which Complex II was formed and oxidation of the hydrogen donor took place. The peroxidatic reaction was stopped by introduction of the proper inhibitor through channel D into chamber 3, and products were collected and partitioned into benzene at the exit of the flow apparatus. To suit the kinetics described by Cormier and Prichard (1968), reaction time (volume of reaction chamber per flow rate) in chamber 1 was half of that allowed in chambers 2 and 3, and the former was varied from 0.2 to 4.0 sec. Deionized, distilled water was used throughout the experiments to minimize autoxidative effects of trace metals. Solutions of peroxidase, hydrogen peroxide, and substituted anilines were prepared at 10^{-4} *M* concentration, using 0.04 *M*, pH 6.0 phosphate buffer as solvent. Care was taken to prepare fresh hydrogen peroxide solutions immediately before each experiment to minimize concentration changes due to decomposition. All experiments were carried out at 25°C. In each experiment, the flow system was operated for a period of time which permitted the consumption of 1 μmol of substituted aniline. Phenylhydroxylamines were detected as their trisodium pentacyanoaminoferroate complexes. A 0.05% (w/v) solution of trisodium pentacyanoaminoferrate was prepared in 0.2 *M* phosphate buffer at pH 7.0. It was introduced into chamber 3 of the flow system. At the outflow, products were accumulated in a vessel that was maintained at 70°C, and the mixture was allowed to stand for 20 min to achieve full color development. The mixture was then cooled to 25°C and analyzed spectrophotometrically, using a Beckman Model DB-GT spectrophotometer.

RESULTS AND DISCUSSION

Evidence for Free Chloroanilino Radicals. Employing various flow rates in the described apparatus, the time required for formation of 4,4′-dichloroazobenzene (4,4′-DCAB) from 4-chloroaniline (4-CA) was determined. Trichloroacetic acid solution (4%, w/v) was introduced into chamber 3 to stop the reaction. Formation of 4,4′-DCAB was detected only when the reaction time in chambers 2 and 3 was 0.6 sec or longer. No 4,4′-DCAB was detected when either peroxidase or H_2O_2 was omitted from the system, proving that enzymatic peroxidation was indeed an essential part of the transformation.

Table I. R_f Values of Some Chloroanilines and Chloroazobenzenes

Compounds	R_f in solvent systems[a] A	B	C
4-chloroaniline		0.34	0.31
3,4-dichloroaniline		0.41	0.35
3,5-dichloroaniline		0.48	0.34
4,4′-dichloroazobenzene	0.60		0.97
3,4′,5-trichloroazobenzene	0.81		0.95
3,3′,4,4′-tetrachloroazobenzene	0.66		0.98
3,3′,5,5′-tetrachloroazobenzene	0.90		0.97

[a] Solvent systems: (A) ligroin 60–70°C; (B) benzene; (C) Hexane:benzene:acetone (7:3:1).

Table II. Mode of Peroxidatic Reaction in the Flow Apparatus

Reactants	Chamber	Sequential reaction	Relative time
Peroxidase H_2O_2	1	Peroxidase + H_2O_2 → Complex I	X
Hydrogen donor AH_2	2	Complex I + AH_2 → Complex II + AH· Complex II + AH_2 → Peroxidase + AH·	2X
Inhibitor	3	Azobenzene formation	2X

AH·, labile intermediate.

In a similar experiment the trichloroacetic acid solution used in chamber 3 to stop the reaction was supplemented with 3,5-dichloroaniline at the concentration of 10^{-4} *M*. This compound was found to resist transformation by horseradish peroxidase (Bartha *et al.*, 1968). When in chambers 2 and 3 the reaction time was 0.6 sec, both the symmetric 4,4′-DCAB and the asymmetric 3,4′,5-trichloroazobenzene were detected. At longer reaction times production of 4,4′-DCAB increased, while that of 3,4′,5-trichloroazobenzene decreased. In neither case was any 3,3′,5,5′-tetrachloroazobenzene detected. These results indicated that the labile intermediate formed during peroxidatic oxidation of 4-CA in chamber 2 was present in chamber 3 at the time when the asymmetric azo compound was produced. It had to be present in a form which permitted its fast reaction with an unchanged aniline. In the flow system used, the proper conditions for such a reaction sequence were met when the flow of each reactant was 0.33 ml/sec. This setting yielded flow rates of 0.66, 0.99, and 1.32 ml/sec in the reaction chambers 1, 2, and 3, respectively. The corresponding reaction times were 0.3, 0.6, and 0.6 sec. These conditions of operation were kept constant in the subsequent experiments.

Since free radicals generated from substrates by peroxidase have been reported (Yamazaki *et al.*, 1960; Yamazaki and Piette, 1961; Cormier and Prichard, 1968), it was reasonable to search for corresponding anilino radicals generated by the peroxidatic oxidation of chloroanilines. The antioxidant 2-(*tert*-butyl)-4-methylphenol was used in an attempt to trap the labile intermediate by formation of a derivative. The compound was used in 10^{-4} *M* concentration in a phosphate buffer solution and was introduced into chamber 3 of the flow system. Other parameters of operation were as stated before. In this semiquantitative experiment, the flow apparatus was operated for a period of 33 sec, permitting the consumption of 1 μmol of each reactant. Gas chromatographic analysis of the products showed that about 25% of the 4-CA had not been oxidized. The amount of 4,4′-DCAB detected accounted for 10% of the amount of 4-CA that had been introduced into the system. Since all products of chloroaniline transformation

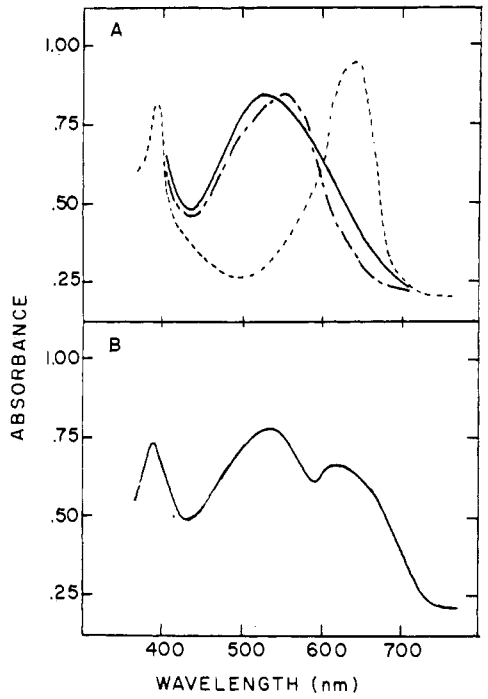

Figure 3. Absorption spectra of trisodium pentacyanoaminoferroate complexes of A: 3,4-dichloroaniline (- - -), 3,4-dichlorophenylhydroxylamine (—), 3,4-dichloronitrosobenzene (— — —); and B: spectrum of product complexes in the peroxidase reaction mixture. Reagent blanks were used in the reference cell

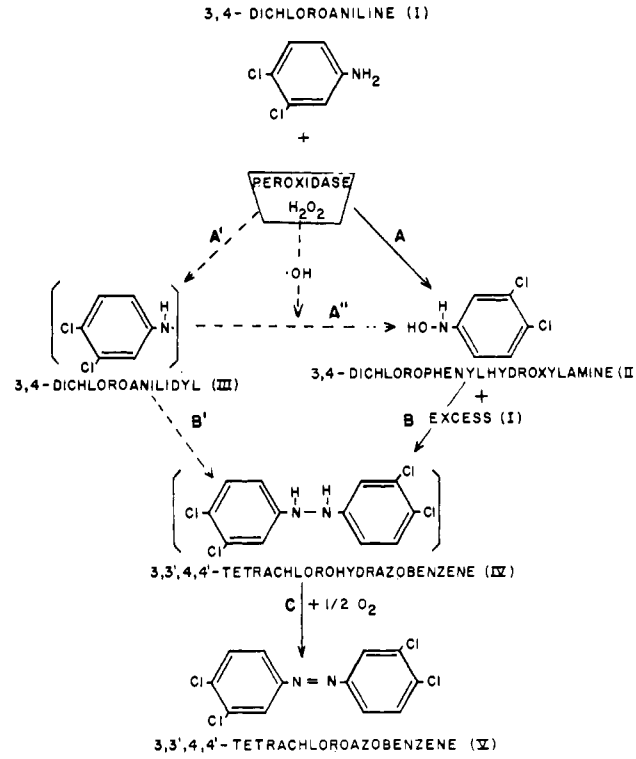

Figure 4. Proposed pathway of 3,3',4,4'-tetrachloroazobenzene formation. For explanation see text

could not be detected, a balance was not obtained. However, a new compound that differed from any of the reactants or the known reaction products appeared. Its gas chromatographic retention time was 420–430 sec. This new compound, hereafter referred to as X, was chromatographed on silica gel thin-layer sheets. When developed with benzene it moved as a single spot with an R_f value of 0.66, and was separated completely from other reaction products.

Compound X was purified by repeated column chromatography using benzene as developing solvent. Fractions containing X were combined and concentrated on a rotary evaporator. Attempts to crystallize it had failed, and it was isolated as an oil. The purified compound exhibited a mass spectrum with a parent ion at *m/e* 326; chlorine was not present. The isotopic analysis by high-resolution mass spectrometry indicated the molecular formula to be $C_{22}H_{30}O_2$. This suggested that two 2-(*tert*-butyl)-4-methylphenoxy radicals had coupled to form compound X. No further attempt was made to characterize this dimer. The antioxidant failed to trap the chloroanilino radical that was suspected of arising from the peroxidatic oxidation of 4-CA.

Another attempt, using 3,4-dichloroaniline (3,4-DCA) as hydrogen donor, was made under the same experimental conditions. Again, part of the 3,4-DCA was oxidized to 3,3',4,4'-tetrachloroazobenzene (3,3',4,4'-TCAB) with the simultaneous oxidative dimerization of the antioxidant.

Oxidative dimerization of 2-(*tert*-butyl)-4-methylphenol is known to occur when the compound is attacked by a free radical (Fieser and Fieser, 1961). Therefore, the presence of free radicals in the flow system can be inferred from the ability of the mixture to initiate the polymerization of the antioxidant. Furthermore, since dimer X was produced only in the complete reaction system, chloroanilino radicals appear to be the initiators of the dimerization. No dimer was produced when any of the reactants (peroxidase, hydrogen peroxide, or chloroaniline) were omitted from the system. This also proved that hydrogen peroxide, subject to homolysis of the weak O–O bond, did not initiate the dimerization reaction. Since it has been reported that amino radicals of the type R–NH· act as polymerization initiators (Walling, 1957), we believe that the chloroanilino radical was the initiator in the present case. Whether or not chloroanilino radical was generated directly by an initial abstraction of one amino hydrogen atom as a result of peroxidatic oxidation of chloroaniline, or indirectly by homolysis of 4,4'-dichlorohydrazobenzene, is unknown. The latter compound was probably present in the reaction mixture, since it was suggested (Holland and Saunders, 1968) as an intermediate in the formation of chloroazobenzene.

Evidence for Chlorophenylhydroxylamine Intermediates. By indirect evidence, earlier experiments (Bordeleau and Bartha, 1970) suggested the possible participation of phenylhydroxylamine analogs in the chloroazobenzene formation pathway. Direct proof was sought using the flow system under the previously described conditions, but with the substitution of trisodium pentacyanoaminoferrate solution in chamber 3 for trichloroacetic acid. Separate tests established that this agent not only formed complexes with arylhydroxylamines, as reported by Boyland and Nery (1964), but also served as reaction terminator by inhibiting peroxidase. Figure 3 shows the results of the experiment in which 3,4-DCA was used as the hydrogen donor. Nitrosobenzene may interfere with the determination of phenylhydroxylamine, but aniline and other aniline derivatives do not. The absorption spectra of reaction product complexes were compared with those of 3,4-DCA, 3,4-dichlorophenylhydroxylamine, and 3,4-dichloronitrosobenzene. The results indicate the presence in the reaction mixture of either 3,4-dichlorophenylhydroxyl-

amine or 3,4-dichloronitrosobenzene, or both. Similar results were obtained when 4-CA served as hydrogen donor for peroxidase. As previous experiments demonstrated that in soil or in aqueous reaction mixtures chloronitrosobenzenes were not the prime precursors of chloroazobenzene formation (Bordeleau and Bartha, 1970), the intermediates in question could be narrowed down to the respective chlorophenylhydroxylamines.

More than one mechanism can be visualized for the peroxidase-mediated production of chlorophenylhydroxylamines from chloroanilines, and the available evidence does not allow a clear choice between these possibilities. Peroxidases have been reported to act also as mixed function oxidases (Dure and Cormier, 1964; Saunders *et al.*, 1964; Evans, 1970; Siegel and Siegel, 1970; Thomas *et al.*, 1970a,b). Consequently, chlorophenylhydroxylamines may arise from direct enzymatic oxidation by abstraction of a hydrogen atom and the attachment of a hydroxy radical to the chloroaniline. The hydroxy radical is supplied by the peroxidase–hydrogen peroxide complex (Chance, 1952); thus the process is completely enzymatic. Another possible route of chlorophenylhydroxylamine formation is the nonenzymatic coupling of hydroxy and chloroanilino radicals. In this case, the enzyme action is required only for the formation of the chloroanilino radical, while hydroxy radical may be generated by the homolysis of hydrogen peroxide. It is less likely that the hydroxy radical is generated by the chloroanilino radical's attack on water, since the H–OH bond (119 kcal/mol) of the latter is much stronger than the HO–OH bond (51 kcal/mol) of the hydrogen peroxide (Kerr and Trotman-Dickenson, 1968–69).

Possible Role of Chlorohydrazobenzenes. The involvement of chlorohydrazobenzenes as intermediates of chloroazobenzene formation has been suggested (Saunders *et al.*, 1964; Holland and Saunders, 1968). When 3,3′,4,4′-tetrachlorohydrazobenzene was incubated in soil, this compound was rapidly transformed to its corresponding chloroazobenzene (Bordeleau *et al.*, 1969). Furthermore, in phosphate buffer solutions, 3,3′,4,4′-tetrachlorohydrazobenzene was quantitatively transformed within minutes to 3,3′,4,4′-TCAB, indicating the great susceptibility of the former compound to undergo autoxidation. Because of the required presence of H_2O_2, autoxidation of 3,3′,4,4′-tetrachlorohydrazobenzene could not be prevented in the flow system. Although theoretical considerations and indirect evidence strongly suggest the existence of chlorohydrazobenzene intermediates, attempts to obtain direct proof of their involvement were unsuccessful.

Proposed Pathway of Chloroazobenzene Formation. Current evidence for the biochemically mediated transformation of chloroanilines to chloroazobenzenes is consistent with the sequence of events illustrated by Figure 4. The pathways of 4-CA and 3,4-DCA transformation are analogous, and only the latter is illustrated. Intermediates that are postulated on the basis of indirect evidence are enclosed in brackets, and all compounds have been assigned Roman numerals to expedite the discussion. The main pathway is indicated by solid arrows.

The peroxidatic oxidation of 3,4-DCA (I) produces compounds II and III, *via* reactions A and A′, respectively. Reaction A″ is also a possible route for the formation of II. Compounds I and II react together to generate 3,3′,4,4′-tetrachlorohydrazobenzene (reaction B, compound IV). Compound IV may also arise from dimerization of two radicals (III) *via* reaction B′. Whichever way it is formed, compound IV undergoes autoxidation (reaction C) to 3,3′,4,4′-TCAB (V). Since the concentration of radicals in solution is much less than the concentration to other reactants (Gould, 1959), it might therefore be expected that the yields of product IV resulting from reaction B′ would be negligible in comparison to the yields of IV *via* reactions A″ and B. Similarly, reaction A is considered to predominate over reaction A′. Following the same line of reasoning, and considering the results of the asymmetric azobenzene formation experiment in that the unchanged 3,5-DCA failed to give the corresponding azobenzene, it is logical to propose that formation of azobenzene results from interaction of compound II with excess aniline (I). Thus the main pathway proceeds by the reactions A, B, and C. This route also accounts for the formation of an asymmetric azobenzene when two differently substituted anilines are incubated simultaneously in biological media (Bartha, 1969; Kearney *et al.*, 1969; Bordeleau and Bartha, 1970).

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Fate of Formothion on Bean Plants in the Greenhouse

Horst H. Sauer

The fate of formothion is studied on bean plants following foliar application. In general, the distribution pattern between the vapor phase, surface, and subsurface area is similar to that of dimethoate. The half-life of formothion breakdown amounts to 1.2 days. Hydrolytic attack causes rapid formothion degradation to approximately equal amounts of dimethoate and *O,O*-dimethyl dithiophosphorylacetic acid. Further breakdown products are dimethoxon, *O,O*-dimethyldithiophosphoric acid, and bis(*O,O*-dimethylthiophosphoryl) disulfide. Only dimethoate and dimethoxon are insecticidally active metabolites. Although their residual amounts are considerably lower after application of Anthio than of dimethoate, and although formothion itself dissipates very fast, the insecticidal efficacy of Anthio is equal to dimethoate. It is concluded that the initial biological action after formothion treatment is caused by formothion itself. The long-term efficacy, however, is generated by potentiation of the insecticidal activity of the Anthio metabolites dimethoate and possibly dimethoxon by synergistic action of *O,O*-dimethyldithiophosphorylacetic acid and bis-(*O,O*-dimethylthiophosphoryl) disulfide.

Anthio is one of the least toxic systemic organophosphorous insecticides with an LD_{50} of 370–400 mg/kg (Klotzsche, 1966). By systemic and contact action it controls a wide range of sucking, mining, and some biting pests on various crops. Its active ingredient is formothion [*O,O*-dimethyl *S*-(*N*-methyl *N*-formylcarbamoylmethyl) phosphorodithioate], which is closely related in its molecular structure to dimethoate. Comparative biological trials in the field under conditions of good agricultural practice as well as in the greenhouse revealed almost identical data of performance of both formothion and dimethoate (Staub, 1964; Wood and Tyson, 1965; Almeida and Cavalcante, 1966; Damiano, 1967; Thompson, 1967; Bassand and Klotzsche, 1970; Jalloul, 1968). Residue investigations, however, analyzing for formothion, dimethoate, and dimethoxon, yielded consistently lower residues in plants treated with formothion (van Hoek, 1966, 1967). Therefore the following study was undertaken to determine quantitatively the fate of formothion applied to bean plants under semi-controlled conditions in comparison to dimethoate. Its aim was to search for an explanation for the almost identical insecticidal action of both formothion and dimethoate, based upon their residual behavior.

MATERIALS AND METHODS

Syntheses of Radio-Labeled Compounds. FORMOTHION-*carbonyl*-^{14}C. To prepare formothion-*carbonyl*-^{14}C, ^{14}C-$BaCO_3$ was treated with concentrated H_2SO_4. The evoluting ^{14}C-CO_2 was converted to ^{14}C-carboxyl acetic acid by a Grignard reaction with CH_3MgI in ether. After dilution with unlabeled acetic acid, bromine was added to yield ^{14}C-carboxylbromoacetic acid, followed by conversion into the corresponding acid chloride by adding phthaloylchloride. The acid chloride was dissolved in trichloroethylene and refluxed with *N*-methylformamide for 2 hr. The solvent was removed at 50°C in vacuum. The crude ^{14}C-bromoacetic acid *N*-methylformamide was dissolved in dioxane and reacted with the sodium salt of dimethylphosphorodithioic acid in water. Purification was done by silica gel column chromatography using ethyl acetate as eluting solvent. The total yield based upon ^{14}C-$BaCO_3$ was 50%. Radiochemical purity was 100% and specific activity was 5.53 mCi/mmol. The material was stored in benzene at 5°C.

Purity of the described labeled compounds was determined by tlc chromatography on silica gel G with ethyl acetate. Visualization of the compounds was done by spraying with potassium iodoplatinate or by treatment with I_2 vapor. Radiochemical purity was determined by tlc–radioscanning on a Berthold Scanner No. 2 (Berthold Frieseke GmbH, 75 Karlsruhe-Durlach, Germany) and by scratching off the silica gel layer in 0.5-cm zones which were transferred into counting vials, extracted with the scintillator solution, and counted as described later.

FORMOTHION-*methoxyl*-^{14}C. ^{14}C-Methanol obtained from ^{14}C-$BaCO_3$ (specific activity 54 mCi/mmol) by reduction of liberated ^{14}C-CO_2 with $LiAlH_4$ in tetrahydrofurfuryloxytetrahydropyrane was reacted with P_2S_5 in shellsol R for 2 hr at 60°C and an additional 2 hr at 80°C. Gaseous reaction products (H_2S) were removed in vacuum. The remaining solution was diluted with benzene and extracted with 1 *N* NH_4OH to yield the ammonium salt ^{14}C-dimethylphosphorodithioic acid. The synthesis of formothion-*methoxyl*-^{14}C was completed by reacting an aqueous solution of the ammonium salt with chloroacetic acid *N*-methylformamide in dioxane for 3 hr at 35°C. Water-soluble by-products were removed from the reaction mixture dissolved in benzene by partition into 2 *N* $KHCO_3$. Purification of the material diluted with unlabeled carrier was done as described for

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