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Comparison of the Metabolism of 5,6-Dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide (Carboxin) in Peanut Plants and Peanut Cell Suspension Cultures

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The metabolism of carboxin in seeds from peanut plants (*Arachis hypogaea* L.) was studied and compared to carboxin metabolism in peanut cell suspension cultures. The following metabolites were identified from both sources by chromatographic and mass spectral properties: 5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide 4-oxide (carboxin sulfoxide), 5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide 4,4-dioxide (carboxin sulfone), malonanilic acid, the β-D-*O*-glucoside of *p*-hydroxymalonanilic acid, and the glucose ester of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid. Oxidation of sulfur and hydrolysis of the carboxamide bond appeared to be the initial metabolic reactions in peanut seeds and peanut cell suspension cultures. The major difference in metabolism between the two systems appeared to be incorporation of ^{14}C into the bound residue. Whereas 2.7% of the applied radioactivity was recovered in the bound residue from peanut cell cultures, 20.7% of the recovered ^{14}C was found in this fraction in peanut seeds. A malonyl transferase activity that catalyzed the formation of malonanilic acid from aniline and malonyl-CoA was detected in cell-free extracts from peanut cell suspension cultures.

Carboxin is a systemic fungicide commonly used as a seed treatment to control various fungi that cause seed and seedling diseases in agronomic and horticultural crops (von Schmeling and Kulka, 1966). It is particularly effective against basidiomycetes (Edgington et al., 1966). The mechanism of action of carboxin appears to be inhibition of mitochrondrial respiration at the site of succinate oxidation (Mathre, 1971).

Carboxin metabolism has been studied in a number of different plant, animal, and soil systems. In most cases, including studies with wheat, barley, and soil, carboxin sulfoxide has been reported as a major metabolite while the corresponding sulfone has only been found in low concentrations (Chin et al., 1970a,b). Oxidation of carboxin to the sulfone was reported to be a major process in a *Pseudomonas aeruginosa* isolate from soil (Balasubramanya et al., 1980). In that system, the sulfone was further metabolized to 2-[(2-hydroxyethyl)sulfonyl]acetic acid and aminophenol. Interestingly, neither carboxin sulfoxide nor carboxin sulfone was found as a urinary metabolite in rats and rabbits (Waring, 1973). Hydroxylation of the aromatic ring to yield the corresponding *o*- and *p*-hydroxy derivatives appeared to be the initial process. These derivatives were isolated from the urine as the corresponding *O*-glucuronides. Hydroxylation of carboxin at the para position also appeared to be a major process in barley, leading to both soluble and bound residues (Briggs et al., 1974). Hydrolysis of the amide bond by an aryl acylamidase from *Bacillus sphaericus* has been reported (Engelhardt et al., 1973).

The purpose of this study was to determine and compare the metabolites of carboxin isolated from seeds of treated peanut plants and peanut cell suspension cultures.

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MATERIALS AND METHODS

Chemicals. Carboxin, [*aniline*-U-^{14}C]carboxin (4.285 mCi/mmol), [*oxathiin*-5,6-^{14}C]carboxin (4.49 mCi/mmol),

carboxin sulfoxide, carboxin sulfone, *p*-hydroxycarboxin, *p*-hydroxycarboxin sulfoxide, 2-acetyl-2-mercaptoethanol, acetanilide, 2-vinylsulfinyl acetanilide, 2-[(2-hydroxyethyl)sulfinyl]acetanilide, oxathiin acid, oxathiin acid sulfoxide, and oxathiin acid sulfone were provided by the Uniroyal Chemical Co. Purity of these compounds was determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Methyl malonanilate was synthesized from aniline and dimethyl malonate by the methods of Patel and Mehta (1960) and Chattaway and Mason (1910). This product was purified by HPLC, and its structure confirmed by electron impact (EI) mass spectrometry. Malonanilic acid was prepared by hydrolysis of methyl malonanilate by the method of Chattaway and Mason (1910).

The β-D-*O*-glucopyranoside tetraacetate of *p*-hydroxymalonanilate methyl ester was synthesized in three steps. *p*-Nitrophenyl-β-D-*O*-glucopyranoside (1 mmol) was acetylated with acetic anhydride (1.8 mL) in the presence of pyridine (0.2 mL) for 60 min at 30 °C. The reaction mixture was concentrated under vacuum, dissolved in 5.0 mL of dichloromethane, and treated with hydrogen (38 psi) in the presence of platinum oxide (20 μg) for 60 min at ambient temperature in a shaking hydrogenator. The resultant β-D-*O*-glucopyranoside tetraacetate of *p*-aminophenol was concentrated and then refluxed for 30 min with dimethyl malonate (1:10 v/v). The β-D-*O*-glucopyranoside tetraacetate of *p*-hydroxymalonanilate methyl ester was purified by HPLC, and the structure confirmed by EI and direct probe chemical ionization (DCI) mass spectrometry.

Derivatization Procedures. Esterifications were performed by bubbling diazomethane into methanolic solutions of samples until a yellow color persisted (Schlenk and Gellerman, 1960). Reaction mixtures were incubated for 15 min at 4 °C with occasional shaking.

Acetylations were performed by dissolving or suspending the samples in acetic anhydride containing 10% pyridine and incubating at 30 °C for 60 min in tubes sealed under nitrogen.

Radioactive Analyses. Radioactivity from treating solutions, peanut plant and cell suspension culture extracts, nonextractable plant residues, column effluent fractions, thin-layer plates, etc. was measured or monitored by methods previously described (Lamoureux and Rusness, 1980).

Mass Spectrometry. Mass spectrometry was performed on a Varian MAT 112S mass spectrometer equipped with a dual electron impact (EI) and chemical ionization (CI) source. Conventional EI and CI spectra were obtained by introducing the sample with a heated solid sample probe.

Direct chemical ionization (DCI) spectra were obtained with an unheated probe equipped with a Vespel tip that was inserted directly into the source of the mass spectrometer in a manner similar to that described by Cotter (1980). Ammonia was used as the reagent gas for CI and DCI mass spectrometry. A Hewlett-Packard 5992A gas chromatograph/mass spectrometer (GC-EI) equipped with an OV-101 capillary column (12 m) and an electron impact source was used in the analyses of several samples. The gas chromatograph was operated with an injector temperature of 200 °C and an initial oven temperature of 120 °C. Three minutes after sample injection, the oven temperature was increased to 375 °C at 10 °C/min.

Chromatographic Methods. Thin-layer chromatograms were developed on 250-μm silica gel HF thin-layer plates (Analab) by using chloroform–acetone–methanol (92:6:2). Products were detected under ultraviolet light and/or with a radiochromatogram scanner.

High-performance liquid chromatography was accomplished with a Beckman Model 334 HPLC system. Three HPLC columns were used: column I, Altex Ultrasphere 5-μm C-18 reverse phase column (4.6 mm i.d. × 25 cm); column II, Waters Associates, Inc., 10-μm C-18 reverse-phase radial compression column (8-mm i.d.); column III, Waters Associates, Inc., 10-μm silica gel normal-phase radial compression column (8-mm i.d.). Two primary solvent elution programs were used with the C-18 columns: With solvent program A, columns were eluted at 1.0 mL/min for 10 min with acetonitrile–acetic acid–water (10:1:89) and then during the next 75 min the solvent composition was changed with a linear gradient to (85:1:14). With solvent program B, columns were eluted at 1.0 mL/min for 10 min with acetonitrile–acetic acid–water (5:1:94), followed by an 80-min linear gradient of acetonitrile–acetic acid–water (5:1:94 to 85:1:14). Other solvent systems used will be described later. Columns I and II were used in different combinations of solvent elution programs and are designated as follows: column I with solvent program A, HPLC system A; column I with solvent program B, HPLC system B; column I with solvent program B (methanol substituted for acetonitrile), HPLC system C; column II with solvent program A, HPLC system D; and column II with solvent program B (methanol substituted for acetonitrile), HPLC system E. Nonradioactive products were detected by ultraviolet absorption at 254 nm, and radioactive products were detected with a Berthold LB503 HPLC radioactivity monitor equipped with a 400-μL flow cell filled with a glass scintillator.

Ion-exchange chromatography was performed with 1-x 13-cm columns of AG 1-X2 anion-exchange resin (200-400 mesh, acetate form) or AG 50W-X2 cation-exchange resin (200–400 mesh, H^+ form) (Bio-Rad Laboratories). The anion-exchange columns were eluted with five bed volumes of water and then with five bed volumes of 6 N acetic acid. The cation exchange columns were eluted with water.

Treatment of Peanut Plants and Extraction of Seeds. Peanut plants were grown in greenhouses in vermiculite irrigated with nutrient solution. The plants were treated with a spray of [*aniline*-U-^{14}C]carboxin 28 and 10 days before harvest of the mature fruit. Each plant was treated with a total of 39.2 μCi of carboxin (112.2 mg of carboxin, specific radioactivity 0.0823 mCi/mmol). All above-ground parts of the plants and the vermiculite were exposed to the spray.

The mature fruit from two peanut plants was harvested and divided into three 200-g lots that were shelled and extracted separately. The extraction and fractionation are outlined in Figure 1. Residues were separated from the extracts by centrifugation after each solvent extraction step. The peanut seeds from each lot were extracted with four 250-mL portions of hexane in an omnimixer (Sorvall, Inc.) for 2 min at 4 °C. The resultant residue was extracted 3 times by stirring with 120-mL portions of dichloromethane. Due to the high specific gravity of dichloromethane, appreciable amounts of insoluble residue remained suspended in the extracts after centrifugation. Therefore, these extracts were combined, concentrated to near dryness, and extracted twice with 50-mL portions of ethyl acetate and centrifuged. The ethyl acetate extracts were pooled and concentrated. The concentrate was applied in 0.4 mL of ethyl acetate to a 0.9 × 32 cm dry silica gel column (silica gel for dry columns, Woelm). The column was eluted with 2% diethyl ether in hexane, 10% diethyl ether in hexane, ethyl acetate, and acetone. The ethyl acetate eluate was pooled, concentrated, and analyzed

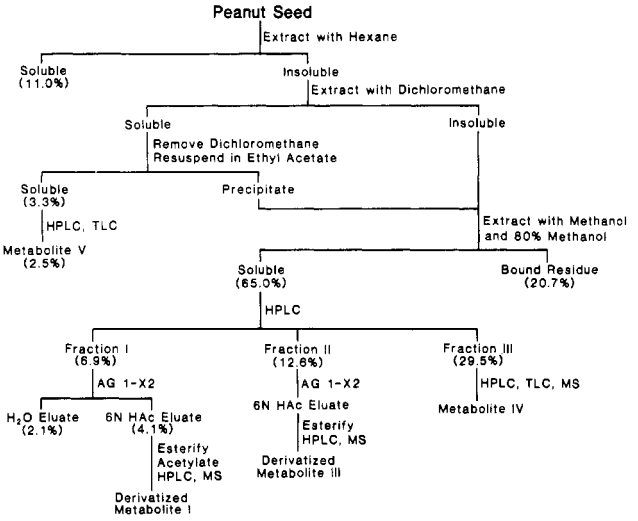

Figure 1. Extraction, purification, and derivatization of radioactive metabolites from seeds of peanut plants treated with [*aniline*-U-^{14}C]carboxin. Values in parentheses are the percent of the total recovered ^{14}C found in this form. Recovery of ^{14}C from peanut seeds was quantitative as determined by combustion analysis of unextracted peanuts.

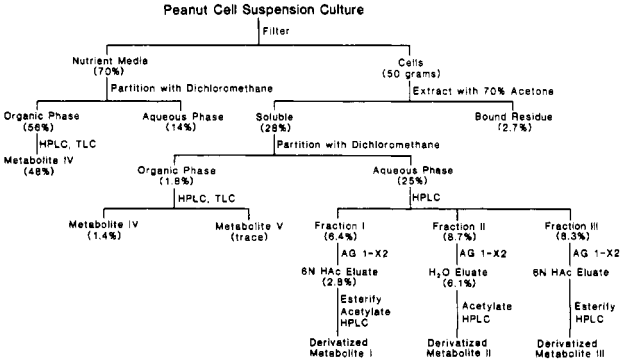

Figure 2. Extraction, purification, and derivatization of radioactive metabolites from peanut cell suspension cultures treated with [*aniline*-U-^{14}C]carboxin. Values in parentheses are the percent of the total ^{14}C applied to the cultures found in this form.

by HPLC system A and by TLC.

The small amount of residue from the concentrated ethyl acetate extract was combined with the residue from the hexane and dichloromethane extractions and extracted once with 250 mL of methanol and 5 times with 250-mL portions of 80% methanol. During the fifth extraction, the residue was stirred at ambient temperatures for 24 h. The residue was then extracted with methanol in a Soxhlet extractor for 22 h. The final residue was brought to a constant weight and assayed for radioactivity. The various methanol extracts, except from the Soxhlet extraction, were pooled, concentrated, and fractionated by HPLC system A into three radioactive fractions (fractions I, II, and III in order of increasing retention time).

Root Treatment of Peanut Plants and Extraction of Plants. To determine whether metabolites observed in peanut cell suspension cultures treated with [*oxathiin*-5,6-^{14}C]carboxin were present in peanut plants, the metabolism of carboxin was examined in root-treated plants. Peanuts were germinated in vermiculite, and after 3 weeks the seedlings were transferred to hydroponic culture in a growth chamber. Two plants were grown in 120-mL jars containing 80 mL of one-fourth strength Hoagland's solution. The temperature during the 14-h light period (light intensity 1600 ft-c) was 25 °C while the temperature during the dark period was 23 °C. The relative humidity was maintained at 40%. After 6 days, the nutrient solution was replaced with 80 mL of 6.3 μM [*oxathiin*-5,6-^{14}C]carboxin (2.16 mCi of ^{14}C) in water. Three days later, the plants were removed from carboxin solution intact, pulverized in liquid nitrogen, and homogenized with 50 mL of cold 70% aqueous acetone for 1 min in a Polytron homogenizer cooled in an ice bath. Following centrifugation, the supernatant was decanted. The extract and insoluble residues were processed by the methods described for the corresponding peanut cell suspension cultures discussed next.

Treatment and Extraction of Peanut Cell Suspension Culture. Peanut cell suspension cultures were developed and maintained as described by Lamoureux et al. (1981). Six days after transfer to flasks containing 50 mL of fresh media, the cultures were treated with [*aniline*-U-^{14}C]carboxin (1.41 mCi/mmol) or [*oxathiin*-5,6-^{14}C]-carboxin (1.70 mCi/mmol). The initial concentration of carboxin in each cell suspension culture was 5 μM. Seven days after treatment, the cultures were filtered and extracted with cold 70% acetone as outlined in Figure 2. The nutrient media was extracted 4 times with equal volumes of dichloromethane, and the dichloromethane phase was analyzed by HPLC system A and by TLC. The cells were homogenized 4 times with a total of approximately 4 volumes of cold 70% acetone/g net weight for 2 min in an omnimixer cooled in an ice bath. The homogenate was centrifuged, and the residue was suspended in acetone and dried at ambient temperature. The supernatant fraction was diluted 1.5-fold with water and extracted 4 times with equal volumes of dichloromethane. The organic phase was analyzed by TLC and HPLC system B. The aqueous phase from cells treated with [*aniline*-U-^{14}C]carboxin was fractionated by HPLC system A. The radioactivity in the most polar fraction (fraction I) was eluted from an AG 1-X2 anion-exchange column with 6 N acetic acid. The metabolite was methylated, acetylated, and purified by HPLC system B by the same methods used for metabolite I isolated from peanut seeds. The metabolite present in peanut cell culture fraction III and the metabolite in fraction II from peanut seeds had identical retention times on HPLC system A.

When peanut cells were treated with [*oxathiin*-5,6-^{14}C]carboxin, the radioactivity in the aqueous phase of dichloromethane-partitioned cell extracts was separated into two fractions by HPLC system A; the most polar fraction contained two peaks. The radioactivity in both fractions eluted from AG 1-X2 anion-exchange columns with water. The second HPLC fraction (fraction II) was further purified with AG 50W-X2 cation-exchange column. Fraction II was acetylated and purified by chromatography on HPLC system B.

Enzyme Extraction and Assay. A crude malonyl transferase enzyme was prepared from two 8-day-old peanut cell suspension cultures. The cells were harvested by centrifugation and resuspended in 50 mL of 0.05 M sodium phosphate buffer (pH 7.0) that was 0.3 M with respect to sucrose and 0.002 M with respect to dithiothreitol. The suspended cells were ruptured by two passages through a French press (10 000 psi). The cell-free supernatant was centrifuged twice at 8000*g* for 15 min to remove the larger particulate matter. The supernatant was then centrifuged twice at 100000*g* for 60 min. The resultant pellets were resuspended in the homogenizing buffer.

The cell-free homogenate and 100000*g* supernatant fractions were assayed for malonyl transferase activity. The reaction mixture consisted of 40 nmol of aniline, 20 nmol of [1,3-^{14}C]malonyl-CoA (0.016 μCi), 20 μg of bovine

serum albumin, and approximately 0.2 mg of protein in a total volume of 0.5 mL of 0.02 M sodium phosphate buffer, pH 7.0. The mixture was incubated for 60 min at 30 °C. The reaction was stopped by addition of 2 drops of concentrated hydrochloric acid and centrifuged for 2 min in a Beckman microfuge B. In the controls, either enzyme or aniline was omitted. Malonanilic acid formation was determined by chromatography of the clarified reaction mixture on HPLC system B. The radioactivity in the peak corresponding to malonanilic acid (31.2 min) was quantitated. Protein concentration was determined by the method of Bradford (1976).

RESULTS AND DISCUSSION

Isolation and Identification of the β-D-*O*-Glucoside of *p*-Hydroxymalonanilic Acid, Metabolite I. Metabolite I was isolated from fraction I of both peanut seeds and peanut cell suspension cultures treated with [*aniline*-U-^{14}C]carboxin, Figures 1 and 2. Fraction I of peanut seeds was separated into two components, one that eluted from AG-1 (acetate) with water and one that eluted with 6 N acetic acid. Only the acidic component was isolated from peanut cell suspension cultures. The acidic product (metabolite I) from both peanut seeds and suspension cultures had identical retention times on HPLC system B before and after esterification with diazomethane. Identical retention times also were observed on HPLC systems B and C after derivatization of metabolite I with both diazomethane and acetic anhydride. The esterified and acetylated derivative of metabolite I cochromatographed with synthetic β-D-*O*-glucopyranoside tetraacetate of *p*-hydroxymalonanilate methyl ester: on HPLC column I eluted with acetonitrile–acetic acid–water (35:1:64), on HPLC column II eluted with methanol–acetic acid–water (55:1:44), and on HPLC column III eluted with dichloromethane–tetrahydrofuran (60:40)—a flow rate of 1.0 mL/min was used in each case. The EI and DCI (ammonia) mass spectra of the synthetic standard and the esterified and acetylated derivative of metabolite I were identical. The EI mass spectra contained ion fragments characteristic of an acetylated glucoside at m/z 331, 271, 229, 187, 169, 127, and 109 (Kochetkov and Chizhov, 1972) but contained very few ions diagnostic of the aglycon. The DCI spectra were characterized by an intense pseudomolecular ion (M + 18) and many ions characteristic of the aglycon, Figure 3A. The even-electron nitrogen-containing glucose acetate ions observed in this spectrum, i.e., ions at m/z 186 and 348, are formed by attachment of neutral ammonia to CI fragment ions in the source (Binkley et al., 1974).

The results indicated that metabolite I was the β-D-*O*-glucopyranoside of *p*-hydroxymalonanilic acid. However, when metabolite I was hydrolyzed for 14 h at 80 °C with 1 N HCl under nitrogen, the radioactive products cochromatographed with authentic *o*-hydroxyaniline and *p*-hydroxyaniline on HPLC system B. This indicated that metabolite I is a mixture of the ortho and para isomers in a ratio of 1:2, respectively.

Isolation and Characterization of a Hexose Ester of 5,6-Dihydro-2-methyl-1,4-oxathiin-3-carboxylic Acid, Metabolite II. Metabolite II was observed in root-fed peanut plants and peanut cell suspension cultures treated with [*oxathiin*-5,6-^{14}C]carboxin. Metabolite II from both sources had identical retention times of 26.2 and 33.4 min in HPLC systems B and C, respectively. Metabolite II was not retained by either a strong anion-exchange resin (AG-1) or a strong cation-exchange resin (AG-50) and was therefore assumed to be nonionic. Metabolite II was isolated from peanut cell suspension cultures. The acetylated derivative was purified by chromatography on HPLC system B (61.6 min) and HPLC system C (68.7 min). Metabolite II was shown to be a hexose ester of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid by GC-EI and CI mass spectrometry of its acetylated derivative. The EI mass spectrum (GC retention time, 15.6 min) was characterized by a weak molecular ion at m/z 490 and a base peak at m/z 160 (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid). A strong fragment ion at m/z 143 represented a loss of OH from the carboxylic acid moiety. Fragment ions at m/z 331, 211, 169, and 109 are characteristic of glucose acetate conjugates (Kochetkov and Chizhov, 1972) and were present in the spectrum of acetylated metabolite II. The CI mass spectrum (Figure 3B) was characterized by a moderately strong M + 18 ion at m/z 508 and strong ions at m/z 178, 160, 143, and 108 that indicated the presence of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid. Fragment ions derived from the acetylated hexose moiety were observed at m/z 331, 271, and 169. In addition, hexose acetate fragments formed ion complexes with neutral ammonia; these ion complexes were observed at m/z 348, 288, 246, and 186 (Binkley et al., 1974).

Although the hexose moiety was not identified, most xenobiotic hexose conjugates isolated from plants have

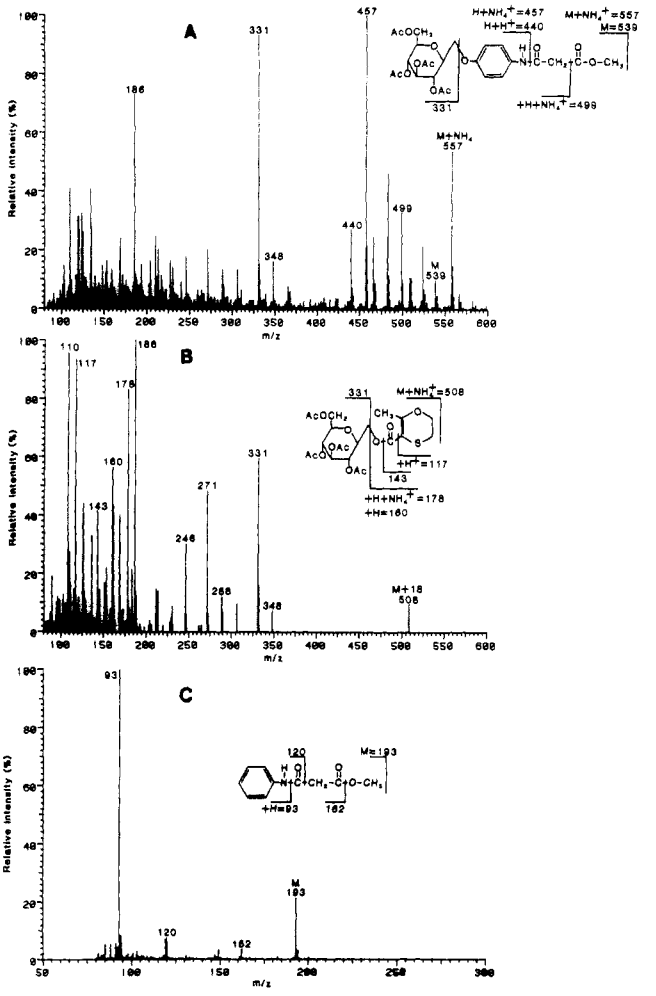

Figure 3. (A) Mass spectrum DCI (NH_3) of metabolite I, β-D-*O*-glucopyranoside of *p*-hydroxymalonanilic acid (acetylated methyl ester) isolated from peanut seeds. (B) Mass spectrum CI (NH_3) of metabolite II, glucose ester of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid (acetylated) isolated from peanut cell suspension culture. (C) Electron impact mass spectrum of metabolite III, malonanilic acid (methyl ester) isolated from peanut seeds.

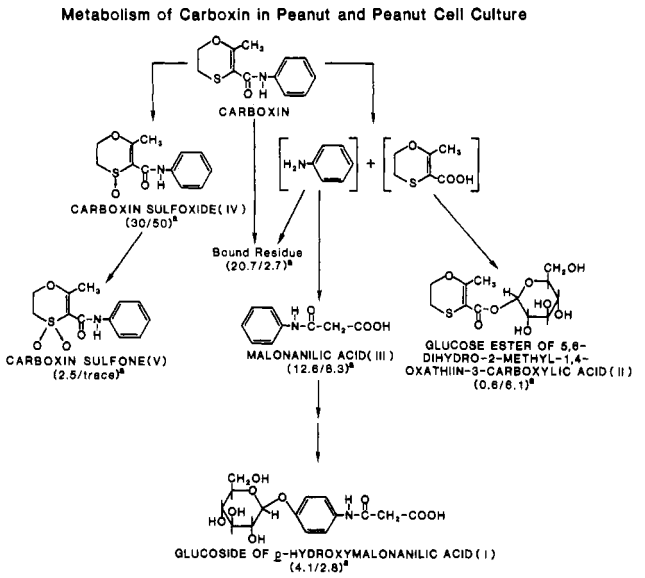

Figure 4. Proposed pathways for carboxin metabolism in peanut plants and peanut cell suspension cultures. [a]Values in parentheses are the percent of the total recovered ^{14}C found in the illustrated form from peanut plants and peanut cell suspension cultures (percent in plants/in cells).

been identified as glucosides or glucose esters (Shimabukuro et al., 1982). Therefore, metabolite II is depicted in both Figures 3B and 4 as being the glucose ester of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid.

Isolation and Identification of Malonanilic Acid, Metabolite III. Metabolite III was isolated from both peanut seeds and peanut cell suspension cultures treated with [*aniline*-U-^{14}C]carboxin by the methods outlined in Figures 1 and 2. This metabolite was eluted from the anion-exchange resin with 6 N acetic acid and was further purified by HPLC system E, esterified, and purified by HPLC system A. Metabolite III and synthetic malonanilic acid had identical chromatographic properties on HPLC system A before and after esterification. The EI mass spectra of metabolite III methyl ester isolated from peanut seeds and synthetic malonanilate methyl ester were identical. The mass spectra were characterized by an intense molecular ion (m/z 193) and a base peak at m/z 93 that corresponds to aniline (Figure 3C). Fragment ions at m/z 162 and 120 represent loss of methoxy and methyl acetate groups, respectively, from the malonyl moiety.

Isolation and Identification of Carboxin Sulfoxide, Metabolite IV. Carboxin sulfoxide was isolated from peanut plants and peanut cell suspension cultures treated with [*aniline*-U-^{14}C]carboxin (Figures 1 and 2) and was also isolated from peanut cell cultures treated with [*oxathiin*-5,6-^{14}C]carboxin. Carboxin sulfoxide from peanut plants (Figure 1) was purified by HPLC system C and identified by cochromatography with authentic carboxin sulfoxide (HPLC system B and TLC) and by EI mass spectrometry. The EI mass spectrum was characterized by a molecular ion at m/z 251 and a base peak at m/z 131 (5,6-dihydro-2-methyl-1,4-oxathiin 4-oxide).

Isolation and Identification of Carboxin Sulfone, Metabolite V. Carboxin sulfone was isolated from the dichloromethane–ethyl acetate extract of peanut seed (Figure 1). Trace amounts of the metabolite were also detected in the dichloromethane phase of the aqueous acetone extracts of peanut cell suspension cultures treated with either [*aniline*-U-^{14}C]- or [*oxathiin*-5,6-^{14}C]carboxin (Figure 2). Its identity was established by cochromatography with authentic carboxin sulfone with HPLC system A (39.2 min) and TLC (R_f 0.48).

Figure 4 summarizes the radioactive metabolites that have been isolated and identified and illustrates a proposed pathway of metabolism of carboxin in peanut seeds and peanut cell suspension cultures. Carboxin sulfoxide was the most abundant metabolite in peanut seeds (30%) and peanut cell suspension cultures (50%). Since only 10% of the carboxin added to autoclaved cell culture media was converted to carboxin sulfoxide after 7 days, it appeared that this oxidation primarily was mediated by the peanut cells rather than the culture media.

Carboxin sulfone was found in trace amounts in peanut cell suspension cultures and represented 2.5% of the total radioactivity recovered from peanut seeds. Both carboxin sulfoxide and carboxin sulfone have been reported as metabolites in wheat and barley (Chin et al., 1970a; Briggs et al., 1974).

Carboxin is hydrolyzed to aniline and 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid in both peanut plants and peanut cell suspension cultures, but neither metabolite was isolated in the unconjugated form. The hexose ester of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid accounted for 0.6% of the radioactivity in extracts of peanut plants and 6.1% in peanut cell suspension cultures treated with [*oxathiin*-5,6-^{14}C]carboxin. The aniline moiety was isolated as malonanilic acid and the β-D-*O*-glucoside of *p*-hydroxymalonanilic acid. Malonanilic acid accounted for 12.6% of the radioactivity recovered from the seeds of peanut plants treated with [*aniline*-U-^{14}C]-carboxin and 8.3% of the radioactivity recovered from peanut cell suspension cultures. The β-D-*O*-glucoside of *p*-hydroxymalonanilic acid accounted for 4.1% and 2.8% of the radioactivity recovered from peanut seeds and peanut cell suspension cultures, respectively.

In Figure 4, the aniline ring is depicted as being hydroxylated after hydrolysis. The present results do not prove that hydroxylation occurs after hydrolysis; however, peanut cell cultures treated with [U-^{14}C]aniline produced radioactive products that were chromatographically identical with malonanilic acid and the β-D-*O*-glucoside of *p*-hydroxymalonanilic acid. Although *p*-hydroxycarboxin was a major water-soluble metabolite in barley seedlings and mature plants (Briggs et al., 1974), it was not observed in these experiments.

A malonyl-CoA transferase enzyme activity capable of malonylating aniline was detected in homogenates of peanut cell suspension cultures. Under the conditions described, the crude homogenate catalyzed a 16% conversion of aniline to malonanilic acid, and the 100000*g* supernatant fraction catalyzed a 13% conversion. The results indicate the presence of a soluble enzyme in peanut cell suspension cultures capable of catalyzing the malonylation of aniline.

Interestingly, it appears that malonanilic acid has plant regulating capabilities. Stimulation of root elongation in rice seedlings treated with malonanilic acid was recently reported. Of 25 substituted malonanilates examined for plant regulating properties in rice and cucumber, unsubstituted malonanilic acid demonstrated the greatest stimulation of root elongation (Shindo and Keto, 1982).

The most obvious difference between the metabolism of carboxin in peanut seeds and peanut cell suspension cultures was the incorporation of ^{14}C into the bound residue. Whereas 2.7% of the applied radioactivity was recovered in the bound residue from cell cultures treated with [*aniline*-U-^{14}C]carboxin, 20.7% of the recovered ^{14}C was found in this fraction in peanut seeds. In other respects the metabolism of carboxin in peanut seeds and peanut cell suspension cultures appears to be very similar.

Oxidation of carboxin to the sulfoxide and hydrolysis of the carboxamide bond appeared to be the two primary metabolic reactions in both peanut seeds and peanut cell suspension cultures. Hydroxylation of the aniline moiety was observed in both systems. The conjugation of carboxin metabolites appeared to be similar in both peanut seeds and cell suspension cultures. Isolation of malonanilic acid and the β-D-*O*-glucoside of *p*-hydroxymalonanilic acid from peanut plants and cell suspension cultures indicated N-malonylation and *O*-glucoside formation of aniline and *p*-hydroxyaniline were common to both systems. The hexose ester of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid was characterized in both peanut plants and peanut cell suspension cultures, which suggested that both systems were capable of forming hexose esters.

It is apparent from the present study and from those described by Lamoureux et al. (1981) that most of the major detoxification pathways present in the peanut plant are also present in peanut cell suspension cultures. Peanut plants and cell suspension cultures also have the capacity to form glutathione conjugates with pentachloronitrobenzene (PCNB) (Lamoureux et al., 1981). The subsequent metabolism of the glutathione conjugates of PCNB appeared to be very similar in peanut roots and cell suspension cultures, with the primary differences being quantitative (Lamoureux et al., 1981). Therefore, peanut cell suspension cultures may be useful as a model system to predict the metabolism of a pesticide in intact peanut plants. Cell cultures can also serve as a convenient source of enzymes for in vitro studies of pesticide metabolism and its regulation.

ACKNOWLEDGMENT

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Registry No. I, 88412-31-1; II, 88412-32-2; III, 15580-32-2; IV, 17757-70-9; V, 5259-88-1; carboxin, 5234-68-4; β-D-*O*-glucopyranoside tetraacetate of *p*-hydroxymalonanilate methyl ester, 88412-33-3; *p*-nitrophenyl-β-D-*O*-glucopyranoside, 2492-87-7; β-D-*O*-glucopyranoside tetraacetate of *p*-aminophenol, 42011-36-9; malonyl transferase, 37257-17-3; aniline, 62-53-3; malonyl-CoA, 524-14-1.

LITERATURE CITED

Balasubramanya, R. H.; Patil, R. B.; Bhat, M. V.; Nagendrappa, G. *J. Environ. Sci. Health, Part B* **1980**, *B15* (5), 485.
Binkley, W. W.; Chizhov, O. S.; Kadentsev, V. I.; Solovyov, A. A. *J. Org. Chem.* **1974**, *39*, 451.
Bradford, M. M. *Anal. Chem.* **1976**, *72*, 248.
Briggs, D. E.; Waring, R. H.; Hackett, A. M. *Pestic. Sci.* **1974**, *5*, 599.
Chattaway, F. D.; Mason, F. A. *J. Chem. Soc.* **1910**, *97*, 339.
Chin, W.-T.; Stone, G. M.; Smith, A. E. *J. Agric. Food Chem.* **1970a** *18*, 709.
Chin, W.-T.; Stone, G. M.; Smith, A. E. *J. Agric. Food Chem.* **1970b**, *18*, 731.
Cotter, R. J. *Anal. Chem.* **1980**, *52* (14), 1589A.
Edgington, L. V.; Walton, G. S.; Miller, P. M. *Science (Washington, D.C.)* **1966**, *152*, 307.
Engelhardt, G.; Wallnofer, P. R.; Plapp, R. *Appl. Microbiol.* **1973**, *26*, 709.
Kochetkov, N. K.; Chizhov, O. S. In "Methods in Carbohydrate Chemistry"; Whistler, R. L., BeMiller, J. N., Eds.; Academic Press: New York, 1972; Vol. VI, p 540.
Lamoureux, G. L.; Gouot, J.-M.; Davis, D. G.; Rusness, D. G. *J. Agric. Food Chem.* **1981**, *29* (5), 996.
Lamoureux, G. L.; Rusness, D. G. *J. Agric. Food Chem.* **1980**, *28*, 1057.
Mathre, D. E. *Pestic. Biochem. Physiol.* **1971**, *1*, 216.
Patel, G. H.; Mehta, C. M. *J. Sci. Ind. Res.* **1960**, *19B*, 436.
Schlenk, H.; Gellerman, J. L. *Anal. Chem.* **1960**, *32*, 1412.
Shimabukuro, R. H.; Lamoureux, G. L.; Frear, D. S. In "Biodegradation of Pesticides"; Matsumura, F.; Murti, K., Eds.; Plenum Press: New York, 1982; p 21.
Shindo, N.; Keto, M. "Abstracts of Papers", 5th International Congress of Pesticide Chemistry, Kyoto, Japan, Aug 1982; International Union of Pure and Applied Chemistry: Oxford, 1982.
von Schmeling, B.; Kulka, M. *Science (Washington, D.C.)* **1966**, *152*, 659.
Waring, R. H. *Xenobiotica* **1973**, *3*, 65.

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