Metabolism of Pentachlorophenol in Cell Suspension Cultures of Soybean (*Glycine max* L.) and Wheat (*Triticum aestivum* L.). General Results and Isolation of Lignin Metabolites

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

Pentachlorophenol (PCP) was administered to cell suspension cultures of soybean and wheat by using standardized procedures for incubation and workup. Total metabolic rates were 73% in soybean and 89% in wheat, with relative standard deviations of ±10–20%. The soluble glucosyl conjugates formed were partially excreted into the growth medium. The wheat "insoluble" metabolite fraction (37% incorporation) was sequentially solubilized by using a Björkman-type procedure. A covalent incorporation of PCP derivatives into lignin was demonstrated by gel permeation chromatography, by ultraviolet and ^{13}C NMR spectra, and by specific chemical degradation.

Pentachlorophenol (PCP) has over many years been used on a large scale for wood protection and a variety of other purposes. In view of the relative persistence and the toxicity of PCP and its impurities or derivatives, the use of PCP has been reduced in recent years. Two extensive recent reviews on the use and environmental properties of PCP are available (Rao, 1978; Crosby et al., 1981). PCP is very phytotoxic, but little information on its plant metabolism exists (Crosby et al., 1981). Whole-plant metabolism studies have been carried out with rice (Haque et al., 1978; Weiss et al., 1982), corn (Cole and Metcalf, 1977), and alfalfa and rye (Gile and Gillett, 1979). In all cases, high metabolic conversion rates and the formation of unidentified polar conjugates and "insoluble" metabolite residues were observed. A number of dechlorinated and/or methoxylated derivatives of PCP were characterized in the rice studies. However, the experiments were carried out under greenhouse conditions so that a participation of the know microbial and abiotic degradation pathways (Rao, 1978; Crosby et al., 1981) remained possible.

The metabolism of PCP has now been studied in sterile cell suspension cultures of soybean and wheat. The main purposes were (i) to assess the reproducibility of a standardized version of previous cell culture techniques (Sandermann et al., 1977; Scheel and Sandermann, 1977; von der Trenck and Sandermann, 1978) and (ii) to characterize the insoluble metabolite fraction.

EXPERIMENTAL SECTION

Materials and General Methods. The previous soybean and wheat cell suspension cultures (Scheel and Sandermann, 1977, 1981a) were used. The cells were grown in 40 mL of B5-medium containing 1 ppm (soybean) or 2 ppm (wheat) of 2,4-D by using 200-mL Erlenmeyer flasks. Uniformly labeled [^{14}C]PCP was purchased from Pathfinder Laboratories, St. Louis, or from Amersham-Buchler, Braunschweig. The purity of these preparations was ≥99% as judged by thin-layer chromatography. Other materials and the general methods used were as previously described (Scheel and Sandermann, 1977, 1981a,b; von der Trenck and Sandermann, 1978).

Radioactivity was determined by liquid scintillation counting using the previous scintillation fluids (Scheel and Sandermann, 1981b) and internal standardization with [^{14}C]toluene. The counting error was generally held below 1%. The ^{13}C NMR spectrum was obtained with a Bruker WP-80 instrument at 20.15 MHz under the previous conditions (von der Trenck et al., 1981). Chemical shift values are given in ppm relative to the tetramethylsilane standard.

Incubation with [^{14}C]PCP. At the usual day of inoculation in late-log growth phase (day 7 for soybean, day 14 for wheat), the contents of five culture flasks were combined, and 2-g aliquots of cells were transferred into 13 (wheat) or 10 (soybean) 200-mL Erlenmeyer flasks containing fresh medium (40 mL). The normal growth conditions (27 °C, 120 rpm, darkness) were maintained for 5 days (soybean) and 12 days (wheat), respectively. Three of the culture flasks were then autoclaved (10 min, 121 °C) and used for the control incubations. [^{14}C]PCP was added to each flask as a solution in 40 μL of methanol. The amount of PCP (40 μg, 0.3–1 μCi) led to a final concentration of 1 ppm. The cultures were then further incubated for 48 h under the normal growth conditions.

Cell Harvest. After the 48-h incubation period, 1-mL aliquots were withdrawn from the cultures in order to examine for bacterial or fungal infection. These aliquots were incubated for 1 week at 37 °C on either standard I nutrient agar (Merck No. 7881) or Czapek-Dox nutrient agar (Merck No. 5460). It should be noted that not all possible types of infection were detected by the method used. The cells were then harvested by filtration of the cultures through a moist preweighed filter paper. The cells were washed with distilled water until the filtrate had a total volume of 100 mL. Aliquots (1 mL) of the filtrates were used to determine the total radioactivity in the growth medium. The weight of the cell mass was determined. The cells were then transferred into a screw-cap vial containing a mixture of 9.6 mL of chloroform, 19.2 mL of methanol, and 3.8 mL of water and stored overnight at −18 °C.

Bligh-Dyer Extraction of Cells. The cell suspensions were treated at ≤0 °C for 5 min with the microtip of the Branson B12 sonifier. Insoluble material was isolated by the above filtration procedure and washed with a mixture of 9.6 mL of chloroform, 19.2 mL of methanol, and 7.6 mL of water. Chloroform (18 mL) was added to the combined filtrates. After overnight storage at 4 °C two solvent phases had formed. These phases were separated and aliquots of 0.5 or 1 mL were removed for determination of radioactivity. The phases were then concentrated to near dryness at ≤40 °C by using a rotary evaporator with a receiving flask cooled to −15 °C. This flask was at the end examined for volatile radioactivity. The near-dry residues were taken up in 5 mL of a mixture of chloro-

Institut für Biologie II, Universität Freiburg, D-7800 Freiburg i.Br., F.R.G.

[1] Present address: Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Vogelsang, F.R.G.

0021-8561/84/1432-1237$01.50/0

Table I. Metabolism of PCP in Cell Suspension Cultures of Wheat and Soybean

	wheat	soybean
wet weight of cells[a]	5.6 ± 0.5	11.3 ± 1.5
wet weight of controls[a]	4.7 ± 0.3	3.3 ± 0.1
total recovery of ^{14}C[b]	92.1 ± 3.3	98.5 ± 10.5
^{14}C in growth medium[c]	6.3 ± 0.9	28.9 ± 3.5
unchanged PCP, cell associated	1.1 ± 0.4	21.1 ± 4.4
unchanged PCP, in growth medium	1.9 ± 0.6	3.3 ± 0.6
polar metabolites, cell associated	47.1 ± 3.6	37.2 ± 6.1
polar metabolites in growth medium	4.4 ± 0.1	24.5 ± 4.6
insoluble residue	37.6 ± 1.8	11.3 ± 2.5

[a] Values are given in grams ± standard deviation. [b] Values are given as percent of applied radioactivity ± standard deviation in this and subsequent lines of the table. [c] ^{14}C in the medium of the heat-inactivated control cultures was 16.7 ± 0.7% (wheat) and 52.3 ± 8.5% (soybean).

form–methanol–water, 1:2:0.8 (v/v/v) by using a sonic bath for dispersion. After centrifugation, aliquots of 50 and 100 μL were withdrawn for determination of radioactivity. The insoluble residue isolated in the filtration step was transferred into preweighed vials and lyophilized, followed by determination of dry weight. Aliquots were combusted for determination of radioactivity by liquid scintillation counting. The values obtained are listed in Table I under "insoluble residue".

Bligh-Dyer Extraction of Growth Medium. Aliquots (10 mL) of the combined filtrates obtained during cell harvest were transferred into a ground-joint graduated flask, and 37.5 mL of chloroform–methanol, 2:1 (v/v), was added. After thorough mixing the flasks were stored overnight at 4 °C. The supernatant was then removed by means of a pipet, and the precipitated material was extracted with a 47.5-mL portion of chloroform–methanol–water, 1:2:0.8 (v/v/v). The final precipitate contained only negligible radioactivity and was discarded. The combined supernatants were diluted with 25-mL portions of chloroform and of water. Phase separation and further workup were as described above for the extraction of cells.

Thin-Layer Chromatography. Aliquots of the various extract phases were applied to precoated silica gel G thin-layer plates (Merck No. 5554) and examined routinely in the following solvent system: *n*-hexane–diethyl ether–formic acid, 70:30:4 (v/v/v). R_{PCP} values quoted under *Results* were determined in this solvent system where PCP migrated with an R_f value of 0.4–0.5, depending on the exact conditions used. Supplementary solvent systems used were cyclohexane–acetone, 5:2 (v/v), and chloroform. The reliability of the routine thin-layer chromatographic separations was ascertained by high-performance liquid chromatography on a Nucleosil-5 C18 column by using various gradient programs between 90% and 10% (v/v) methanol in water and Berthold Model LB 503 radiomonitor. The thin-layer distributions of radioactivity were determined quantitatively by using an automatic scanning system (Berthold Model LB2832). This instrument was found to work accurately by a comparison with radioactivity profiles obtained by cutting the thin-layer plates, followed by scintillation counting. Polar metabolites were defined as having a lower thin-layer chromatographic mobility than PCP. Metabolites migrating faster than PCP were termed nonpolar metabolites.

Isolation of Lignin Metabolites. The protocol used was adopted from literature procedures (Björkman, 1956; Crawford, 1981). Wheat cells (150 g of wet weight) were incubated with 1 ppm of [^{14}C]PCP by a scaled-up version of the above incubation procedure (48 h). The cells were then harvested by filtration, washed with 200 mL of water, and lyophilized. The dry residue (21 g) was extracted twice with 200-mL portions of water (2 × 8 h, 80 °C), followed by lyophilization and Soxhlet extraction with benzene–ethanol, 1:1 (v/v; 200 mL, 24 h). This was followed by Soxhlet extraction with 200 mL of ethanol (24 h). The remaining residue was further treated with five 100-mL portions of water (5 × 4 h, 80 °C). At this point, 80% of the initially applied radioactivity had been extracted. The insoluble residue was lyophilized and thoroughly ground in a Fritsch type 601 ball mill by using 1-cm steel balls in toluene suspension and under a nitrogen atmosphere (84 h, 25 °C). The ground material was isolated by centrifugation (50000 *g*, 20 min) and washed with two 60-mL portions of ethyl acetate (2 × 10 min, 50 °C). The toluene and ethyl acetate extracts contained <0.1% of the applied radioactivity. The remaining residue was dried in vacuo and then dispersed in 100 mL of 90% aqueous dioxane. Incubation in this solvent was for 1 week at 25 °C in a shaking water bath. This extraction was repeated twice, leaving a final insoluble residue. The dioxane–water extracts were combined, and polysaccharides were precipitated by slowly adding benzene and neutral aluminum oxide (Woelm) in small portions to final amounts of 80 mL and 2 g, respectively, per L of extract. The dioxane–water extract was then cleared by centrifugation and concentrated by means of a rotary evaporator. Subsequent lyophilization yielded the Björkman lignin fraction (650 mg). The amount of incorporated radioactivity (about 5% of the initial insoluble residue) was determined in Bray's scintillation fluid. The Björkman lignin fraction was solubilized in 5 mL of dimethyl formamide for subsequent chromatography on Sephadex LH-60 (von der Trenck et al., 1979, 1981).

RESULTS

PCP Metabolism in Wheat and Soybean Cell Cultures. The present protocol for incubation and workup was simplified from previous procedures (Sandermann et al., 1977; Scheel and Sandermann, 1977; von der Trenck and Sandermann, 1978) in order to rapidly perform multiple parallel incubations for a statistical evaluation. The results are summarized in Table I and were obtained with relative standard deviations of ±10–20%. Polar metabolites were formed in total amounts of 50–60% of the applied radioactivity in both wheat and soybean cultures. These metabolites were present in the methanol–water phases and, in lower amounts, also in the chloroform phases of the Bligh-Dyer extraction steps. About 40% of the soluble soybean metabolites was found in the external growth medium. This value was 9% in the case of wheat cells. The latter cell type incorporated a remarkable 37.6% of the applied radioactivity into the insoluble residue. Total metabolic conversion rates were 89% in wheat and 73% in soybean.

Nonpolar metabolites occurred at <0.2% of the applied radioactivity and were not further studied. The radioactivity in the various fractions isolated from the heat-inactivated control incubations consisted in all cases of unchanged [^{14}C]PCP.

Characterization of Soluble Metabolites. The polar metabolites were treated with β-glucosidase (Scheel and Sandermann, 1981a) and the ether-extractable products were examined by thin-layer chromatography. A sharply defined product with R_{PCP} = 1.0 was released as the predominant aglycon from the soybean metabolites. A heterogeneous aglycon mixture with major peaks at R_{PCP} = 0.02 and 0.42 and minor peaks at R_{PCP} = 0.25, 0.69, and 1.0 was released from the wheat metabolites. Various tested isomeric trichloro- and tetrachlorophenols had R_{PCP} values of between 0.65 and 1.3, while 2,3,5,6-tetrachloro-

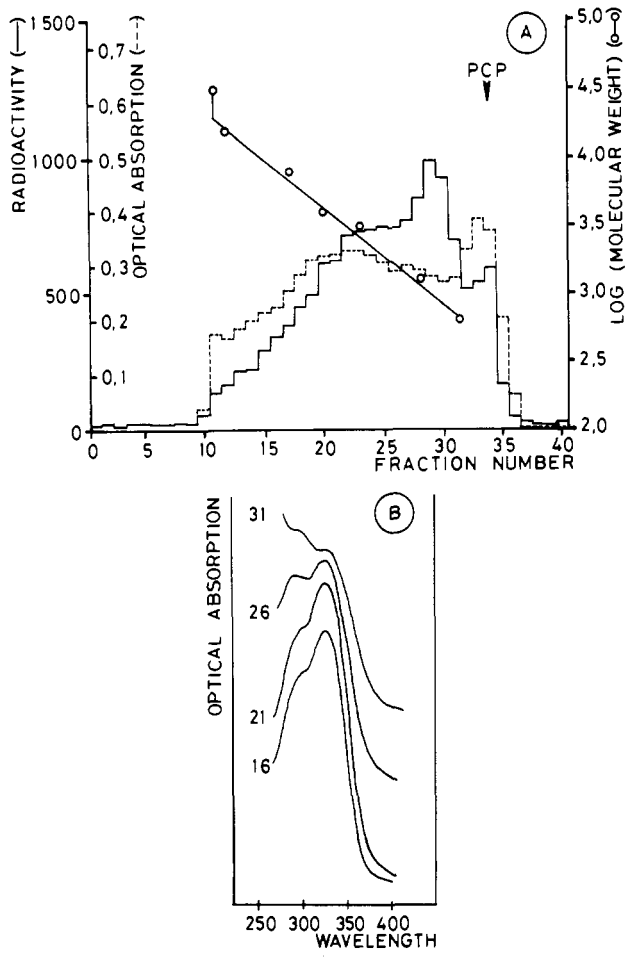

Figure 1. (A) Gel permeation chromatography of the wheat Björkman lignin fraction on a column (103 × 2.3 cm) of Sephadex LH-60. The solvent used was dimethylformamide, and fractions of 8.7 mL were collected. Aliquots of 1 mL were counted for 5 min. Radioactivity [(—) cpm/mL] and UV absorption at 280 nm (---) are plotted against the fraction number. The elution position of free PCP is indicated by an arrow. In addition, a calibration curve was determined in independent runs using polystyrene molecular weight markers (O). (B) UV spectra of column fractions 16, 21, 26, and 31. Optical absorption was measured vs. wavelength (nm).

hydroquinone had R_{PCP} = 0.66 and 3,4,5,6-tetrachlorocatechol had R_{PCP} = 0.42. The identity of the wheat aglycons has not been elucidated. A very similar product pattern was obtained upon chemical degradation of the wheat insoluble metabolite fractions (see below).

The soybean polar metabolites were further fractionated on Bio-Gel P-2 and reversed-phase HPLC. Mass spectroscopy, specific enzymatic reactions, and chemical synthesis then led to the conclusive identification of PCP-β-D-glucopyranoside and its malonyl derivative as major metabolites. Preliminary data indicate that these conjugates are also formed by the wheat cells (Th. von der Trenck, J. Kaul, R. Schmitt, E. Schaller, and H. Sandermann, unpublished results).

Characterization of Lignin Metabolites. A soluble lignin metabolite fraction containing 5% of the radioactivity of the initial insoluble residue could be isolated by the Björkman procedure (Björkman, 1956; Crawford, 1981). In order to exclude the presence of inclusion compounds, the lignin metabolite fraction was chromatographed on a column of Sephadex LH-60 in dimethylformamide (von der Trenck et al., 1979, 1981). The linear separation range of the column used was between polystyrene molecular weights of 650–20 000. The ultraviolet elution profile in-

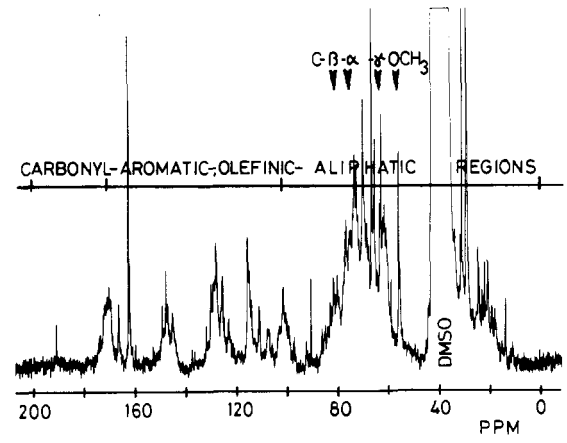

Figure 2. ^{13}C NMR spectrum of the wheat Björkman lignin metabolite fraction (500 mg in 2.5 mL of dimethyl sulfoxide-d_6). The spectral areas corresponding to carbonyl C atoms, to aromatic and olefinic C atoms, and to aliphatic C atoms are marked. In addition, the peaks of cinnamyl methoxy carbons and of the α-, β-, and γ-carbon atoms of phenylpropanoid lignin side chains are marked by arrows.

dicated that polydisperse lignin species were present over the entire molecular weight range (Figure 1A). The incorporated radioactivity gave an approximately parallel elution curve, indicating true copolymerization. The UV spectra of several eluted fractions are shown in Figure 1B. A relatively large amount (500 mg) of the Björkman lignin metabolite fraction was employed to obtain the ^{13}C NMR spectrum shown in Figure 2. This spectrum closely resembled a ^{13}C NMR spectrum previously published for milled lignin from a Bermuda grass (Himmelsbach and Barton, 1980). The spectral assignments given in Figure 2 are based upon literature data (Lüdemann and Nimz, 1974; Nimz et al., 1974, 1975; Himmelsbach and Barton, 1980). The spectrum of Figure 2 displays the basic features of lignin spectra in the spectral regions corresponding to carbonyl C atoms as well as the region corresponding to aromatic and olefinic and aliphatic C atoms. The peak corresponding to cinnamly methoxy groups is marked at 55.5 ppm. Furthermore, the locations of the "fingerprint" signals for lignin (Nimz et al., 1975) are marked at 63.8, 75.5, and 81.9 ppm. These signals correspond to the γ-C, α-C, and β-C atom, respectively, of lignin phenylpropanoid side chains.

Amounts of 10–500 mg of the final insoluble residue, of the Björkman lignin fraction and of the peak fractions of Figure 1A were subjected to ether cleavage by 1–5 mL of the standard HBr–acetic acid reagent ("Organikum. Organisch-Chemisches Grundpraktikum", 1963). In each case, ether extraction and thin-layer chromatography led to major product peaks at R_{PCP} = 0.03, 0.27, and 0.43 and minor product peaks at P_{PCP} = 0.68 and 1.0. In control experiments, the HBr–acetic acid reagent was found not to attack free PCP and to lead to complete cleavage of the model compound, benzohydroquinone monobenzyl ether. The products released from the wheat insoluble metabolite fractions have so far not been identified, although they appeared to resemble the aglycons released from the wheat polar metabolite fraction (see above). A partial release of these split products from the insoluble and lignin fractions also occurred upon alkaline (0.1 N NaOH, 1 h, 50 °C) or acid hydrolysis (1 N HCl, 5 h, 80 °C).

DISCUSSION

Test Procedure. Recent reviews (Mumma and Hamilton, 1979; Mumma and Davidonis, 1983) have emphasized the need to standardize the procedures used to determine

the metabolism of environmental chemicals by cultured plant cells. In a previous study of 2,4-D metabolism in the present wheat and soybean cultures (Scheel and Sandermann, 1981a) the ethanol extraction procedure of Mumma and Hamilton (1979) was used and an average relative error of ±10–20% was determined. The present standard deviations were in the same range although a different procedure involving Bligh-Dyer extraction was employed. It is concluded that plant cell culture techniques allow a rapid and reproducible determination of metabolic rates. The metabolism of [^{14}C]PCP in wheat and soybean cell suspension cultures has furthermore been examined in a rigorous ring test program involving three independent laboratories. The interlaboratory data agreed to better than ±25%, and this agreement was also found in a ring test with [^{14}C]diethyl hexylphthalate (Scheel et al., 1984).

Characterization of Soluble Metabolites. The identification of the β-D-glucopyranoside and the malonyl β-D-glucopyranoside as major soybean metabolites of PCP will be reported elsewhere. Both conjugates also predominated in the polar metabolite fraction released in high amounts into the external growth medium of the soybean cells. The release of these highly polar conjugates for which a vacuole localization would be presumed (Marty et al., 1980; Schmitt and Sandermann, 1982) is unusual and may be related to the uncoupling effect of PCP. The efflux of metabolites was much smaller in the wheat cell cultures. The applied concentration of 1 ppm of PCP was not growth inhibitory for either the wheat or the soybean cultures. While the mechanism for the metabolite efflux is unknown at present, a more detailed study has shown that all of the extracellular soybean conjugates of PCP were originally cell associated (Langebartels and Harms, 1984).

Characterization of Lignin Metabolites. The present study was focussed upon the insoluble metabolite fraction because the state of knowledge about such metabolites is poor [reviewed by Sandermann et al. (1983)]. Insoluble metabolite residues had been encountered in all previous whole-plant studies of PCP metabolism (Cole and Metcalf, 1977, Haque et al., 1978; Gile and Gillett, 1979; Weiss et al., 1982). A drastic alkali extraction procedure had indicated that radioactivity from [^{14}C]PCP was associated with the cellulose and lignin fractions of rice plants (Weiss et al., 1982). The formation of lignin metabolites from PCP is further supported by the present study. A defined Björkman lignin metabolite fraction could be isolated from the initial wheat insoluble residue in 5% yield based upon initial amount of radioactivity. The reported yields of the Björkman procedure are up to 30–50% based on the initial amount of lignin (Lai and Sarkanen, 1971). The radioactivity in the final insoluble residue that was not solubilized in the Björkman procedure could be partially solubilized with a hot sodium dodecyl sulfate solution. This reagent does not dissolve lignin (Scheel and Sandermann, 1981b). The wheat insoluble residue of Table I therefore appears to also comprise unidentified nonlignin components.

The parallel size distributions of UV absorption and of incorporated radioactivity in Figure 1A argue against an incorporation into carbohydrate material. They rather indicate that a covalent incorporation of PCP-derived radioactivity into lignin had occurred by some random mechanism. The lignin nature of the isolated material was supported by the UV spectra of Figure 1B and the ^{13}C NMR spectrum of Figure 2. The UV spectra displayed the double peak that is typical for grass lignins (Goldschmid, 1971) and which has previously been found for wheat straw Björkman lignin and for a lignin fraction solubilized from the present wheat cells (Scheel and Sandermann, 1981b). There was a relative increase of absorption at 280 nm in going from fraction 16 to fraction 31 (cf. Figure 1B), indicating heterogeneity of the sample.

The ^{13}C NMR spectrum of Figure 2 closely resembled the reported ^{13}C NMR spectra for milled lignin from Bermuda grass (Himmelsbach and Barton, 1980) and from soybean cell suspension cultures (Nimz et al., 1975). The amount of incorporated PCP was too low to be detected by ^{13}C NMR spectroscopy. The assignment of most of the spectral peaks of Figure 2 appeared possible on the basis of the literature data (Lüdemann and Nimz, 1974; Nimz et al., 1974, 1975; Himmelsbach and Barton, 1980). However, no detailed interpretation is given because the multitude and intensity of the peaks in the aliphatic spectral region indicated complications by the presence of carbohydrate material. The same observation has been made in the case of the lignins from soybean cells (Nimz et al., 1975) and from Bermuda grass (Himmelsbach and Barton, 1980). The spectral assignments given in Figure 2 were taken from published reports (Lüdemann and Nimz, 1974; Nimz et al., 1975; Himmelsbach and Barton, 1980) and provide clear evidence for the presence of lignin.

Radioactivity from [^{14}C]PCP has recently also been found to be copolymerized into spruce lignin. Spruce twigs were incubated with [^{14}C]PCP for 3 weeks by a modified "Tauchtrieb" technique (Freudenberg et al., 1955), lignin was isolated by the Björkman procedure, and copolymerization was demonstrated by gel permeation chromatography on Sephadex LH-60 in dimethylformamide (Toloczyki and Sandermann, 1983).

In the case of chlorinated anilines, a major mechanism for lignin incorporation consisted of a nucleophilic addition of the anilines to lignol quinonemethides (Still et al., 1981; von der Trenck et al., 1981). This mechanism could in principle also apply to PCP. The resulting benzylether linkage should be susceptible to the standard HBr–acetic acid reagent for ether cleavage ("Organikum. Organisch-Chemisches Grundpraktikum", 1963). However, PCP appeared to be only a minor split product, a number of more polar PCP derivatives being released instead. Similar polar products were also obtained by β-glucosidase digestion of the wheat soluble metabolite fraction. The structures of the released PCP derivatives remain to be elucidated. It is concluded that the incorporation of PCP-derived radioactivity into wheat lignin proceeds by a more complex mechanism than that described for chlorinated anilines.

In summary, the present report provides another example for the copolymerization of an environmental chemical into plant lignin. Previous examples where the crucial gel permeation step has been performed were chlorinated anilines (von der Trenck et al., 1981), 2,4-D, and its 4-hydroxy derivatives (Scheel and Sandermann, 1981b) and benzo[*a*]pyrene quinones (von der Trenck and Sandermann, 1981).

ACKNOWLEDGMENT

We thank D. Hunkler, Institut für Organische Chemie der Universität Freiburg, for the ^{13}C NMR spectrum of Figure 2.

Registry No. PCP, 87-86-5; PCP β-D-glucopyranoside, 28217-33-6.

LITERATURE CITED

Björkman, A. *Sven. Papperstidn.* **1956**, *59*, 477–485.

Cole, L. K.; Metcalf, R. L. In "Terrestrial Microcosms and Environmental Chemistry"; Witt, J. M., Gillett, J. W., Wyatt, J., Eds.; Oregon State University: Corvallis, OR, 1977; pp 57–73.

Crawford, R. L. "Lignin Biodegradation and Transformation"; Wiley: New York, 1981; pp 28–31.
Crosby, D. G.: Beynon, K. I.; Greve, P. A.; Korte, F.; Still, G. G.; Vonk, J. W. *Pure Appl. Chem.* **1981**, *53*, 1051–1080.
Freudenberg, K.; Reznik, H.; Fuchs, W.; Reichert, M. *Naturwissenschaften* **1955**, *42*, 29–35.
Gile, J. D.; Gillett, J. W. *J. Agric. Food Chem.* **1979**, *27*, 1159–1164.
Goldschmid, O. In "Lignins. Occurrence, Formation, Structure and Reactions"; Sarkanen, K. V.; Ludwig, C. H., Eds.; Wiley: New York, 1971; pp 241–266.
Haque, A.; Scheunert, I.; Korte, F. *Chemosphere* **1978**, *7*, 65–69.
Himmelsbach, D. S.; Barton, F. E. *J. Agric. Food Chem.* **1980**, *28*, 1203–1208.
Lai, Y. Z.; Sarkanen, K. V. In "Lignins. Occurrence, Formation, Structure and Reactions"; Sarkanen, K. V.; Ludwig, C. H., Eds.; Wiley: New York, 1971; pp 165–240.
Langebartels, C.; Harms, H. *Z. Pflanzenphysiol.* **1984**, *113*, 201–211.
Lüdemann, H.-D.; Nimz, H. *Makromol. Chem.* **1974**, *175*, 2409–2422.
Marty, F.; Branton, D.; Leigh, R. A. In "The Biochemistry of Plants. A Comprehensive Treatise"; Stumpf, P. K.; Conn, E. E., Eds.; Academic Press: New York, Vol. 1, 1980; pp 625–658.
Mumma, R. O.; Davidonis, G. H. In "Progress in Pesticide Biochemistry and Toxicology"; Hutson, D. H.; Roberts, T. R., Eds.; Wiley: New York, 1983; Vol. 3, pp 255–278.
Mumma, R. O.; Hamilton, R. H. In "Xenobiotic Metabolism. In Vitro Methods"; Paulson, G. D.; Frear, D. S.; Marks, E. P., Eds.; American Chemical Society: Washington, DC, 1979; ACS Symp. Ser. No. 97, pp 35–76.
Nimz, H.; Ebel, J.; Grisebach, H. *Z. Naturforsch., C. Biosci.* **1975**, *30C*, 442–444.
Nimz, H.; Mogharab, I.; Lüdemann, H.-D. *Makromol. Chem.* **1974**, *175*, 2563–2575.
"Organikum. Organish-Chemisches Grundpraktikum"; Autorenkollektiv der Techischen Universität Dresden, Eds.; VEB Deutscher Verlag der Wissenschaften: Berlin, 1963; pp 162–163.
Rao, K. R., Ed. "Pentachlorophenol. Chemistry, Pharmacology and Environmental Toxicology"; Plenum Press: New York, 1978.
Sandermann, H.; Diesperger, H.; Scheel, D. In "Plant Tissue Culture and Its Bio-Technological Application"; Barz, W.; Reinhard, E.; Zenk, M. H., Ed.; Springer-Verlag: Berlin, 1977; pp 178–196.
Sandermann, H.; Scheel, D.; von der Trenck, T. *J. Appl. Polym. Sci.: Appl. Polym. Symp.* **1983**, *37*, 407–420.
Scheel, D.; Sandermann, H. *Planta* **1977**, *133*, 315–320.
Scheel, D.; Sandermann, H. *Planta* **1981a**, *152*, 248–252.
Scheel, D.; Sandermann, H. *Planta* **1981b**, *152*, 253–258.
Scheel, D.; von der Trenck, K. T.; Sandermann, H.; Langebartels, C.; Harms, H.; Schuphan, I.; Haque, A.; Ebing, W. In "Methoden zur ökotoxikologischen Bewertung von Umweltchemikalien"; Führ, F., Ed.; Spezielle Berichte der Kernforschungsanlage Jülich: Jülich, 1984; Vol. 3, in press.
Schmitt, R.; Sandermann, H. *Z. Naturforsch., C: Biosci.* **1982**, *37C*, 772–777.
Still, G. G.; Balba, H. M.; Mansager, E. R. *J. Agric. Food Chem.* **1981**, *29*, 739–746.
Toloczyki, C.; Sandermann, H., Institut für Biologie II, Universität Freiburg, unpublished results, 1983.
von der Trenck, K. T.; Hunkler, D.; Sandermann, H. *Z. Naturforsch. C: Biosci.* **1981**, *36C*, 714–720.
von der Trenck, K. T.; Sandermann, H. *Planta* **1978**, *141*, 245–251.
von der Trenck, T.; Sandermann, H. *FEBS Lett.* **1981**, *125*, 72–76.
von der Trenck, T.; Scheel, D.; Sandermann, H. *Hoppe Seyler's Z. Physiol. Chem.* **1979**, *360*, 1199.
Weiss, U. M.; Moza, P.; Scheunert, I.; Haque, A.; Korte, F. *J. Agric. Food Chem.* **1982**, *30*, 1186–1190.

Received for review February 6, 1984. Revised manuscript received May 31, 1984. Accepted July 10, 1984. This work has been supported by BMFT and in part by DFG and the Fonds der Chemischen Industrie.

Effects of Pesticide Treatments on the Carotenoid Pigments of Lettuce

Jean Rouchaud,* Chantal Moons, and Joseph A. Meyer

Plots of lettuce were grown in the field in soils treated with one of the herbicides propyzamide or chlorpropham or with their mixture; other plots of lettuce were treated with one of the fungicides benomyl, iprodione, or vinclozolin, and four harvests were made. The treated lettuces, or the ones grown in treated soils, were compared to the untreated lettuces grown in untreated soils. The concentration of total carotene was higher in the lettuces treated with one of the herbicides propyzamide or chlorpropham or with their mixture or with the fungicide iprodione; it was generally the same for the lettuces treated with one of the fungicides benomyl or vinclozolin. Each of the β-cryptoxanthin, lutein, violaxanthin, and neoxanthin contents was increased by each of the propyzamide, chlorpropham, propyzamide plus chlorpropham, and iprodione treatments but not by the benomyl and vinclozolin treatments.

Selected urea herbicides and organophosphoro insecticides incorporated into the soil can either increase or decrease the carotene contents of the carrots grown in these soils (Rouchaud et al., 1982, 1983). The herbicide metachlor decreases the carotene content of sorghum (Wilkinson, 1981). The growth regulator ethephon enhances the β-carotene and the lycopene content of tomato (Buescher and Doherty, 1978). Hormone treatment enhances carotenoid accumulation in wheat leaf (Duysen and Freeman, 1976).

At our knowledge, very few studies have been made about the influence of the pesticide treatments on the provitamin A content of the lettuce. In the present work, we studied the influence of two herbicides applied separately or as a mixture. These are propyzamide, i.e., 3,5-dichloro-*N*-(1,1-dimethylpropynyl)benzamide, and chlorpropham, i.e., isopropyl 3-chlorophenylcarbamate. Three

Laboratory of Phytopathology, Université Catholique de Louvain, 3, Place Croix du Sud, SCI. 15D, 1348 Louvain-la-Neuve, Belgium.

0021-8561/84/1432-1241$01.50/0