Bull. Environ. Contam. Toxicol. (1992) 49:821–826

Environmental Contamination and Toxicology

Characterization of Monochlorinated Biphenyl Products Formed by Paul's Scarlet Rose Cells

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In previous work (Fletcher et al. 1987), it was shown that during a 76-hr exposure axenic plant cultures metabolized 83% of the provided ^{14}C-2-chlorobiphenyl with the metabolized ^{14}C distributed approximately evenly between a methanol-H_2O fraction and the insoluble residue. Thin layer chromatograms of the methanol-H_2O fraction showed that the product(s) was not the parent PCB, but the identity of the product(s) were not established, nor have they been established in similar studies employing combined plant-microbe systems (Moza et al. 1974, 1976, 1979). The present investigation was conducted to determine the PCB-product(s) formed from carbon-14 labeled 2-chlorobiphenyl provided to 11 d-old tissue culture cells of Rosa sp. cv Paul's Scarlet.

MATERIALS AND METHODS

Methanol-H_2O fractions analyzed in the present study came from previous work (Fletcher et al. 1987) in which Paul's Scarlet Rose cultures exposed to ^{14}C-2-chlorobiphenyl for 72 hr had been homogenized and subjected to solvent extraction. Following phase separation of the chloroform-methanol-H_2O mix, the chloroform and methanol-H_2O fractions were sampled for ^{14}C analyses (Fletcher et al. 1987), and the remainder stored in glass bottles at −10°C. The analyses reported in the present communication were initiated by drying the stored methanol-H_2O fraction under nitrogen at 40°C and resuspending in 50% methanol-H_2O. Individual aliquots ranging from 50 to 100 μL were analyzed by reverse-phase liquid chromatography using a Waters Associates M-273 microprocessor controlled gradient HPLC system, equipped with a Waters M-480 variable wavelength detector and a Waters resolve C_{18} 5μm Radial-PAK column. Separation was accomplished using a gradient protocol with H_2O as solvent A and methanol as solvent B. The gradient consisted of 20% methanol (0 to

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30 min), increasing from 20-80% methanol (30 to 40 min), and 80% methanol thereafter. The flow rate was 1.0 mL/min. The eluent was monitored by measuring absorbance at 280 nm, and 1.0 mL fractions were collected. Fifty μL aliquots of each fraction were assayed for radioactivity. Fractions possessing the majority of the ^{14}C (Figure 1, peak B) were pooled and combined with samples from 8 replicate runs. The pooled sample (80 mL) was concentrated under a nitrogen stream and diluted to 20 mL with 0.2 M Na acetate buffer, pH 4.5. Fifteen mL of the buffered sample were reacted with 5,000 units of β-glucuronidase (Sigma Type H-1) for 24 hr at 37°C in a shaking water bath. This enzyme was used according to the procedures of Dodge et al. (1979) where they showed that biphenyl conjugates synthesized from biphenyl by 12 different species of fungi were hydrolyzed when extracts were incubated with β-glucuronidase. One unit of enzyme liberated 1 μg/h of phenolphthalein from phenolphthalein glucuronide at 37°C and pH of 5.0. The remaining 5 mL of the buffered sample served as a control, and was incubated for 24 h at 37°C without the β-glucuronidase. Following the incubation the control and enzyme treated samples were extracted with 13 and 52 mL of ethyl acetate, respectively. The ethyl acetate extracts were reduced almost to dryness and redissolved in 1 mL of acetonitrile. Aliquots (100 μL) were then injected onto a μ Bondapak C_{18} 10μm Radial-PAK column which was subjected to an acetonitrile -H_2O step gradient of CH_3CN-H_2O at a rate of 1.5 mL/min. The gradient consisted of 30% CH_3CN (0 to 35 min), increasing from 30 to 70% CH_3CN (35 to 50 min), and 70% CH_3CN thereafter. Both the absorbance at 280 nm and the radioactivity of the effluent was monitored as described above. The fractions possessing the greatest amount of ^{14}C were pooled and the CH_3CN evaporated off under a nitrogen stream. The remaining sample was extracted with ethyl acetate. Concentrated samples of the ethyl acetate extract were analyzed using a Hewlett-Packard 5985 B GC/MS system equiped with a 25 m J and W Scientific DB-S column (0.25 μm film thickness, 0.25 mm ID). Aliquots (5-7 μL) of ethyl acetate extracts were injected with a 5 min time delay to clear the sample solvent from the column. After ramp start conditions were 50°C for 1 min, 30°C/min until 110°C, then 10°C/min until 280°C.

RESULTS AND DISCUSSION

The majority of the ^{14}C present in the methanol-H_2O fraction eluted as a single peak at approximately 48 min through the run (Figure 1). This radioactivity was associated with a relatively small proportion of the total organic material passing through the column since there was substantial absorption by components eluting at other times, but not by materials eluting at 48 min.

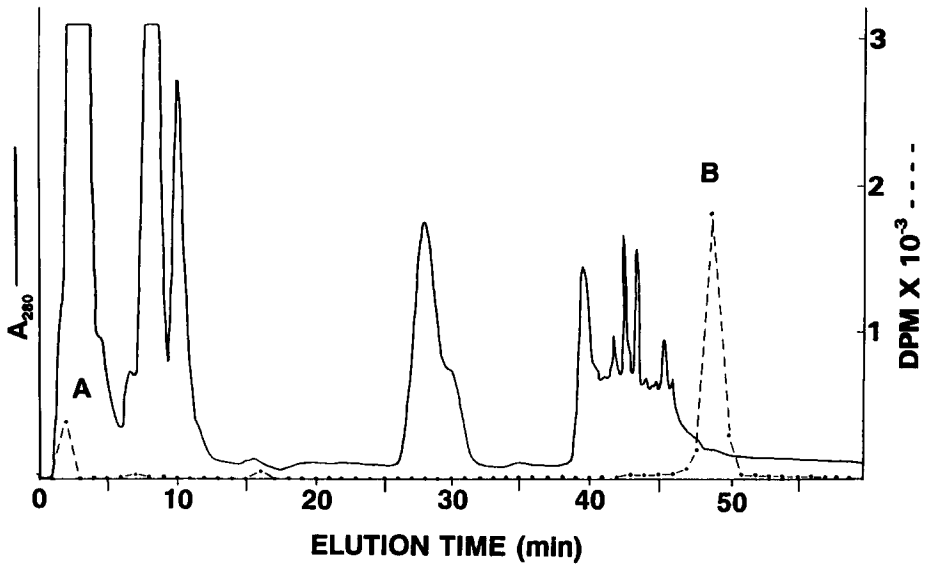

Figure 1. HPLC elution profile of methanol-H_2O soluble metabolites recovered from rose cells following a 76-hr incubation with ^{14}C-2-chlorobiphenyl.

When the radioactive material eluting at 48 min (Figure 1) was reacted with β-glucuronidase for 24 h, approximately 90% of the radioactivity was recovered in the ethylacetate phase of an H_2O-ethylacetate phase separation. In contrast, control material (48 min eluent, Figure 1, not treated with β-glucuronidase) partitioned in a 65 to 35 ratio between H_2O and ethylacetate. This indicated that the enzyme had cleaved a sugar moiety from a polar, H_2O soluble compound and formed a less polar product, presumably a hydroxylated derivative of the parent PCB provided to the rose cells. This interpretation of the results suggested that when rose cells were provided with 2-chlorobiphenyl, the compound was first hydroxylated and subsequently glycosylated. This contention was investigated further by comparing the chromatographic properties of the enzyme product with that of control material which had not been subjected to β-glucuronidase. All of the radioactivity in the control material eluted as a single fraction (Figure 2, peak A), whereas the ^{14}C, in the enzyme treated material was eluted at two distinctly different times (Figure 2, peaks B and C). The elution time of the B peak approximated that of the material eluting at A on the control chromatogram. The C peak which possessed the majority of the ^{14}C, and eluted at a much later time, appeared to be the product of β-glucuronidase reaction since it was not present on the control chromatogram. When the material eluting as peak C was subjected to mass spectral analysis it was shown that the fragmentation pattern observed at

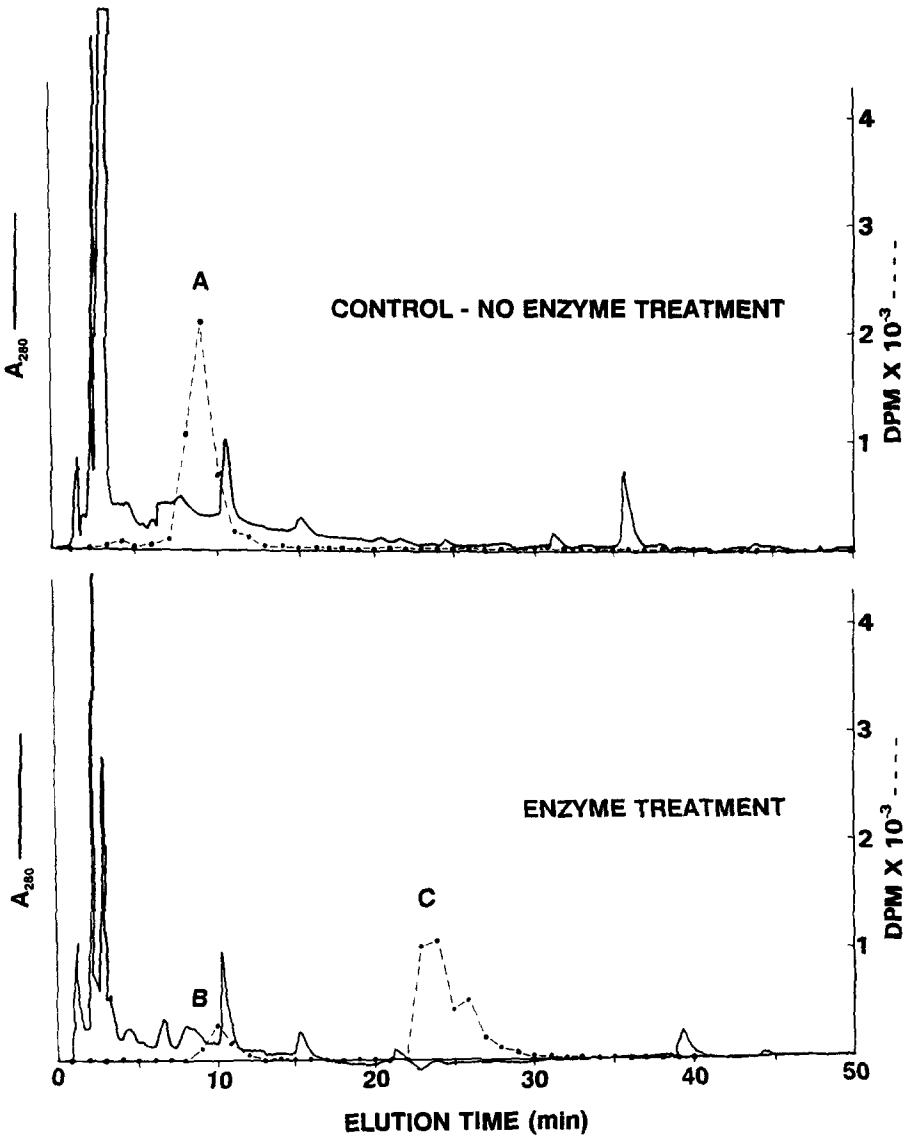

Figure 2. HPLC elution profile of control and β-glucuoronidase treated radioactive material which had been partially purified from rose extracts (Figure 1, peak B).

204 m/e (Figure 3) was identical to that of a single hydroxylated monochlorobiphenyl as demonstrated in our laboratory and as reported in the literature (Safe and Hutzinger 1973).

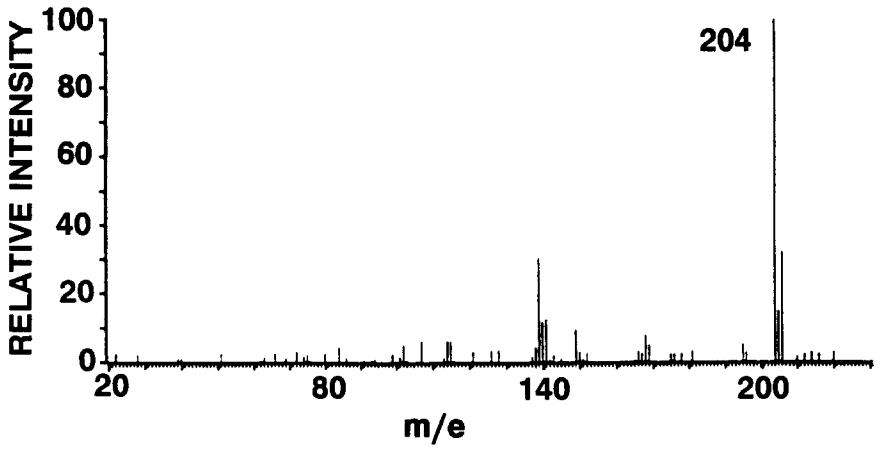

Figure 3. Electron-impact mass spectra of partially purified radioactive material (Figure 2, Peak C) recovered from rose cells.

This investigation demonstrated for the first time that plant cells are capable of hydroxylating and glycosylating a chlorinated biphenyl in a manner similar to what has been reported for animals (Sandermann 1982). The physiology of rapidly growing, nonphotosynthetic tissue culture cells used in our research is comparable to that of cells present in the young roots of intact plants. Considering the large biomass and spatial distribution of plant roots, the metabolism of PCBs by plants in natural habitats may be considerable. The fibrous roots of plants extend laterally and vertically for several feet, and since their fine roots are continuously growing and dying, it follows that the plant root serves as a dynamic probe of the soil. The production and subsequent release of hydroxylated PCBs by either living or dead roots would favor microbial degradation of the compounds. Plant species with the greatest capacity to carry out these events are unknown, an issue to be resolved by screening studies in progress in our laboratory.

Acknowledgements. We thank John Laing and the University of Oklahoma, Department of Chemistry, for the GC/MS analyses which they provided.

REFERENCES

Fletcher JS, AW Groeger, JC McFarlane (1987) Metabolism of 2-chlorobiphenyl by suspension cultures of Paul's Scarlet rose. Bull Environ Contam Toxicol 39: 960-965

Dodge RH, Cerniglia CE and Gibson DT (1979) Fungal metabolism of biphenyl. Biochem J 178:223-230

Moza P, Weisgerber W, Klein W, Korte F (1974) Metabolism of 2,2'-dichlorobiphenyl-^{14}C in two plant-water-soil systems. Bull Environ Contam Toxicol 12:541-546

Moza P, Kilzer L, Weisgerber I, Klein W (1976) Metabolism of 2,5,4'-trichlorobiphenyl-^{14}C and 2,4,6,2',4'-pentachlorobiphenyl-^{14}C in the marsh plant Veronica beccabunga. Bull Environ Contam Toxicol 16:454-463

Moza P, Scheunert I, Klein W, Korte F (1979) Studies with 2,4'5'-trichlorobiphenyl-^{14}C and 2,2',4,4',6-pentachlorobiphenyl-^{14}C in carrots, sugar beets, and soil. J Agric Food Chem 27:1120-1124

Safe S, Hutzinger O (1973) Mass spectrometry of pesticides and pollutants. CRC Press, Cleveland, Ohio

Sandermann H (1982) Metabolism of environmental chemicals: A comparison of plant and liver enzyme systems. In: Klekowski EJ (ed) Environmental Mutagensis, Carcinogensis, and Plant Biology, vol I. Praeger, New York, pp 2-32

Received October 10, 1991; accepted July 13, 1992.