Handbook; Stahl, E., Ed.; Academic: New York, 1965.
Culter, H. G. "A Fresh Look at the Wheat Coleoptile Bioassay". Proceedings of the 11th Annual Meeting Plant Growth Regulator Society of America, 1984; pp 1-9.
Delcambe, L.; Peypoux, F.; Guinand, M.; Michel, G. "Iturin and Iturinic Acids". *Rev. Ferment. Ind. Aliment.* **1976**, *31*(6), 147-51.
Doddrell, D.; Pegg, T.; Bendall, M. R. "Distortionless Enhancement of NMR Signals by Polarization Transfer". *J. Magn. Reson.* **1982**, *48*, 323.
Frank, H.; Woiwode, W.; Nicholson, G. "Determination of the Rate of Acidic Catalyed Racemization of Protein Amino Acids". *Bayer Liebigs Ann. Chem.* **1981**, 354.
Garbay-Jaureguiberry, C.; Roques, B. P.; Delcambe, L.; Peypoux, F.; Michel, G. "NMR Conformational Study of Iturin A, An Antibiotic from *Bacillus subtilis*". *FEBS Lett.* **1978**, *93*, 151.
Isogai, A.; Takayama, S.; Murakoshi, S.; Suzuki, A. "Structures of β-Amino Acids in Iturin A". *Tetrahedron Lett.* **1982**, *23*, 3065-3068.
Korzybski, T.; Kowszyk-Gindifer, Z.; Kurylowicz, W. "Section C: Antibiotics Isolated from the Genus *Bacillus* (*Bacillaceae*)". In *Antiobiotics-Origin, Nature and Properties*; American Society for Microbiology: Washington, DC 1978; Vol. III.
McKeen, C.; Reilly, C.; Pusey, P. "Production and Partial Characterization of Antifungal Substances Antagonistic to *Monilinia fructicola* from *Bacillus subtilis*". *Phytopathology* **1986**, *76*, 136-139.
Nagai, U.; Besson, F.; Peypoux, F. "Absolute Configuration of an Iturinic Acid as Determined by CD Spectrum of its DNP-P-Methoxyanilides". *Tetrahedron Lett.* **1979**, 2359-2360.
Peypoux, F.; Guinaud, M.; Michel, G.; Delcambe, L.; Das, B.; Lederer, E. "Structure of Iturine A, a Peptidolipid Antibiotic from *Bacillus subtilis*". *Biochemistry* **1978**, *17*(19), 3992-3996.
Pusey, P. L.; Wilson, C. L. "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis*". *Plant Dis.* **1984**, *68*(9), 753-756.
Quentin, M. J.; Besson, F.; Peypoux, F.; Michel, G. "Action of Peptidolipidic Antibiotics of the Iturin Group On Erythrocytes: Effects of Some Lipids on Hemolysis". *Biochim. Biophys. Acta* **1982**, *684*, 207-211.
Wilson, C. L.; Pusey, P. L. "Potential for Biological Control of Post Harvest Plant Diseases". *Plant Dis.* **1985**, *69*(5), 375-378.
Zehr, E. I. "Control of Brown Rot in Peach Orchards". *Plant Dis.* **1982**, *66*, 1101-1105.

Received for review June 15, 1987. Accepted October 23, 1987. Mention of firm names does not imply endorsement by the U.S. Department of Agriculture of the products. No discrimination or preference is intended. The use of firm names and products is for identification only.

Metabolism of Pentachlorophenol in Cell Suspension Cultures of Wheat (*Triticum aestivum* L.). Tetrachlorocatechol as a Primary Metabolite

Wolfgang Schäfer[1] and Heinrich Sandermann, Jr.*[2]

Wheat cell suspension cultures were incubated with [U-^{14}C]pentachlorophenol (PCP; 1 ppm, 48 h, 27 °C). Soluble metabolites were formed in ~50% yield, another ~31% of the applied radioactivity being incorporated into the "insoluble" residue. The soluble metabolite fraction, and its β-D-glucoside conjugate components, the total "insoluble" residue, and its lignin components, were all found to contain a novel polar PCP derivative besides smaller amounts of tetrachlorohydroquinone and PCP. The novel derivative also predominated in intact wheat plants and was identified as tetrachlorocatechol by TLC, HPLC, GC, and EI as well as CI mass spectroscopy. Tetrachlorocatechol is a potential mutagen, so that the soluble and "insoluble" conjugates formed in wheat from PCP may present a toxicological hazard.

Pesticide metabolism in plants often leads to significant incorporation of the radioactive label into the insoluble residue fraction. The lignin component of "insoluble" plant cell wall fractions has been recognized to be a major site of covalent binding, as demonstrated for 2,4-D (Scheel and Sandermann, 1981), benzo[*a*]pyrene quinones (Trenck and Sandermann, 1981), chlorinated anilines (Trenck et al., 1981; Arjmand and Sandermann, 1985a, 1986), and pentachlorophenol (PCP; Scheel et al., 1984). A number of additional reports on the lignin incorporation of pesticide chemicals exist (Baldwin, 1977; Roberts, 1984). However, these studies failed to establish the covalent nature of binding and/or to exclude labeling by refixation of radioactive carbon dioxide [see Sandermann et al. (1983)].

A stepwise approach has been suggested for the toxicological evaluation of plant insoluble residues (Huber and Otto, 1983; Kovacz, 1986). The nature of the bound radioactive material should be elucidated for residues corresponding to more than 10% of the applied radioactive pesticide (Huber and Otto, 1983; Kovacz, 1986). Pentachlorophenol has previously been shown to be incorporated to 37% into the insoluble residue of cultured wheat cells, the lignin cell wall component representing a major binding site (Sandermann et al., 1984; Scheel et al., 1984). High rates of incorporation into the insoluble residue have also been observed in parallel studies with wheat cell cultures (Langebartels and Harms, 1984; Harms and Langebartels, 1986; Schuphan et al. 1984) and in whole plant studies employing [U-^{14}C]pentachlorophenol, e.g. in rice (Haque et al., 1978; Weiss et al., 1982), corn (Cole and Metcalf, 1977), alfalfa and rye (Gile and Gillett, 1979), and wheat

Institut fuer Biochemische Pflanzenpathologie, Gesellschaft fuer Strahlen- und Umweltforschung mbH Muenchen, Ingolstaedter Landstrasse 1, D-8042 Neuherberg, FRG, and Institut fuer Biologie II, Universitaet Freiburg, D-7800 Freiburg, FRG.
[1] Present address: Universitaetsfrauenklinik, Endokrinologisches Labor, Universitaet Freiburg.
[2] Address correspondence to this author at the Institut fuer Biochemische Pflanzenpathologie.

0021-8561/88/1436-0370$01.50/0

(Langebartels and Harms, 1985; Langebartels et al., 1986). The purpose of the present study was to elucidate the mechanism for incorporation of pentachlorophenol into the insoluble metabolite fraction of wheat cells, studying the soluble conjugates for comparison. Pentachlorophenol was only to a low degree incorporated as such. Incorporation into lignin and other PCP metabolites of wheat appeared to proceed mainly via the primary metabolite, tetrachlorocatechol, a potential mutagen.

EXPERIMENTAL SECTION

Materials. Wheat cell cultures were grown and incubated as previously described (Sandermann et al., 1984; Scheel et al., 1984). PCP-β-D-glucoside was synthesized by a previous procedure (Schmitt et al., 1985). Analytical-grade *o*- and *p*-chloranil (2,3,4,5-tetrachloro-*o*-benzoquinone and 2,3,5,6-tetrachloro-*p*-benzoquinone, respectively) were purchased from Fluka, Neu-Ulm, FRG. Tetrachlorocatechol (192–193 °C) was synthesized by a published procedure (Rott et al., 1979). Tetrachlorohydroquinone (235–237 °C) was synthesized by the same procedure. PCP-acetate (144–148 °C), tetrachlorocatechol diacetate (188–192 °C), and tetrachlorohydroquinone diacetate (not determined) were synthesized by a standard acetylation procedure (Arjmand and Sandermann, 1985b; Rott et al., 1979). [U-^{14}C]PCP (30–40 mCi/mmol) was supplied by Amersham-Buchler, Braunschweig, FRG, or CEA, Gif-sur-Yvette, France. For radiochemical synthesis of tetrachlorocatechol, a published procedure (Deichmann and Schäfer, 1942) was adopted as follows: [U-^{14}C]PCP (6.6 mg, 10 μCi) was dissolved in 1 mL of 0.1 N NaOH, the resultant mixture was cooled in ice, and 5 mL of fuming HNO_3 (Merck No. 450) was added. After 20 min 100 mL water was added to the dark red solution, followed by extraction with diethyl ether and drying of the organic phase with Na_2SO_4. TLC in solvent system A and HPLC showed that PCP had been converted 95% to a 3:1 mixture of *o*- and *p*-chloranil. The crude product was used for lignin copolymerization and also for synthesis of [U-^{14}C]tetrachlorocatechol/hydroquinone by adoption of a published procedure (Rott et al., 1979). A portion of the oxidation product (3 mg) was dissolved in 0.5 mL of ethanol, and 5 mg of $NaBH_4$ was added. After addition of 5 mL of water and acidification with HCl the product was extracted with diethyl ether. TLC in solvent system A showed tetrachlorocatechol and tetrachlorohydroquinone to be present in a ratio of about 3:1. This material was employed for copolymerization into lignin.

[U-^{14}C]Pentachlorothiophenol was synthesized by adoption of a published procedure (Renner and Nguyen, 1981), working under a nitrogen atmosphere. $NaHS \cdot H_2O$ (22 mg) was dissolved at 50 °C in 0.2 mL of ethylene glycol and the resultant mixture added to a boiling solution of [U-^{14}C]hexachlorobenzene (38.4 mg 10 μCi) in 0.8 mL of pyridine. After refluxing for 5 min, the reaction was stopped by cooling and addition of 3.4 mL of water. The precipitate was removed by centrifugation. Fuming HCl (0.6 mL) was added. The resulting white precipitate was thoroughly washed with oxygen-free water and acetone. The dried reaction product (yield 62%) was characterized in solvent system B (R_f 0.24, R_f(disulfide) 0.73). The melting point of the final product was determined after a nonradioactive synthesis to be 230–236 °C (lit. mp 238–239 °C; Renner and Nguyen, 1981).

Chromatographic and General Methods. TLC was performed on precoated silica gel G plates (Merck No. 5554) using the following solvent systems: (A) *n*-hexane/diethyl ether/HCOOH, 70:30:4 (v/v/v); (B) ethyl acetate/acetic acid/water, 18:1:1 (v/v/v). Detection of compounds and of radioactivity as well as other general methods was as previously described (Scheel et al., 1984; Arjmand and Sandermann, 1985a, b, 1986).

The HPLC separation of cleavage products prepared from the soluble metabolite or the Björkman lignin fraction was performed with the previously described instruments (Arjmand and Sanderman, 1985a, 1986). A Lichrosorb Si 60 column (250 × 4.6 mm) was used for isocratic development with *n*-hexane/propanol-2, 9:1 (v/v; 1.5 mL/min).

For the GC separation of the same cleavage products a Packard Model 437 gas chromatograph with attached radiodetector or, alternatively, flame ionization detector was used with a packed SE-30 column (2 m × 3 mm). The injector and detector temperatures were 250 °C, and the column was held at a constant temperature of 150 °C. Helium at 70 mL/min served as the carrier gas.

Isolation of Soluble Conjugate Metabolites. For small-scale analytical work the previous extraction with chloroform/methanol and purification procedures (Scheel et al., 1984; Schmitt et al., 1985) were employed. For large-scale isolation of soluble metabolites another method (Arjmand and Sandermann, 1985b) was adopted as follows. Two 2-L flasks containing 400 mL of wheat cell suspension cultures (68 g of cellular wet weight each) were incubated with 1 ppm [U-^{14}C]PCP (8 μCi; 48 h, 27 °C). The cells were harvested by filtration and suspended in 1 L of 80% aqueous acetone at 0 °C. Portions (250 mL) of the cell suspension were sonicated (Branson Sonifier, Model 1312, microtip B 12, 6 min). Cell debris (insoluble fraction) were isolated by filtration. The bound radioactivity associated with this fraction (31.6% of applied radioactivity) was determined by combustion as described (Scheel and Sandermann, 1981). The cellular aqueous acetone filtrates (52.5% of applied radioactivity) were partitioned with *n*-hexane (600 mL). The *n*-hexane phase was discarded since it contained only 0.7% of the applied radioactivity. The pH was adjusted to 3.0, followed by extraction with two 400-mL portions of ethyl acetate. The organic phase contained 25.7% of the applied radioactivity. The aqueous phase was further extracted with 250 mL of butanol-1, resulting in the extraction of another 20.1% of the applied radioactivity. TLC analysis (solvent system B) of the ethyl acetate and butanol-1 extracts showed practically identical profiles. Of the radioactivity 48% migrated as a single peak with R_f 0.29, close to the PCP-β-D-glucoside standard (R_f 0.32). The remaining radioactivity was retained near the origin (R_f <0.06). Further studies were carried out with the ethyl acetate extract.

Isolation of Björkman Lignin Metabolites. Wheat cells (330 g wet weight) were incubated for 48 h with 1 ppm [U-^{14}C]PCP, harvested by filtration, and washed with 600 mL of water. The cells were lyophilized, and the dried material (48 g) was subjected to the previous extraction and grinding sequence (Scheel et al., 1984). The purified Björkman lignin metabolite fraction was dissolved in 5 mL of dimethylformamide for further study. Where indicated, the final residue, which was not solubilized by 90% aqueous dioxane, was also further studied.

Cleavage of Soluble Conjugates. β-Glucosidase digestion was performed on the total soluble metabolite fraction following the published protocol (Schmitt et al., 1985).

The more detailed characterization of cleavage products started from the cellular ethyl acetate extract. This solution was brought to dryness (40 °C) and dissolved in 0.5 mL of water, adjusting the pH value to ~8.0 by addition of 2 N NaOH. The aqueous solution was applied to a column (0.4 × 2.3 cm) of DEAE–Sephacel in water

(Pharmacia, Freiburg; acetate form). Elution was first with 10 mL of water and then with a linear gradient between 0 and 1 M aqueous ammonium acetate (total of 30 mL). Fractions of 0.5 mL were collected. Most of the radioactivity (75%) appeared as a broad peak between fractions 42 to 74. These fractions were combined and hydrolyzed (4 N HCl, 100 °C, 2 h). The hydrolyzate was lyophilized and immediately acetylated by the previous procedure (Arjmand and Sandermann, 1985b), followed by removal of reagents under vacuum. The residue was dissolved in ethyl acetate and fractionated by preparative TLC in solvent system A. The major peak of radioactivity (85% of applied radioactivity) appeared at R_f 0.30 and cochromatographed with authentic tetrachlorocatechol diacetate. This material was eluted with ethyl acetate.

Cleavage of Björkman Lignin Metabolites. Portions of the Björkman lignin metabolites (60 mg in 3 mL of dimethylformamide) were treated with 1 mL of 47% HBr in acetic acid in sealed tubes (150 °C, 1 h; Scheel et al., 1984). Reagents were removed under vacuum at <40 °C. The residue was taken up in 15 mL of water, and 4 N HCl was added to give pH 1–2. This solution was extracted with six 15-mL portions of diethyl ether. The organic extract was dried with Na_2SO_4 and then brought to complete dryness at <40 °C, followed by acetylation and preparative TLC as described above for cleavage of soluble conjugates. About half of the applied radioactivity remained at the origin and was not further studied. The remaining radioactivity appeared in the area of the tetrachlorocatechol diacetate standard (R_f 0.30). This material was eluted with ethyl acetate. Further purification of the cleavage product was by HPLC and GC as described under Results.

Enzymatic Preparation of Lignin Copolymers. The lignin copolymers were prepared on the basis of a previously described procedure (Trenck and Sandermann, 1981; Trenck et al., 1981). Some modifications had to be introduced in order to meet the specific demands of the investigated xenobiotic for an optimal incorporation. The reaction media usually consisted of 50 mM sodium phosphate buffer, pH 7.5. In the case of 2,4-D a series of preliminary experiments showed highest incorporation at pH 5.5 (data not shown). Since a solution of *o*- and *p*-chloranil in phosphate buffer darkened within seconds, the reaction was in this case carried out in 5% aqueous acetone. The reaction media were depleted of oxygen by vacuum filtration and bubbling of nitrogen gas for 60 min. In a typical experiment, solutions of (a) 1 g (5.55 mmol) of coniferyl alcohol and 26 mg of horseradish peroxidase (Sigma No. P 8250), (b) 0.63 mL (5.55 mmol) of 30% hydrogen peroxide, and (c) 5.55 mmol (1–10 μCi) of U-^{14}C-labeled test chemical, in a solution of 240 mL in each case, were pumped simultaneously and separately into a well-stirred solution of (d) 8.8 mg of horseradish peroxidase and 17.6 mg (0.11 mmol) of vanillyl alcohol in 120 mL of reaction medium.

In the copolymerization with PCP and *o*/*p*-chloranil solutions, solutions (c) and (d) were united in order to maintain a high substrate concentration from the beginning of the reaction.

The pumping time usually was 1 h, allowing 1 or 2 h more to achieve a higher degree of polymerization. Parallel control experiments omitting solution (a) were carried out in order to check for non-lignin polymer products (analysis by TLC as well as gel permeation chromatography; see Results).

In the case of pentachlorothiophenol, a two-step procedure (Trenck et al., 1981) had to be applied since the thiol was rapidly oxidized by H_2O_2. The following solutions were prepared: (A) 150 mg (0.83 mmol) of coniferyl alcohol and 3.9 mg of peroxidase in 36 mL of reaction medium (containing 1.8 mL of acetone); (B) 0.83 mmol of 30% H_2O_2 in 36 mL of reaction medium; (C) 0.087 mmol (5.9 μCi) of U-^{14}C-labeled pentachlorothiophenol in 12 mL of dioxane; (D) 1.35 mg of peroxidase and 2.7 mg of vanillyl alcohol in 42 mL of reaction medium. At first solutions (A) and (B) were pumped into solution (D) within 8 min. After another 4 min the remaining H_2O_2 was decomposed by addition of excess catalase (Sigma No. C-10; 20.000 U). Subsequently, solution (C) was added within 30 s, and the mixture was stirred for another 90 min.

All lignin copolymers prepared were isolated by centrifugation and thoroughly washed as described (Trenck and Sandermann, 1981; Trenck et al., 1981). The samples were then lyophilized, dissolved in dimethylformamide, and fractionated by gel permeation chromatography on Sephadex LH-60 as described (Trenck and Sandermann, 1981; Trenck et al., 1981).

Oxidation of PCP. The following published methods of oxidation were used: (i) a Fenton-type reagent consisting of $H_2O_2/FeCl_2$/ADP, using 1.3 μM [U-^{14}C]PCP (Floyd et al., 1984); (ii) horseradish peroxidase/H_2O_2 (Öberg and Paul, 1985) using 75 μM [U-^{14}C]PCP with inclusion of 10% (v/v) ethanol in the assay buffer. Aliquots from the incubation mixtures were directly used for TLC in solvent system A.

RESULTS

Isolation and Cleavage of Soluble PCP Metabolites. Wheat cell suspension cultures were incubated with [*ring*-U-^{14}C]PCP. Soluble as well as insoluble cell fractions were isolated by adoption of previous procedures (Sandermann et al., 1984; Scheel et al., 1984). The cellular fractionation procedure is summarized in the flow scheme shown in Figure 1.

The insoluble residue contained 31.6% of the applied radioactivity, 7.3% being present in the growth medium. TLC of the cell extract and of the growth medium showed that free PCP had nearly completely disappeared. The initial cellular acetone/water extract contained 52.5% of the applied radioactivity. On the basis of β-glucosidase digestion [cf. Scheel et al. (1984)], β-D-glucosides were the major metabolite fraction (about 50% of the soluble metabolite fraction). Another 45% of the soluble metabolite fraction appeared as a peak at R_f 0.03 (solvent system B). This material has not been characterized. The presence of β-D-glucosides was further indicated by the following evidence: Upon HPLC, the acetylated soluble metabolite fraction appeared to about 80% as a sharp peak with a retention time of 16.8 min. This retention time was similar to that of authentic PCP-β-D-glucoside tetraacetate (17 min, determined in independent experiments). The EI mass spectrum of the HPLC peak material had fragments at *m*/*z* 109, 127, 169, 271, and 331. These mass values are typical for hexoside conjugates (Frear et al., 1983; Schmitt et al., 1985). In addition, a peak at *m*/*z* 248 was present that on the basis of the peak fine pattern apparently originated from the tetrachlorocatechol aglycon. This mass fragment was also present in the EI mass spectrum of authentic acetylated tetrachlorocatechol (see below). A distinct minor metabolite (1.3% of the initial radioactivity) occurred in the growth medium and had R_f 0.58 upon TLC in solvent system B. This metabolite has not been further characterized.

The soluble metabolite fraction released the previously described aglycons (Scheel et al., 1984) upon digestion with β-glucosidase or acid hydrolysis (1 N HCl, 100 °C, 3 h)

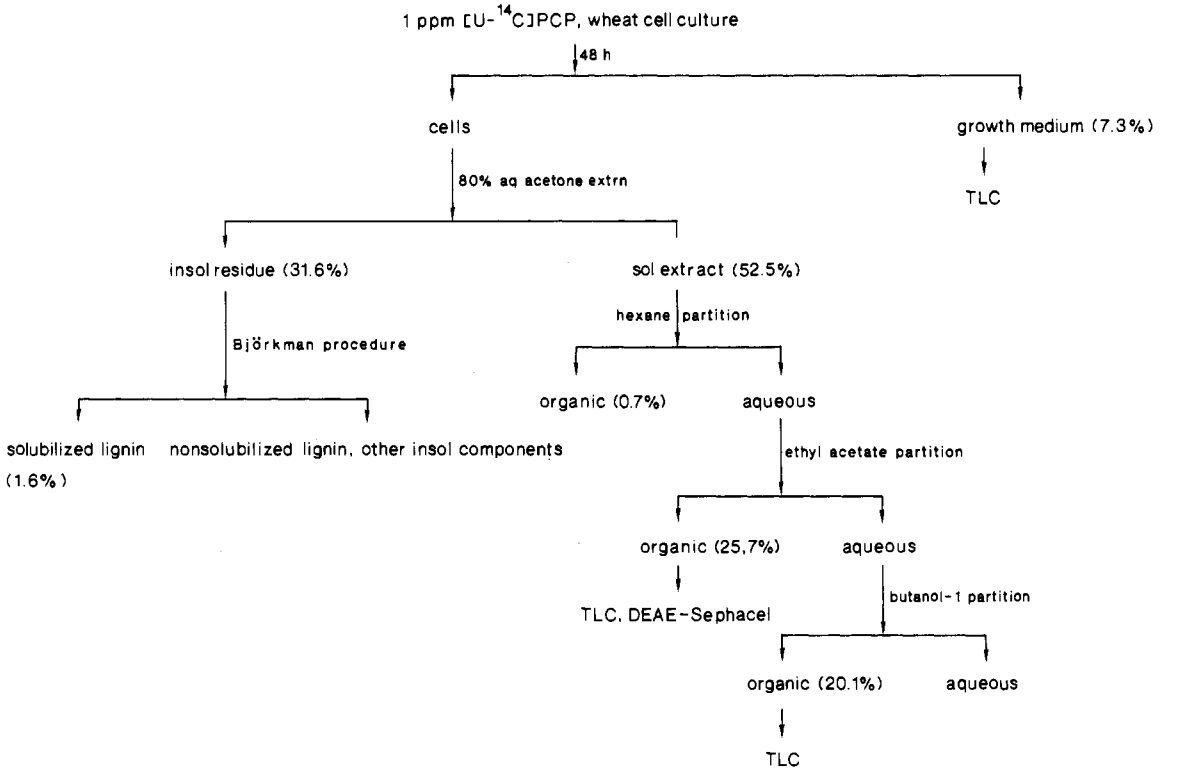

Figure 1. Summary of isolation and characterization methods used for the study of PCP metabolism in wheat cell suspension cultures. Percentages are expressed in terms of applied radioactivity.

under the previous conditions (Scheel et al., 1984). The major released aglycon (90%) comigrated with authentic tetrachlorocatechol upon TLC in solvent system A (R_f 0.16). Minor portions (~5%) comigrated with the PCP (R_f 0.45) and tetrachlorohydroquinone (R_f 0.30) standards. After acetylation, the major aglycon comigrated with the acetylated tetrachlorocatechol standard (R_f 0.30; TLC solvent system A). For large-scale isolation of the aglycons, the soluble metabolites were purified by chromatography on DEAE–Sephacel and hydrolyzed (4 N HCl, 100 °C, 2 h). The cleavage products were acetylated and purified by preparative TLC (solvent system A). The major peak of radioactivity (90%; R_f 0.30) was eluted and further purified by HPLC (Figure 2). This material was further identified as tetrachlorocatechol diacetate as described below.

Isolation and Cleavage of Lignin Metabolites of PCP. The total insoluble metabolite residue remaining after 80% aqueous acetone extraction was processed as described (Scheel et al., 1984) in order to obtain the dioxane/water (9:1, v/v) soluble Björkman lignin fraction. This material (650 mg) contained 5% of the initial total insoluble radioactivity and was used for further study. The test of a 20-μL aliquot with the phloroglucinol/HCl reagent (Freudenberg, 1968) gave a reddish color typical for lignin. The metabolite material has previously been further characterized as lignin by various methods including ^{13}C NMR spectroscopy (Scheel et al., 1984). A few experiments were also carried out with the material not dissolved by 90% aqueous dioxane. This material contained nonsolubilized lignin and cell wall polysaccharides as well as other insoluble components.

Hydrolysis of the Björkman lignin fraction with the HBr/acetic acid reagent [cf. Scheel et al. (1984)] released 31% of the employed radioactivity in the form of products, which upon TLC in solvent system A comigrated with tetrachlorocatechol (R_f 0.16; 70%), tetrachlorohydroquinone (R_f 0.30; 5%), and PCP (R_f 0.45; 25%). Cleavage

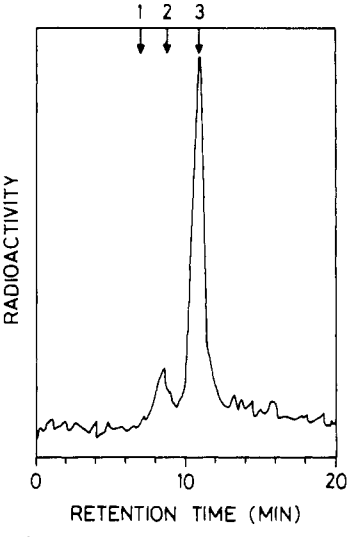

Figure 2. HPLC separation of cleavage products prepared from the soluble metabolite or the Björkman lignin fraction. The radioactivity profile of the cleavage products is shown, and both profiles were practically identical. The positions of the ultraviolet peaks of the following reference compounds are indicated at the top of the graph: 1, PCP-acetate; 2, tetrachlorohydroquinone diacetate; 3, tetrachlorocatechol diacetate.

in a yield of about 30% was also obtained when the lignin solvent dimethylformamide rather than acetic acid was used with HBr. Treatment with 1 N HCl or 1 N NaOH for 1 h at 100 °C gave only 15% liberation of radioactivity, with PCP being the predominant product. A control treatment (100 °C, 1 h) with water released exclusively [^{14}C]PCP in 5% yield. The latter results pointed to the presence of some loosely bound PCP in the lignin fraction.

The major cleavage product was isolated as described in the Experimental Section and further purified by HPLC to give a profile practically identical with that shown in Figure 2.

Table I. Copolymerization of U-^{14}C-Labeled Xenobiotics into Artificial Lignin[a]

expt no.	xenobiotic	molar ratio of coniferyl alcohol to xenobiotic	polymer yield, % rel to coniferyl alcohol	incorpn of radioactivity, mol %
1	PCP	10:1	32	0.1
2	PCP	2:1	39	0.8
3	PCP	1:1	50	0.8
4	o/p-chloranil	10:6	60	7.4
5	tetrachlorohydroquinone/catechol	10:1	91	6.0
6	tetrachlorohydroquinone/catechol	1:1	65	50
7	2,4-D	2:1	70	1.0
8	2,4-dichlorophenol	1:1	16	24
9	pentachlorothiophenol	10:1	70	1.4

[a] The experimental conditions used are described in the Experimental Section. In the experiments involving the water-insoluble substances, o/p-chloranil and pentachlorothiophenol, 5% (v/v) acetone and 10% (v/v) dioxane, respectively, were added as solubilizing agents. Variations in the amounts of coniferyl alcohol (4.4–6.6 μmol/mL), hydrogen peroxide (2.5–6.6 μmol/mL), or horseradish peroxidase (18–41 μg/mL) were without much influence on the incorporation rates observed (data not shown). In experiments 1–4 and 9 the reaction volumes were reduced to 15% of the standard size.

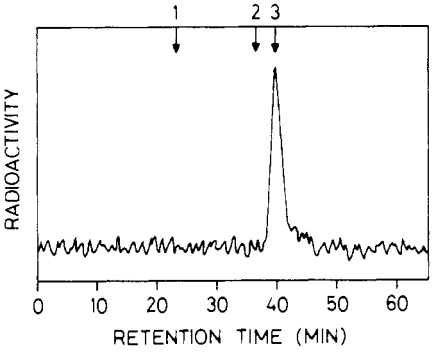

Figure 3. GC separation of cleavage products prepared from the soluble metabolite or the Björkman lignin fraction. The radioactivity profile of the cleavage products is shown, and both profiles were practically identical. In independent experiments, flame ionization detection after capillary GC showed the same peak pattern (data not shown). The elution positions of the following reference compounds are indicated at the top of the graph: 1, PCP-acetate; 2, tetrachlorohydrooquinone diacetate; 3, tetrachlorocatechol diacetate.

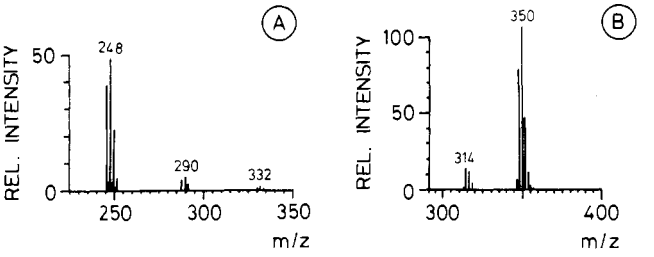

Figure 4. Mass spectra of the acetylated cleavage product derived from the soluble metabolite fraction after purification by HPLC and GC. (A) Electron impact mass spectrum obtained on a Finnigan 4000 instrument with an ionization energy of 30 eV (direct inlet). The base peak (100%; not shown) was located at m/z 43. (B) Chemical ionization mass spectrum obtained on a Varian 44-S instrument using ammonia as reagent gas and an ionization energy of 170 eV (GC inlet). In both cases, authentic tetrachlorocatechol diacetate gave identical spectra.

Identification of Tetrachlorocatechol. The acetylated cleavage products from the soluble as well as the lignin metabolite fraction had upon HPLC the same retention time as the acetylated tetrachlorocatechol standard (Figure 2). Both derivatives were further analyzed by GC where radioactivity as well as flame ionization detection showed single sharp peaks again corresponding to the acetylated tetrachlorocatechol standard (Figure 3). Further study was by EI mass spectroscopy (Figure 4A) and CI mass spectroscopy (Figure 4B). In both cases the spectra were identical with those of the acetylated tetrachlorocatechol standard. The CI mass fragment at m/z 350 corresponded to $[M + NH_4]$, and the peak pattern indicated the presence of four chlorine atoms. The EI mass spectrum showed the molecular ion at m/z 332, the fragments at m/z 290 and 248 corresponding to the successive loss of two acetyl residues.

Enzymatic Preparation of Lignin Conjugates. [^{14}C]PCP and several derivatives were compared with regard to their ability to copolymerize into enzymatically prepared lignin. The incorporation data are summarized in Table I. In all cases, the products were analyzed by gel permeation chromatography on Sephadex LH-60 (Figure 5A,B). Control products prepared by the standard procedure in the absence of coniferyl alcohol were also studied by gel permeation chromatography (Figure 5B). Figure 5A,B is representative for all xenobiotics studied in Table I and for pentachlorothiophenol (see below). Results similar to those listed in Table I were also obtained

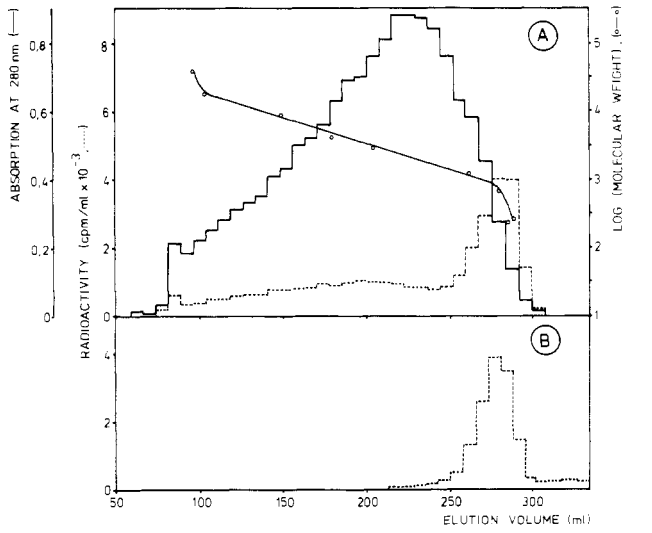

Figure 5. Gel permeation profiles of polymeric products derived from [U-^{14}C]tetrachlorocatechol with (A) or without (B) use of coniferyl alcohol. Catalytic amounts of vanillyl alcohol were present in both cases (see the Experimental Section). Product amounts of about 25 mg were applied in dimethylformamide to a column of Sephadex LH-60 under the previously described conditions (Scheel et al., 1984). UV absorption at 280 nm (—) and/or radioactivity (- - -) is plotted against elution volume (mL). The molecular weight calibration curve shown (o—o) was obtained as previously described (Scheel et al., 1984).

in a number of independent experiments carried out, with each of the xenobiotics varying the scale of the reaction or details of the experimental protocol. TLC in solvent

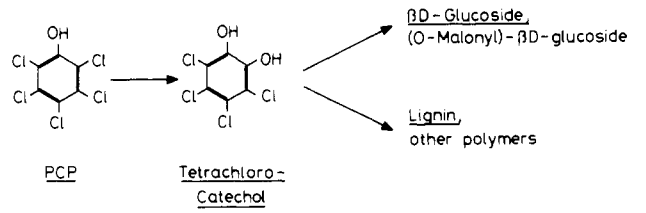

Figure 6. Proposed metabolic pathway of PCP in wheat cell cultures.

system A was routinely applied to all eluted column fractions (cf. Figure 5) in order to differentiate between the parent xenobiotic and reaction products. The incorporation values of Table I refer only to truly copolymerized radioactivity.

In agreement with the in vivo result, PCP itself was a poor lignin precursor, whereas the derived quinone and catechol derivatives were efficiently incorporated into lignin. The herbicide 2,4-D was studied as a reference compound and was also poorly incorporated in vitro (Table I) although a significant incorporation into the insoluble residue and lignin had been observed in vivo (Scheel and Sandermann, 1981). The derived 2,4-dichlorophenol was well incorporated in vitro.

The copolymers obtained from the tetrachlorohydroquinone and -catechol precursors were treated with the HBr/acetic acid reagent. Products cochromatographed with the tetrachlorohydroquinone and -catechol standards upon TLC (solvent system A), but the degradation reaction has not been further characterized.

For further comparison, the more nucleophilic thio analogue of [U-^{14}C]PCP was synthesized and tested in the in vitro lignin synthetic system. Since the thiol was rapidly converted to the disulfide by H_2O_2, the previous two-step procedure employing catalase for decomposition of H_2O_2 (Trenck et al., 1981) had to be employed for copolymerization (see the Experimental Section). At a molar ratio of coniferyl alcohol to xenobiotic of 10:1, a 1.4% incorporation of radioactivity was obtained, the yield of lignin being 70% (relative to the initial amount of coniferyl alcohol). The gel permeation profile was similar to that of Figure 5A while the control without coniferyl alcohol was similar to Figure 5B. TLC controls further indicated true copolymerization.

Enzymatic Synthesis of Tetrachlorocatechol. Radioactively labeled PCP was treated with a Fenton reagent and with horseradish peroxidase as described in the Experimental Section. In both cases, two major products comigrating with tetrachlorocatechol (R_f 0.16) and the derived *o*-quinone (R_f 0.10/0.31) were formed in 10–20% total yield (TLC analysis, solvent system A). No evidence for product formation in the area of tetrachlorohydroquinone and the derived *p*-quinone (R_f 0.25–0.50) was obtained.

DISCUSSION

Tetrachlorocatechol as a Primary PCP Metabolite. On the basis of TLC, HPLC, and GC chromatographies as well as EI and CI mass spectroscopy, the major PCP-derived component in the total soluble and insoluble metabolite fractions as well as in the enriched β-D-glucoside and lignin fractions was identified as tetrachlorocatechol. The corresponding metabolic pathway is shown in Figure 6. In view of the presence of high *O*-malonyl transferase activity in the wheat cell cultures used (Schmitt et al., 1985), the *O*-malonyl-β-D-glucosyl conjugates of tetrachlorocatechol were probably also present, without being detectable by the methods employed here. An independent TLC analysis has shown the presence of a metabolite comigrating with *O*-malonyl-β-D-glucosyl-PCP (Langebartels, 1986).

On the basis of TLC data, tetrachlorocatechol was also the major PCP derivative released by acid hydrolysis from the final insoluble residue not solubilized by 90% aqueous dioxane. Part of the radioactivity of the final insoluble residue (37%) was soluble in 1% aqueous SDS (100 °C, 10 min), indicating binding to nonlignin polymers [cf. Scheel and Sandermann (1981)]. A parallel study has indicated binding of PCP to hemicelluloses, proteins, and pectins in cultured wheat cells as well as intact wheat plants (Langebartels and Harms, 1985). TLC analysis in solvent system A indicated that tetrachlorocatechol was the predominant radioactive component of the various soluble as well as the insoluble metabolite fractions (Langebartels et al., 1986; Langebartels and Harms, personal communication). Preliminary evidence for the presence of tetrachlorocatechol and tetrachlorobenzoquinone as PCP metabolites in rice plants has previously been published (Weiss et al., 1982). Methoxytetrachlorophenol was reported as a major component of the soluble conjugates formed from PCP in soybean plants (Casterline et al., 1985). All the quoted results on tetrachlorocatechol formation provide an interesting contrast to soybean cell suspension cultures where the incorporation rate into the insoluble residue was very low and where PCP was directly converted in high yield to β-D-glucosyl and *O*-malonyl-β-D-glucosyl conjugates (Schmitt et al., 1985).

Enzymatic Aspects. The transformation reaction with Fenton's reagent as well as peroxidase suggested that hydroxyl radicals or other activated oxygen species formed tetrachlorocatechol from PCP. A specific conversion of phenols to *o*-quinones and catechols has also been reported for a mushroom polyphenol oxidase working in chloroform (Kazandjian and Klibanov, 1985). It remains an interesting question why the reaction took place in wheat cells but not in soybean cells. The conversion of PCP to tetrachlorocatechol may resemble the radical-type conversion of benzo[*a*]pyrene to benzo[*a*]pyrene quinones by plant microsomes (Trenck and Sandermann, 1980) as well as by rat liver microsomal lipid peroxidation products (Morgenstern et al., 1981). NADPH-induced lipid peroxidation in liver microsomes also converted aldrin to dieldrin (Lang and Maier, 1986), a reaction often described in plant systems (Dohn and Krieger, 1981; Dennis and Kennedy, 1986). The listed observations may point to a role of reactions involving activated oxygen and radical species rather than specific cytochrome P-450 enzymes in plant pesticide metabolism. The previous proposal (Weiss et al., 1982) of the formation of tetrachlorocatechol via hydroxylation of the primary PCP metabolite 2,3,4,5-tetrachlorophenol appears unlikely in view of the present results.

Photochemical as well as microbial and mammalian transformation of PCP have previously been found to lead to varying proportions of tetrachlorocatechol and tetrachlorohydroquinone and further conversion to *o*- and *p*-chloranil (Ahlborg, 1978; Crosby, 1981; Engelhardt et al., 1986; Renner and Mücke, 1986; van Ommen et al., 1986). In the rat microsomal studies (Ahlborg, 1978; van Ommen et al., 1986), the conversion of PCP to tetrachlorohydroquinone and tetrachlorocatechol appeared to be catalyzed by cytochrome P-450.

Lignin Incorporation. The high incorporation of [U-^{14}C]PCP into wheat lignin (Scheel et al., 1984) is shown here to proceed primarily via tetrachlorocatechol, perhaps with participation of *o*-chloranil. In the case of the in-

secticide Zectran [4-(dimethylamino)-3,5-dimethylphenyl methylcarbamate], it has similarly been proposed that catechol and hydroquinone derivatives serve as efficient lignin precursors (Williams et al., 1964). In the case of benzo[a]pyrene, incorporation into lignin required prior microsomal oxidation to benzo[a]pyrene quinones (Trenck and Sandermann, 1981).

PCP was only poorly incorporated into the enzymatically prepared lignin copolymers. The herbicide 2,4-D also was not well copolymerized although the derived 2,4-dichlorophenol was well incorporated (Table I). The poor incorporation of PCP and 2,4-D under the conditions of Table I is difficult to understand since both xenobiotics are readily converted to a variety of soluble and insoluble conjugates in vivo.

Several hypotheses may explain the low incorporation rates observed. One possible explanation derives from the fact that cleavage of labile lignin linkages may occur under the present workup conditions. A preferred 4-hydroxybenzyl substitution pattern by only certain of the chemicals of Table I could explain the results obtained [cf. Sandermann et al. (1983)].

The nucleophilic thio derivative of PCP was found to be significantly incorporated into artificial lignin although a discontinuous two-step incorporation procedure was employed. This result agrees with the previously observed high incorporation of pentachlorothiophenol into the insoluble residue of peanut plants (Rusness and Lamoureux, 1980). Thio-PCP may be formed from an initial glutathione-*S* conjugate, and may be the intermediate responsible for the high labeling of plant insoluble residues and lignin by the fungicide pentachloronitrobenzene (Rusness and Lamoureux, 1980; Langebartels et al., 1986). The previously unidentified in vivo metabolite I of PCP (Schmitt et al., 1985) has been characterized as a glutathione-derived conjugate on the basis of its resistance toward various glycosidases and acid hydrolysis as well as cochromatography and coelectrophoresis with free glutathione and reference glutathione-*S* conjugates (Kaul, 1984).

Tetrachlorocatechol as a Potential Mutagen. Tetrachlorohydroquinone has been found to be mutagenic for fibroblasts, to cause strand breaks in a model DNA system, and to bind covalently to DNA (Witte et al., 1985). More recently, the covalent binding of radioactive PCP to rat microsomal proteins and to DNA was shown to proceed via tetrachlorohydroquinone and tetrachlorocatechol (van Ommen et al., 1986). Catechols and hydroquinones are moreover known to undergo a redox cycle leading to superoxide anion and derived activated oxygen species that ultimately cause strand breaks in DNA (Irons and Sawahata, 1985; Sandermann, 1987). In view of the quoted results, tetrachlorocatechol may be concluded to be a potential mutagen.

The soluble and insoluble conjugates derived from PCP may present a toxicological hazard if tetrachlorocatechol becomes bioavailable in the intestinal tract of animals and humans [cf. Huber and Otto (1982), Kovacz (1986), Sandermann (1987), and Sandermann et al., (1983)]. The fungal bioavailability of the enzymatically prepared lignin copolymer containing [U-^{14}C]tetrachloro-*o*-quinone (cf. Table I) was observed to be high. The white rot fungus, *Phanerochaete chrysosporium*, converted 47% of the polymer-bound radioactivity to [^{14}C]CO_2 in a 30-day fermentation experiment under previous conditions (Arjmand and Sandermann, 1985a; Arjmand et al., 1985).

ACKNOWLEDGMENT

The help of Dr. P. Beyer in radio-GC and of M. Weber and Dr. J. Wörth in MS is gratefully acknowledged.

Registry No. PCP, 87-86-5; tetrachlorocatechol, 1198-55-6.

LITERATURE CITED

Ahlborg, U. G. "Dechlorination of pentachlorophenol *in vivo* and *in vitro*". In *Pentachlorophenol, Chemistry, Pharmacology and Environmental Toxicology*; Rao, K. R., Ed.; Plenum: New York, 1978; pp 115–130.

Arjmand, M.; Sandermann, H. "Mineralization of Chloroaniline/Lignin Conjugates and of Free Chloroanilines by the White Rot Fungus, *Phanerochaete chrysosporium*". *J. Agric. Food Chem.* **1985a**, *33*, 1055–1060.

Arjmand, M.; Sandermann, H. "Metabolism of DDT and Related Compounds in Cell Suspension Cultures of Soybean (*Glycine max* L.) and Wheat (*Triticum aestivum* L.)". *Pesticide Biochem. Physiol.* **1985b**, *23*, 389–397.

Arjmand, M.; Sandermann, H. "Plant Biochemistry of Xenobiotics. Mineralization of Chloroaniline/Lignin Metabolites from Wheat by the White-Rot Fungus, *Phanerochaete chrysosporium*". *Z. Naturforsch, C: Biosci.* **1986**, *41C*, 206–214.

Arjmand, M.; Schäfer, W.; Sandermann, H. Universität Freiburg, 1985, unpublished work.

Baldwin, B. C. "Xenobiotic Metabolism in Plants". In *Drug Metabolism—from Microbe to Man*". Parke, D. V., Smith, R. L., Eds.; Taylor and Francis: London, 1977; pp 191–217.

Casterline, J. L., Jr.; Barnett, N. M.; Ku, Y. "Uptake, Translocation and Transformation of Pentachlorophenol in Soybean and Spinach Plants". *Environ. Res.* **1985**, *37*, 101–118.

Cole, L. K.; Metcalf, R. L. "Predictive Environmental Toxicology of Pesticides in the Air, Soil, Water and Biota of Terrestrial Model Ecosystems". In *Terrestrial Microcosms and Environmental Chemistry*; Witt, J. M., Gillett, J. W., Wyatt, J., Eds.; Oregon State University: Corvallis, OR, 1977; pp 57–73.

Crosby, D. G; Beynon, K. I.; Greve, P. A.; Korte, F.; Still, G. G.; Vonk, J. W. "Environmental Chemistry of Pentachlorophenol". *Pure Appl. Chem.* **1981**, *53*, 1051–1080.

Deichmann, W.; Schäfer, L. J. "Spectroscopic Estimation of Pentachlorophenol in Tissues and Water". *Ind. Eng. Chem. Anal.* **1942**, *14*, 310–312.

Dennis, S.; Kennedy, I. R. "Monooxygenase from Soybean Root Nodules: Aldrin Epoxidase and Cinnamic Acid 4-Hydroxylase". *Pesticide Biochem. Physiol.* **1986**, *26*, 29–35.

Dohn, D. R.; Krieger, R. I. "Oxidative Metabolism of Foreign Compounds by Higher Plants". *Drug Metab. Rev.* **1981**, *12*, 19–157.

Engelhardt, G.; Wallnöfer, P. R.; Mücke, W.; Renner, G. "Transformations of Pentachlorophenol. Part II. Transformations under Environmental Conditions". *Toxicol. Environ. Chem.* **1986**, *11*, 233–252.

Floyd, R. A.; Watson, J. J.; Wong, P. K. "Sensitive Assay of Hydroxyl Free Radical Formation Utilizing High Pressure Liquid Chromatography with Electrochemical Detection of Phenol and Salicylate Hydroxylation Products". *J. Biochem. Biophys. Methods* **1984**, *10*, 221–235.

Frear, D. S.; Mansager, E. R.; Swanson, H. R.; Tanaka, F. S. "Metribuzin Metabolism in Tomato: Isolation and Identification of N-Glucoside Conjugates". *Pesticide Biochem. Physiol.* **1983**, *19*, 270–281.

Freudenberg, K. "The Constitution and Biosynthesis of Lignin" In *Constitution and Biosynthesis of Lignin*; Freudenberg, K., Neish, A. C., Eds.; Springer-Verlag: Berlin, 1968; pp 48–122.

Gile, J. D.; Gillett, J. W. "Fate of Selected Fungicides in a Terrestrial Laboratory Ecosystem". *J. Agric. Food Chem.* **1979**, *27*, 1159–1164.

Haque, A.; Scheunert, I.; Korte, F. "Isolation and Identification of a Metabolite of Pentachlorophenol-^{14}C in Rice Plants". *Chemosphere* **1978**, *1*, 65–69.

Harms, H.; Langebartels, C. "Standardized Plant Cell Suspension Test Systems for an Ecotoxicologic Evaluation of the Metabolic Fate of Xenobiotics". *Plant Sci.* **1986**, *45*, 157–165.

Huber, R.; Otto, S. "Bound Pesticide Residues in Plants". In *IUPAC Pesticide Chemistry*; Miyamoto, J., Kearney, P. C., Eds.; Pergamon: Oxford, 1983; Vol. 3, pp 357–362.

Irons, R. D.; Sawahata, T. "Phenols, Catechols and Quinones". In *Bioactivation of Foreign Compounds*; Anders, M. W., Ed.; Academic: New York, 1985; pp 259–281.

Kaul, J. Universität Freiburg, 1984, unpublished results.

Kazandjian, R. Z.; Klibanow, A. M. "Regioselective Oxidation of Phenols Catalyzed by Polyphenol Oxidase in Chloroform". *J. Am. Chem. Soc.* **1985**, *107*, 5442–5450.

Kovacs, M. F. "Regulatory Aspects of Bound Residues (Chemistry)". In *Residue Reviews*; Gunther, F. A., Gunther, I. D., Eds.; Springer-Verlag: Berlin, 1986; Vol. 97, pp 1–17.

Lang, B.; Maier, P. "Lipid Peroxidation Dependent Aldrin Epoxidation in Liver Microsomes, Hepatocytes and Granulation Tissue Cells". *Biochem. Biophys. Res. Commun.* **1986**, *138*, 24–32.

Langebartels, C.; Harms, H. "Metabolism of Pentachlorophenol in Cell Suspension Cultures of Soybean and Wheat: Pentachlorophenol Glucoside Formation". *Z. Pflanzenphysiol.* **1984**, *113*, 201–211.

Langebartels, C.; Harms, H. "Analysis for Nonextractable (Bound) Residues of Pentachlorophenol in Plant Cells Using a Cell Wall Fractionation Procedure". *Ecotoxicol. Environ. Safety* **1985**, *10*, 268–279.

Langebartels, C.; Harms, H. Bundesforschungsanstalt für Landwirtschaft, Braunschweig, private communication, 1986.

Langebartels, C.; Harms, H.; Arjmand, M.; Schäfer, W.; Sandermann, H. "Cell Wall Components as Binding Sites for Xenobiotics". In *Cell Walls* '86. *Proceedings of the Fourth Cell Wall Meeting*; Vian, B., Reis, D., Goldberg, R., Eds.; Université Pierre et Marie Curie, École Normale Supérieure: Paris, 1986; pp 158–161.

Morgenstern, R.; DePierre, J. W.; Lind, C.; Guthenberg, C.; Mannervik, B.; Ernster, L. "Benzo(a)pyrene Quinones Can be Generated by Lipid Peroxidation and are Conjugated with Glutathione by Glutathione S-Transferase B from Rat Liver". *Biochem. Biophys. Res. Commun.* **1981**, *99*, 682–690.

Öberg, L. G.; Paul, K. G. "The Transformation of Chlorophenols by Lactoperoxidase". *Biochim. Biophys. Acta* **1985**, *842*, 30–38.

Renner, G.; Nguyen, P.-T. "Sulfur-Containing Metabolites of the Fungicides Pentachloronitrobenzene (PCNB) and Hexachlorobenzene (HCB)". *Chemosphere* **1981**, *10*, 1215–1222.

Renner, G.; Mücke, W. "Transformations of Pentachlorophenol". *Toxicol. Environ. Chem.* **1986**, *11*, 9–29.

Roberts, T. R. "Non-Extractable Pesticide Residues in Soils and Plants". *Pure Appl. Chem.* **1984**, *56*, 945–956.

Rott, B.; Nitz, S.; Korte, F. "Microbial Decomposition of Sodium Pentachlorophenolate". *J. Agric. Food Chem.* **1979**, *27*, 306–310.

Rusness, D. G.; Lamoureux, G. L. "Pentachloronitrobenzene Metabolism in Peanut". *J. Agric. Food Chem.* **1980**, *28*, 1070–1077.

Sandermann, H. "Mutagenic Activation of Xenobiotics by Plant Enzymes". *Mutat. Res.* **1988**, in press.

Sandermann, H.; Scheel, D.; Trenck, V. D. T. "Metabolism of Environmental Chemicals by Plants—Copolymerization into Lignin". *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1983**, *37*, 407–420.

Sandermann, H.; Scheel, D.; Trenck, V. D. T. "Use of Plant Cell Cultures to Study the Metabolism of Environmental Chemicals". *Ecotoxicol. Environ. Safety* **1984**, *8*, 167–182.

Scheel, D.; Sandermann, H. "Metabolism of 2,4-Dichlorophenoxyacetic Acid in Cell Suspension Cultures of Soybean (*Glycine max* L.) and Wheat (*Triticum aestivum* L)". *Planta* **1981**, *152*, 253–258.

Scheel, D.; Schäfer, W.; Sandermann, H. "Metabolism of Pentachlorophenol in Cell Suspension Cultures of Soybean (*Glycine max* L.) and Wheat (*Triticum aestivum* L.). General Results and Isolation of Lignin Metabolites". *J. Agric. Food Chem.* **1984**, *32*, 1237–1241.

Schmitt, R.; Kaul, J.; Trenck, V. D. T.; Schaller, E.; Sandermann, H. "β-D-Glucosyl- and *o*-Malonyl-β-D-Glucosyl- Conjugates of Pentachlorophenol in Soybean and Wheat. Identification and Enzymatic Synthesis". *Pesticide Biochem. Physiol.* **1985**, *24*, 77–85.

Schuphan, I.; Haque, A.; Ebing, W. "Ecochemical Assessment of Environmental Chemicals. Part I: Standard Screening Procedure to Evaluate Chemicals in Plant Cell Cultures". *Chemosphere* **1984**, *13*, 301–313.

Trenck, V. D. T.; Sandermann, H. "Oxygenation of Benzo[*a*]-pyrene by Plant Microsomal Fractions". *FEBS Lett.* **1980**, *119*, 227–231.

Trenck, V. D. T.; Sandermann, H. "Incorporation of Benzo[*a*]-pyrene Quinones into Lignin". *FEBS Lett.* **1981**, *125*, 72–76.

Trenck, V. D. T.; Hunkler, D.; Sandermann, H. "Incorporation of Chlorinated Anilines into Lignin". *Z. Naturforsch., C: Biosci.* **1981**, *36c*, 714–720.

Van Ommen, B.; Adang, A.; Müller, F. "The Mirosomal Metabolism of Pentachlorophenol and Its Covalent Binding to Protein and DNA". *Chem. Biol. Interact.* **1986**, *60*, 1–11.

Weiss, U. M.; Moza, P.; Scheunert, I.; Haque, A.; Korte, F. "Fate of [^{14}C]Pentachlorophenol in Rice Plants under Controlled Conditions". *J. Agric. Food Chem.* **1982**, *30*, 1186–1190.

Williams, E.; Weikle, R. W.; Reddemann, C. T. "Identification of Metabolites of Zectran Insecticide in Broccoli". *J. Agric. Food Chem.* **1964**, *12*, 453–457.

Witte, I.; Juhl, U.; Butte, W. "DNA-Damaging Properties and Cytotoxicity in Human Fibroblasts of Tetrachlorohydroquinone, a Pentachlorophenol Metabolite". *Mutat. Res.* **1985**, *145*, 71–75.

Received for review December 29, 1986. Accepted September 21, 1987. This work has been supported by the Deutsche Forschungsgemeinschaft (Sa 180/15-5) and in part by the Fonds der Chemischen Industrie.