Metabolism of the Insecticide Phoxim in Plants and Cell Suspension Cultures of Soybean

Hans-Ulrich Höhl and Wolfgang Barz*

Institute of Plant Biochemistry and Biotechnology, Westfälische Wilhelms-Universität Münster, Hindenburgplatz 55, 48143 Münster, Germany

The metabolism of phoxim (*Z*-α-[[(diethoxyphosphinothioyl)oxy]imino]phenylacetonitrile), Volaton, was investigated using heterotrophic cell suspension cultures of soybean (*Glycine max* L.) and isolated organs (roots, stems, cotyledons, and leaves) of aseptically grown soybean plants. In both systems phoxim was first hydrolyzed to the corresponding oxime, which was then reduced to a primary nitrile amine. The primary amino group was *N*-malonylated as the terminal step of the catabolic sequence. The structure of this terminal metabolite was elucidated by spectroscopic (UV, IR, NMR, and MS) methods and chemical synthesis. In the plant no organ-specific differences in phoxim metabolism were observed. In the cell culture system phoxim was quantitatively converted to the *N*-malonate within 18–20 h, whereas in plant organs such extensive conversion could not be observed even within incubation times of about 5 days. The *N*-malonate was found to be excreted from the cultured cells into the medium; this effect could not be shown for the plant tissues.

Keywords: *Organothiophosphate metabolism; soybean plants and cell suspension cultures; conjugation reactions; N-malonylation; O-glucoside formation*

INTRODUCTION

Phoxim is a nonphytotoxic organothiophosphoric acid ester insecticide that is used against mites, beetles, and ants. The toxicity of phoxim results from a chemical modification of a seryl group in the active site of acetylcholinesterase. Until now only a few papers have described the metabolic behavior of phoxim and its degradation in plants. Known reactions are photocatalyzed dimerizations and oxidative desulfurizations of phoxim (Dräger, 1969, 1971) as well as hydrolysis of the ester bond, yielding an oximinophenylacetonitrile which may undergo Beckmann rearrangements under acidic conditions (Mason and Meloan, 1976). The oximinophenylacetonitrile was also conjugated to the corresponding glucoside and gentiobioside in *Lycopersicon esculentum* (Dräger, 1969).

The use of plant cell cultures for investigations on xenobiotic metabolism offers several advantages such as reliable sterility and rapid uptake of substrates. Plant metabolic reactions can thus be differentiated from abiotic transformations (Gohre and Miller, 1986) (i.e. influence of light, autoxidation) and from microbial reactions. The metabolism of phoxim has now been studied in heterotrophic cell suspension cultures of soybean (*Glycine max* L.) and in dissected organs derived from aseptically grown soybean plants. The main objectives were (a) to isolate and characterize metabolites and (b) to compare these two test systems with regard to their ability and their efficiency in metabolizing exogenously applied xenobiotics.

MATERIALS AND METHODS

Reagents. Samples of [*phenyl*-U-^{14}C]phoxim, specific radioactivity 1.19 MBq/mg, unlabeled phoxim, oximinophenylacetonitrile, and β-1-*O*-oximinophenylacetonitrile glucoside were kindly donated by Bayer AG (Leverkusen, Germany).

Procedures. *Cell Suspension Cultures.* Heterotrophic cell suspension cultures of soybean (*G. max* L.) were cultivated on B5 medium (Gamborg et al., 1976) in the dark on a rotary shaker (120 rpm, 26 °C). Cells were transferred weekly to fresh medium with an inoculum of 2 g fr wt/40 mL of nutrient medium.

Plants. Soybean seeds, variety Effi (Kleinwanzlebener Saatzucht KWS, Einbeck, Germany), were first defatted in ethanol (70%, 1 min) and then surface-sterilized in NaOCl (8%, 15 min). After five or six washes in sterile water, the seeds were germinated on sterile vermiculite in 1 L Erlenmeyer flasks, first in the dark and, after formation of shoots, in the light (6000 lx, 26 °C). The flasks were stoppered with silicone foam lids to allow sufficient gas exchange.

Feeding of Cell Cultures. Fifty microliters of [^{14}C]phoxim diluted with unlabeled phoxim to 18.5 kBq/40 mL of nutrient medium in a DMSO/phoxim (1:1 v/v) solution was applied on the fourth day of the growth cycle. To avoid thermal decomposition of phoxim, it had to be sterilized by passage through a membrane filter (type SM 16241, Sartorius, Germany).

After incubation, cells were separated from the culture medium by filtration and washed slightly for some seconds with petroleum ether (40–60 °C bp) to detect cell surface absorbed phoxim. With the same intention the culture vessels were rinsed twice with petroleum ether. After homogenization of the cells in 20 mL of acetone (−20 °C) (Ultraturrax, Janke + Kunkel, Germany), the extract was taken to dryness, redissolved in 5 mL of water, and extracted three times with CH_2Cl_2. The organic fractions which contained unmetabolized phoxim were pooled, concentrated, and redissolved in 100 μL of methanol. The aqueous fractions were acidified to pH 1.5 and again extracted with CH_2Cl_2 to extract acidic conjugates. The remaining aqueous solution contained glucosylated phoxim metabolites.

Feeding of Plants. Sterile soybean plants were cut in a Petri dish to separate leaves, stems, and roots, respectively. Plant organs were weighed and placed in 200 mL Erlenmeyer flasks containing 40 mL of B5 medium and preincubated for 5 days in the light (8000 lx, 26 °C). After contaminated flasks were eliminated, 50 μL of [^{14}C]phoxim, diluted with unlabeled material to 18.5 kBq in a DMSO/phoxim (1:1 v/v) solution, was applied.

Harvest and extraction procedures of the plant organs were the same as described for the plant cell cultures. Tissues were washed with petroleum ether (40–60 °C bp) and homogenized in a chilled mortar. Because of the high chlorophyll content of the tissues, the acetone homogenates were first extracted

* Author to whom correspondence should be adressed.

0021-8561/95/1443-1052$09.00/0

with petroleum ether (40–60 °C bp), and phase separation could be achieved by adding water dropwise to the acetone extracts.

Synthesis of Malonic Acid Methyl Ester Mono(phenylcyanomethylamide). *(I)* Phenylacetonitrileamine was synthesized according to the Strecker synthesis of amino acids. Nine grams of KCN, 7.5 g of NH_4Cl, and 25.5 mL of NH_3 were dissolved in a minimum volume of water (bidistilled). A solution of 15 g of benzaldehyde in methanol (1:2) was added, and the resulting emulsion was stirred for 4 h. After extraction with ether (three times), the combined organic phases were evaporated under reduced pressure to yield a yellow oil. HCl (10%) was added, and the mixture was treated in an ultrasonic bath until complete solution had occurred. The remaining benzaldehyde was removed by washing with ether, and the acidic phase was slowly evaporated under reduced pressure. Phenylacetonitrileamine crystallized in the form of its hydrochloride; the crystals (mp 175 °C) were dried by suction.

(II) $KO_2CCH_2CO_2CH_3$ (commercially available) (500 mg) was dissolved in 10 mL of CH_2Cl_2, and triphenylphosphine/C_2Cl_6 (2 g each) were added. The resulting acid chloride $ClOCCH_2CO_2CH_3$ (bp 66 °C, 22 Torr) was distilled and used at once for the next reaction.

(III) To prepare the free amine, 500 mg of phenylacetonitrileamine hydrochloride was dissolved in ethanol and 500 mg of Na_2CO_3 was added. The suspension was treated in an ultrasonic bath and extracted with ether. The resulting amine had to be used immediately for the next step because it is quite unstable to oxygen, acids, and bases. The distilled $ClOCCH_2CO_2CH_3$ (430 mg) was carefully added to the phenylacetonitrileamine (840 mg)/ether solution; addition of tertiary amines or pyridine should be avoided because of self-acylation of the $ClOCCH_2CO_2CH_3$. The reaction starts rapidly, resulting in a precipitation of the hydrochloride. After filtration, the organic phase was concentrated and stored at 4 °C. Crystals were obtained by suction, and the malonic acid methyl ester mono(phenylcyanomethylamide) was recrystallized from ether/CH_2Cl_2 (−20 °C): IR (KBr) wn (cm^{-1}) 3350 N-H, 3000 C-H, 1735 C=O, 1650 amide I, 1620 amide II, 1290 C-N, 750 *out of plane*; 690; 300 MHz ^1H NMR ($CDCl_3$) δ 3.42 (*s*, 2H, methylene protons), 3.76 (*s*, 3H, methyl ester), 6.10 (*d*, ^3J = 8 Hz, 1H, benzylic proton), 7.44 (*m*, 5H, phenyl protons), 8.02 (*s*, 1H, amide); EI-MS, *m/z* 232 ($M^{\bullet+}$, molecular ion), 201 ($M^{\bullet+} - OCH_3$; 31), 173 ($M^{\bullet+} - CO_2CH_3$; 59), 159 ($M^{\bullet+} - CH_2CO_2CH_3$; 73), 155 ($M^{\bullet+} - C_6H_5$; 77), 131 [$M^{\bullet+} - COCH_2CO_2CH_3$; 101; base peak), 116 ($M^{\bullet+} - C_6H_5CHCN$; 116), 101 ($COCH_2CO_2CH_3$), 77 ($C_6H_5$), 59 ($CO_2CH_3$).

Synthesis of Malonic Acid Mono(phenylcyanomethylamide). *(I) Synthesis of malonic acid monobenzyl ester* was performed with some modifications according to the procedure of Rosa and Neish (1968): 34.2 g of diethyl malonate, dissolved in 100 mL of benzylic alcohol, was slowly added to a solution of 12 g of KOH in 200 mL of benzylic alcohol. The resulting suspension was diluted by addition of benzylic alcohol, yielding a viscous syrup. After vigorous stirring (12 h), the suspension was diluted by addition of 1 L of ethyl acetate and stirred again. The suspension was separated on a glass funnel, and the gummy residue was suspended in ether. Malonic acid monobenzyl ester was crystallized from CH_2Cl_2/petroleum ether (bp 40–60 °C) (1:1): 300 MHz ^1H NMR (D_2O) δ 3.35 (*s*, 2H, methylene protons of malonic acid), 5.20 (*s*, 2H, benzylic protons), 7.42, 7.44, 7.45, 7.47 (*m*, 5H, aromatic protons).

(II) Synthesis of Malonic Acid Monobenzyl Ester Mono(phenylcyanomethylamide). Six grams of phenylacetonitrileamine hydrochloride and 8.25 g of malonic acid monobenzyl ester were suspended in 120 mL of CH_2Cl_2 (ultrasonic bath), whereby dicyclohexylcarbodiimide (38 °C) was slowly added. The suspension was stirred for 12 h, the reaction was stopped by addition of water, and 150 mL of ethyl acetate was added. The suspension was taken to dryness and the residue dissolved in CH_2Cl_2 (ultrasonic bath). Petroleum ether (bp 40–60 °C) was added to induce crystallization. After crystallization of dicyclohexylurea, the malonic acid monobenzyl ester mono(phenylcyanomethylamide) crystallized step by step over a period of 3 days after successive addition of petroleum ether (bp 40–60 °C): 300 MHz ^1H NMR ($CDCl_3$) δ 3.43 (*s*, 2H, methylene protons of malonic acid ester), 5.15 (*s*, 2H, benzylic protons of benzyl ester), 6.85 (*d*, ^3J = 8 Hz, 1H, benzylic proton of nitrileamine), 7.30–7.47 (*m*, 10H, 2 aromatic rings), 8.00 (*d*, ^3J = 8 Hz, 1H, amide proton); EI-MS, *m/z* 308 ($M^{\bullet+}$, molecular ion), 282 ($M^{\bullet+} - 26$, CN), 217 ($M^{\bullet+} - 91$, C_7H_7), 173 ($M^{\bullet+} - 135$, $CO_2CH_2C_6H_5$), 131 ($C_6H_5CHCNNH$), 116 (C_6H_5-CHCN), tropylium rearrangement 91 (C_7H_7, base peak), 65 ($C_7H_7 - C_2H_2$), phenylic cleavage 77 (C_6H_5), 51 ($C_6H_5 - C_2H_2$).

(III) Hydrogenolysis of Malonic Acid Monobenzyl Ester Mono(phenylcyanoethylamide). Malonic acid monobenzyl ester mono(phenylcyanomethylamide) (1.75 g) was cleaved by catalytic hydrogenation in 120 mL of ethanol over 240 mg of Pd/C (10%, 10 min). Reductive cleavage of toluene was monitored by TLC (SS-II). The suspension was separated on a D4 microfunnel and carefully (ice bath) taken to dryness to avoid thermal decarboxylation of the β-keto acid. Phenylcyanomethylaminomalonic acid precipitated in the form of white flocks and was recrystallized from ethanol/CH_2Cl_2: 300 MHz ^1H NMR (CD_3OD) δ 3.34 (*s*, 2H, methylene protons of malonic acid amide), 6.12 (*d*, ^3J = 8.02 Hz, 1H, benzylic proton), 7.42–7.46 (*mc*, 5H, aromatic ring), 7.51 (*d*, ^3J = 8.02 Hz, 1H, amide proton).

Spectroscopic analysis of tetrasilyl-β-1-*O*-oximinophenylacetonitrileamine glucoside: GC–MS, *m/z* 596 ($M^{\bullet+}$, molecular ion), 581 ($M^{\bullet+} - CH_3$, 15), 437 ($M^{\bullet+} - C_6H_5NCC{=}NO$, 145), 451 ($M^{\bullet+} - C_6H_5NCC{=}NOCH_2$, 159), fragments of persilylated glucose moiety, 103, 117, 129, 131, 133, 147, 169, 189, 191, 204, 217, 229, 231, 243, 257, 271, 291, 305, 319, 331.

Apparatus. *Measurement of Radioactivity.* Aliquots of liquid samples were added to 10 mL of Hydroluma scintillation cocktail (Fa. Bertold-Frieseke, Wildbach, Germany) and measured in a Betaszint BF 5000 (Fa. Bertold-Frieseke). Quenching effects were corrected by internal standardization. Thin-layer plates were scanned in an LB 2723 scanner (Fa. Bertold-Frieseke). The gel of radioactive zones was scraped off from the plates and suspended in 1 mL of methanol, and then 10 mL of Hydroluma scintillation cocktail was added.

Chromatography. Thin-layer chromatography was carried out with precoated silica gel GF_{254} (Fa. Merck, Germany) using the following solvent systems (ss) (v/v): SS-I, $CHCl_3$/*n*-hexane (1:1); SS-II, acetonitrile/ethyl acetate/formic acid (82:9:9); SS-III, ethyl acetate/methyl ethyl ketone/formic acid/water (5:3:3:1). Anion exchange chromatography was performed with Dowex 1X8 (mesh 100–200, formate form). Reversed-phase high-performance liquid chromatography (HPLC) analyses were performed with Latek HPLC equipment coupled with a RAMONA (Raytest) scintillation counting system. Chromatograms were developed using a linear gradient of acetic acid (3%) (A)/acetonitrile (B) with 80% (A) to 40% (A) in (A + B) within 25 min of monitoring the UV absorption at 254 nm. Middle-pressure liquid chromatography (MPLC) was performed with a Duramat pump (Chemie und Filter GmbH, Germany) using a LiChroprep Si 60/B column (40–60 μm) (Merck), flow 50 μL/min (8 bar) at a detection wavelength of 250 nm.

Spectroscopy. UV–vis spectra were recorded on an Uvicon 725 photometer (Fa. Kontron, Germany). KBr pellet spectra were recorded on a Perkin-Elmer 298 IR-spectrophotometer. ^1H-NMR spectra were performed on a Bruker WM 300 MHz NMR spectrometer at room temperature. Samples were dissolved in $CDCl_3$ (99.9%) or in CD_3OD (99.8%), respectively. Tetramethylsilane was used for internal standardization. Electron impact mass spectra (EI-MS) were obtained with a Varian MAT 312 mass spectrometer. Samples were analyzed directly utilizing the solid probe. Gas–liquid mass spectrometry (GC–MS) was performed with a Finnigan 4015 GC–MS spectrometer connected to a 9610 gas chromatograph. Analyses were carried out with a fused silica quartz SE 30 CB column. Helium (2 mL/min) served as a carrier gas, and a temperature program with an injector temperature of 230 °C was used as follows: from 90 (1 min) to 200 °C at 10 °C/min, from 200 to 300 °C at 8 °C/min, and held at 300 °C for 20 min.

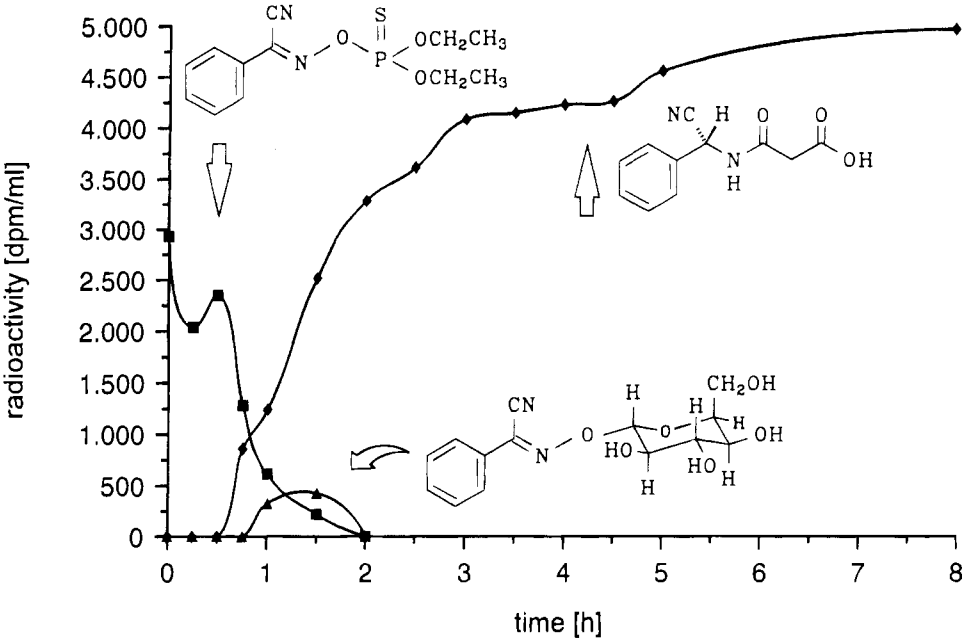

Figure 1. Kinetics of metabolite formation in cell suspension cultures of soybean after application of [*phenyl*-U-^{14}C]phoxim (18.5 kBq/40 mL, 4.3 mM).

RESULTS

(I) Phoxim Metabolism in Soybean Cell Suspension Cultures. Preliminary tests (data not shown) indicated that no significant *in vitro* destruction of phoxim occurred under the cell culture incubation conditions. Furthermore, application of a phoxim/DMSO (1:1) solution up to a final concentration of 4.2 mM showed no detrimental effect on the growth kinetics of the cell culture. However, the employment of ethanol or 2-methoxyethanol as solvent inhibited growth. Because of the low hydrophilicity of phoxim (solubility 7 mg/L of water) the substance immediately absorbed to cell surfaces after application, whereas solubilized phoxim could never be detected in the cell culture medium. After about 5 h, the absorbed phoxim had nearly quantitatively diffused into the cells. Beginning 30 min after substrate application, one metabolite could be detected by HPLC in the medium whose concentration increased with incubation time (Figure 1). This metabolite could only be extracted after acidification of the medium; therefore, it was assumed that this product might be an acidic derivate of phoxim. This hypothesis was supported by Dowex 1X8-anion exchange chromatography; the metabolite could be eluted with 6 N formic acid but not with water and 2 N formic acid, respectively. Oximinophenylacetonitrile as a possible candidate for this metabolite could be excluded after TLC cochromatography (SS-III) with reference material. The metabolite was further purified by MPLC and, after methylation, by HPLC (R_t = 11.15 min). In both chromatography systems elution of the metabolite was monitored at 254 nm. Structural elucidation by ^{1}H NMR and GC–MS revealed a methylated malonic acid mono(phenylcyanomethylamide) (structure **4**, Figure 3). This structure was proven by chemical synthesis (see Materials and Methods) and by comparison of the UV spectral data (λ_{max} = 254 nm) and melting points (mp isolated metabolite, 103 ± 0.5 °C; mp synthetic product, 110 ± 0.5 °C).

TLC analysis of cell extracts of soybean cell suspension cultures also indicated the transient accumulation of another metabolite (SS-I, R_f = 0; SS-II, R_f = 0.63; SS-III, R_f = 0.80) in a time range of 30–120 min after application of phoxim (Figure 1). Because of the low transient concentration of this metabolite, structural identification could only be achieved by GC–MS (see Materials and Methods). Furthermore, using a reference standard, this substance could be identified as β-1-*O*-oximinophenylacetonitrileamine glucoside (compound **5**, Figure 3).

(II) Phoxim Metabolism in Soybean Plants. Dissected organs (roots, leaves, stems, and cotyledons) of aseptically grown soybean plants were incubated with [*phenyl*-U-^{14}C]phoxim, and the formation of metabolites was determined at regular time intervals (Figure 2). Extracts of incubation medium and tissues were subjected to TLC (SS-I and SS-III), and these analyses showed high amounts of residual phoxim on the plant surfaces over long incubation periods. These data indicate a very slow diffusion of phoxim through the cuticle and plant cell walls. Even after 7 days significant amounts of unmetabolized phoxim were detected. The radioactivity found in the medium quantitatively consisted of unmetabolized phoxim. The only metabolic product identified (TLC, HPLC) in the tissue extracts over an incubation time of about 7 days was phenylacetonitrileamidomalonic acid. This product was identified by HPLC cochromatography using the synthetic reference. β-1-*O*-Oximinophenylacetonitrileamine glucoside could not be detected either in the cell extract or in the surrounding medium. In this experiment no excretion of polar metabolites into the medium could be observed. The extent of phoxim degradation and product formation in the dissected organs of soybean plant is depicted in Figure 2. Furthermore, in agreement with the data for the phoxim metabolism in soybean cell cultures no incorporation of radioactivity in cell wall bound structures of soybean plant organs was found.

DISCUSSION

The comparison of phoxim metabolism in soybean cell suspension cultures and dissected organs of aseptically grown plants (leaves, cotyledons, roots, and stems)

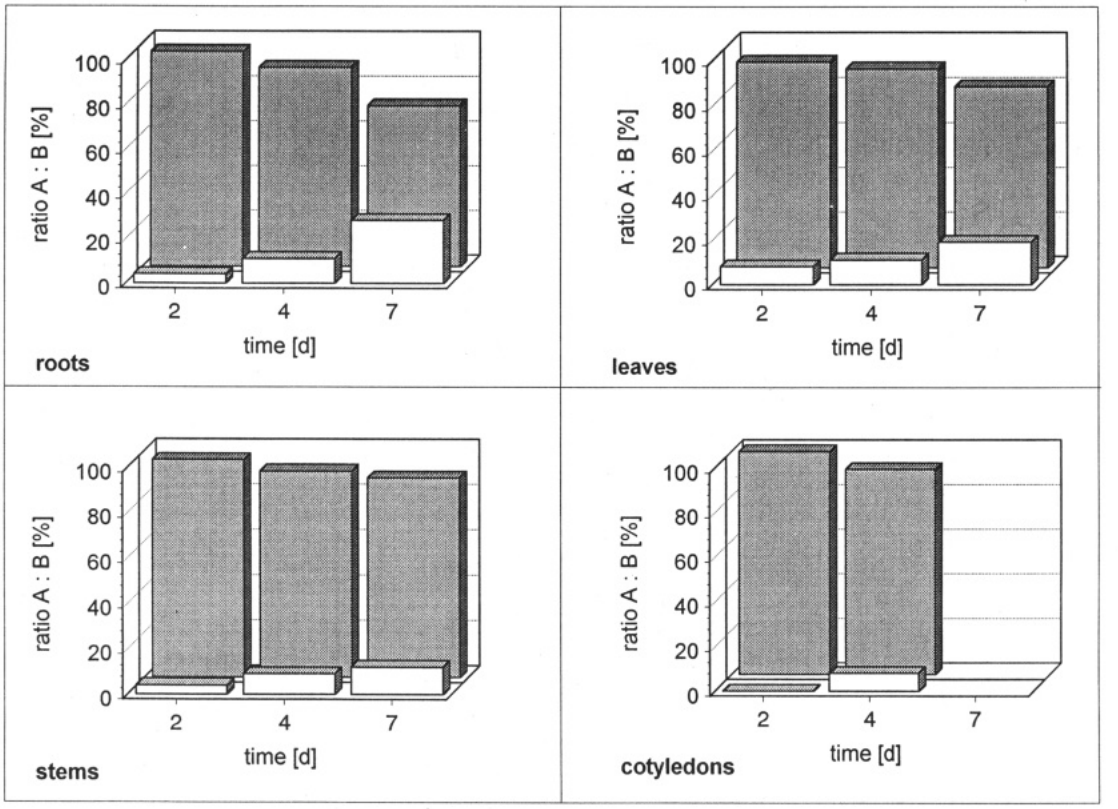

Figure 2. Ratio of unmetabolized phoxim (**A**, shaded bars) to major metabolite malonic acid mono(phenylcyanomethylamide) (**B**, white bars) in cell extracts of sterile plant organs (leaves, cotyledons, stems, and roots). [*phenyl*-U-^{14}C]Phoxim (18.5 kBq/per assay, 4.3 mM) was incubated with the tissue samples for the indicated time periods and radioactivity in compounds **A** and **B** was determined by HPLC scintillation counting in the cell extracts.

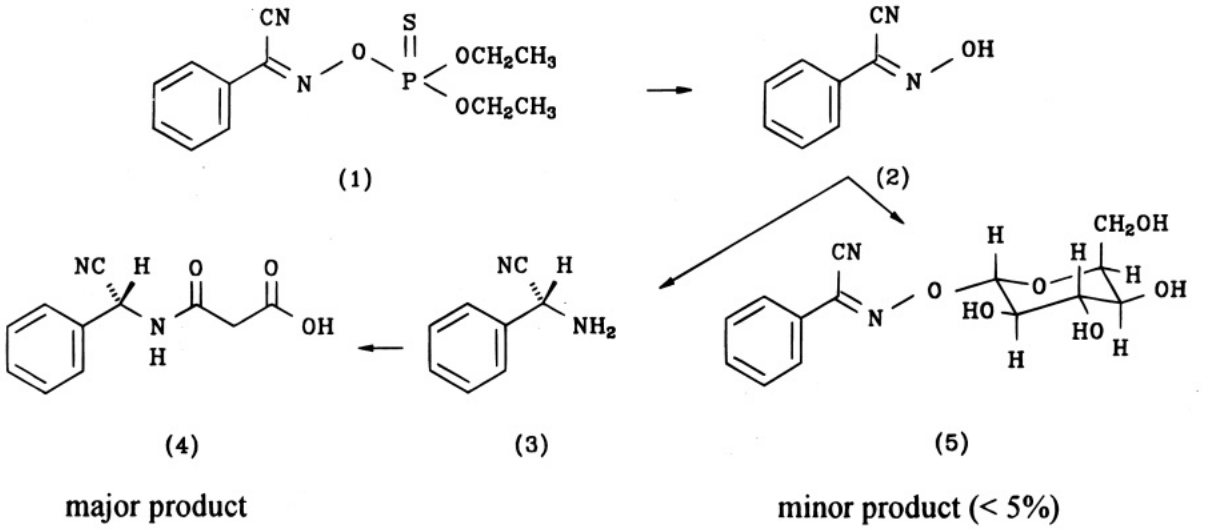

Figure 3. Metabolic pathway of phoxim catabolism in plants and cell suspension cultures of soybean (*G. max* L.). The oxime (**2**) and the primary amine (**3**) did not accumulate in detectable amounts.

clearly demonstrates the high efficiency of the cultured cells for xenobiotic catabolism. Although phoxim metabolism was found to be qualitatively identical in the two systems, maximum conversion in the dissected organs only occurred to some 34% in 7 days. In contrast, the cell cultures nearly quantitatively transformed the substrate to the *N*-malonate product within a few hours. This high rate of conversion appears to be facilitated by the almost instant adsorption of the lipophilic substrate to cell surfaces. This behavior known for lipophilic organothiophosphates (Lohmann and Hagedorn, 1985) may have occurred with the dissected organs also, but the much smaller surface area of this material and the reduced rate of uptake through differentiated tissue decreased the rate of conversion.

In general, rapid uptake and efficient conversion of substrates are characteristic traits of cell cultures which render such systems versatile for plant investigations on xenobiotics (Ebing et al., 1986; Harms and Langebartels, 1986; Barz et al., 1990). Major differences in xenobiotic metabolism of the two experimental systems are only quantitative but not qualitative, essentially as shown in other cases (Schneider et al., 1984; Swisher and Weiner, 1986; Cole and Owen, 1987). Furthermore, the qualitative organ-specific routes of phoxim metabolism were not observed with the dissected plant tissues.

The main product of phoxim metabolism in soybean, an *N*-malonic acid conjugate of phenylacetonitrileamine (compound **4**, Figure 3), adds to the rapidly increasing number of natural and xenobiotic plant *N*-malonates (Harborne, 1985; Winkler and Sandermann, 1989; Barz et al., 1990). Formation of this structure, elucidated by spectroscopic techniques and chemical synthesis, requires first phoxim cleavage at the ester bond to yield the oxime (**2**) (Figure 3) and a subsequent, presently unknown, reduction process to the primary amine (**3**). The former product is supported by the low but transient accumulation of the oxime glucoside (**5**), whereas no free nitrileamine (**3**) could be detected. This fact indicates rapid conversion of the amine (**3**) to the *N*-malonate (**4**), in agreement with other amine substrates (Owen and Donzel, 1986; Brown and Neighbors, 1987; Gareis et al., 1992).

The phenylacetonitrileamine (**3**) represents an amino acid derivative, and it is to be expected that the reduction process of the oxime (**2**) yielding **3** proceeds stereospecifically. In case the D-configurated compound should have been formed, the *N*-malonylation reaction would be equivalent to the well-known plant detoxification process of D-amino acids to *N*-malonyl derivatives (Zenk and Scherf, 1964; Barz et al., 1990). This assumption has meanwhile been demonstrated to be correct (Höhl and Barz, unpublished results).

In contrast to other plants where xenobiotic *N*- or *O*-malonyl conjugates are stored in vacuoles (Matern et al., 1983), soybean cells preferentially excrete these products into the cell culture medium. In agreement with several published studies (Scheel et al., 1984; Gareis et al., 1992) our data on the isolation of **4** exclusively from the extracellular compartment again document this special feature of soybean cells. Furthermore, these cells failed to incorporate any xenobiotic material into insoluble cell wall structures, which are quite often a deposit for xenobiotic residues (Gareis et al., 1992).

ACKNOWLEDGMENT

Financial support by Minister für Wissenschaft und Forschung, Düsseldorf, and assistance by Bayer AG, Leverkusen, are gratefully acknowledged. We thank Dr. J. R. Jansen for help with chemical synthesis and spectroscopic analyses.

ABBREVIATIONS USED

bp, boiling point; mp, melting point; DMSO, dimethyl sulfoxide; fr wt, fresh weight; ppm, parts per million; TLC, thin-layer chromatography; wn, wavenumber.

LITERATURE CITED

Barz, W.; Jordan, M.; Metschulat, G. Bioconversion of Xenobiotics (Pesticides) in Plant Cell Cultures. In *Progress in Plant Cellular and Molecular Biology*; Nijkamp, H. J. J., van der Plas, L. H. W., van Aartrijk, J., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1990; pp 631–639.

Brown, H. M.; Neighbors, S. M. Soybean Metabolism of Chlorimuron Ethyl: Physiological Basis for Soybean Selectivity. *Pestic. Biochem. Physiol.* **1987**, *29*, 112–120.

Cole, D. J.; Owen, W. J. Metabolism of Metalaxyl in Cell Suspension Cultures of *Lactuca sativa* L. and *Vitis vinifera* L. *Pestic. Biochem. Physiol.* **1987**, *28*, 354–361.

Dräger, G. Gas Chromatographic Method for the Determination of Valexon Residues in Plants. *Pflanzenschutz-Nachr. Bayer* **1969**, *22*, 311–317.

Dräger, G. Examination of the Metabolism of Phoxim (Bay 77488). *Pflanzenschutz-Nachr. Bayer* **1971**, *24*, 243–255.

Ebing, W.; Haque, A.; Schuphan, I.; Harms, H.; Langebartels, C.; Scheel, D.; von der Trenck, K. T.; Sandermann, H., Jr. Economical Assessment of Environmental Chemicals: Draft Guideline of the Test Procedure to Evaluate Metabolism and Degradation of Chemicals by Plant Cell Cultures. *Chemosphere* **1984**, *13*, 947–957.

Gamborg, O. L.; Murashige, T.; Thorpe, T. A.; Vasil, I. K. Plant Tissue Culture Media. *In Vitro* **1976**, *12*, 473–478.

Gareis, Ch.; Rivero, Ch.; Schuphan, I.; Schmidt, B. Plant Metabolism of Xenobiotics. Comparison of the Metabolism of 3,4-Dichloroaniline in Soybean Excised Leaves and Soybean Cell Cultures. *Z. Naturforsch.* **1992**, *47C*, 823–829.

Gohre, K.; Miller, G. C. Photooxidation of Thioether Pesticides on Soil Surfaces. *J. Agric. Food Chem.* **1986**, *34*, 709–713.

Harms, H.; Langebartels, C. Standardized Plant Cell Suspension Test for an Ecotoxicologic Evaluation of the Metabolic Fate of Xenobiotics. *Plant Sci.* **1986**, *45* (3), 157–166.

Harborne, J. B. The Natural Distribution in Angiosperms of Anthocyanins Acylated with Aliphatic Dicarboxylic Acids. *Phytochemistry* **1986**, *25*, 1887–1894.

Lohmann, E.; Hagedorn, H. The effect of parathion on green algae. *Appl. Microbiol. Biotechnol.* **1985**, *22*, 268–272.

Mason, W. A.; Meloan, C. E. Degradation Products of Phoxim (Bay 77488) on Stored Wheat. *J. Agric. Food Chem.* **1976**, *24*, 229–304.

Matern, U.; Heller, W.; Himmelspach, K. Conformational Changes of Apigenin 7-O-(6-O-malonylglucoside), a Vacuolar Pigment from Parsley, with Solvent Composition and Proton Concentration. *Eur. J. Biochem.* **1983**, *133*, 439–448.

Owen, W. J.; Donzel, B. Oxidative Degradation of Chlortoluron, Propiconazole, and Metalaxyl in Suspension Cultures of Various Crop Plants. *Pestic. Biochem. Physiol.* **1986**, *26*, 75–89.

Rosa, N.; Neish, A. C. Formation and Occurence of N-malonylphenylalanine and related compounds in plants. *Can. J. Biochem.* **1968**, *46*, 797–806.

Schneider, B.; Schütte, H. R.; Tewes, A. Comparative Investigations on the Metabolism of 2-(2,4-Dichlorophenoxy)-Isobutyric Acid in Plants and Cell Suspension Cultures of *Lycopersicon esculentum*. *Plant Physiol.* **1984**, *76*, 989–992.

Shimabukuro, R. H.; Walsh, W. C.; Jacobson, A. Aryl-O-glucoside of Diclofop. A detoxication Product in Wheat Shoots and Wild Oak Cell Suspension Culture. *J. Agric. Food Chem.* **1987**, *35*, 393–397.

Swisher, B. A.; Weimer, M. R. Comparative Detoxification of Chlorsulfuron in Leaf Disks and Cell Cultures of Two Perennial Weeds. *Weed Sci.* **1986**, *34*, 507–512.

Winkler, R.; Sandermann, H. Plant metabolism of chlorinated anilines: Isolation and identification of N-glucosyl and N-malonyl conjugates. *Pestic. Biochem. Physiol.* **1989**, *33*, 239–248.

Zenk, M. H.; Scherf, H. Spread of D-Tryptophan Conjugation Mechanisms in the Plant World. *Planta* **1960**, *62*, 350–354.

Received for review February 8, 1994. Revised manuscript received November 23, 1994. Accepted January 10, 1995.[⊗]

JF940063Z

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1995.