Chloramben Metabolism in Plants: Isolation and Identification of Glucose Ester

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A reported chloramben-X conjugate in giant foxtail (*Setaria* sp.) was purified and identified as methyl 3-amino-2,5-dichlorobenzoate (chloramben methyl ester). Additional studies established that the chloramben methyl ester was an artifact formed by transesterification of an unknown ester metabolite during the clean up of methanol extracts on Florisil columns. The unknown metabolite was isolated from [*carboxy*-^{14}C]chloramben-treated giant foxtail and barley (*Hordeum vulgare* L.) seedling tissues and identified as the glucose ester, α-D-glucosyl 3-amino-2,5-dichlorobenzoate. Differential metabolism studies with excised tissues of chloramben-sensitive and tolerant seedlings suggested that levels of the glucose ester metabolite, the previously identified *N*-glucoside conjugate, and the bound chloramben residues were significant factors in chloramben phytotoxicity and selectivity.

Chloramben (3-amino-2,5-dichlorobenzoic acid) is a selective preemergence herbicide used for the control of annual seedling grasses and broadleaf weeds in soybeans [*Glycine max* (L.) Merr.] and other crops. Colby et al. (1964) first reported the presence of an unknown, polar chloramben conjugate (chloramben-X) in tomato (*Lycopersicon esculentum* Mill). In subsequent studies with tolerant and susceptible plant species, Colby (1965, 1966) tentatively identified chloramben-X as an *N*-glycoside and suggested that the formation of this conjugate was a selective detoxification mechanism. Swanson et al. (1966b) unequivocally identified chloramben-X as the *N*-glucoside, and the in vitro biosynthesis of the *N*-glucoside has been demonstrated (Frear et al., 1967; Frear, 1968). A second unknown chloramben conjugate was reported by Stoller and Wax (1968). Unfortunately, this conjugate was also designated as chloramben-X. Stoller (1968, 1969) demonstrated that chloramben phytotoxicity and selectivity were correlated with the rate and extent of conjugate formation. In susceptible species, levels of chloramben-X increased, residual concentrations of unreacted chloramben increased, and the capacity for *N*-glucoside formation decreased.

The objectives of this study were to isolate and identify the second chloramben-X conjugate reported by Stoller and Wax (1968) and to determine the possible significance of this conjugate in chloramben phytotoxicity and selectivity.

MATERIALS AND METHODS

[^{14}C]Chloramben, [^{14}C]Chloramben *N*-Glucoside, and Reference Compounds. Chloramben, chloramben methyl ester, chloramben amide, and [carboxy-^{14}C]-chloramben (sp act., 2.19 mCi/mmol) were provided by Amchem Products, Inc. [*carboxy*-^{14}C]Chloramben *N*-glucoside was isolated from 4-day-old etiolated barley shoots (50 g fresh weight) treated in the dark for 24 h with 19 mL of 1.58×10^{-5} M [*carboxy*-^{14}C]chloramben (2.19 μCi/μmol). [^{14}C]Chloramben *N*-glucoside purification (≃98% radiochemical purity) was achieved by TLC separation with solvent system 4 (Table I) developed two times, elution with MeOH, and rechromatography in solvent system 1 (Table I).

Chloramben-X Purification. A partially purified sample of chloramben-X (Stoller, 1968, 1969) was provided by Dr. Stoller. It was dissolved in a minimum volume of methanol (1–2 mL), placed on a 1 × 90 cm column of Sephadex LH-20, and eluted with MeOH at 0.5 mL/min. The column eluate was monitored at 254 nm and collected in 3-mL fractions. Purified chloramben-X eluted as a single sharp peak (33–39 mL).

Table I. Thin-Layer Chromatography of Chloramben Metabolites and Reference Compounds

compound	solvent system ($R_f \times 100$)[a]				
	1	2	3	4	5
chloramben	43	25	62	13	68
chloramben amide	67	78	33	58	65
chloramben methyl ester	73	90	70	80	77
chloramben-X	73	90	70	80	77
N-glucosyl chloramben	29	0	3	5	12
unknown metabolite	38[b]	63	7	51	40

[a] 1 = *n*-BuOH-EtOH-NH_4OH (2:1:1); 2 = MeOAc-2-PrOH-H_2O (18:1:1); 3 = benzene-dioxane-HOAc (90:25:4); 4 = $CHCl_3$-MeOH-H_2O (65:25:4); 5 = $CHCl_3$-MeOH-H_2O-HOAc (65:25:4:2). [b] Decomposition to chloramben and chloramben amide (ammonolysis).

Plant Materials and Treatment. Seeds were germinated in the dark at 25 °C on moist paper toweling. Tissue sections (roots, shoots or hypocotyls) from 3- to 4-day-old seedlings were evenly distributed on the bottom of beakers or trays, wet with a minimum volume of treating solution, and incubated in the dark at 25 °C.

Metabolite Extraction and Isolation. Procedures developed for metabolite isolation and purification are summarized in Figure 1. [^{14}C]Chloramben-treated tissues were homogenized for 2 min in MeOH (5 mL/g fresh weight) with an Omnimixer. Homogenates were vacuum-filtered, and the residue was washed with additional MeOH until negligible ^{14}C was detected in the filtrate. Combined methanol filtrates were concentrated in vacuo at 30 °C with a rotary evaporator. The aqueous concentrate was placed on a 1.5 × 20 cm Amberlite XAD-2 column and eluted successively with distilled water (100 mL) and 95% EtOH (100 mL). The ethanol eluate was concentrated in vacuo at 30 °C and placed on a 1.0 × 10 cm DEAE cellulose (acetate) column. The metabolite was eluted from the column with distilled water. Unreacted chloramben, the *N*-glucoside metabolite, and other anions were adsorbed. The water eluate was lyophilized, dissolved in a small volume of 95% EtOH, streaked on preparative 2 × 20 cm TLC plates (500 μm Silica gel HF), and chromatographed with solvent system 4 (Table I). Separated metabolite zones were located with a Packard 7201 radiochromatogram scanner and fluorescence quenching,

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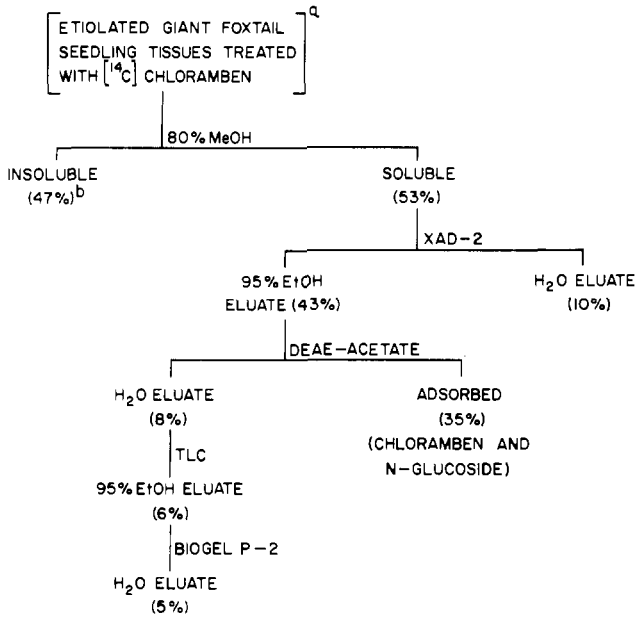

Figure 1. Chloramben metabolite extraction and isolation. (*a*) Four-day-old etiolated giant foxtail seedling tissues (65 g fresh weight) treated in the dark in a 30 × 19 × 5 cm enameled tray with 80 mL of 4.66 × 10^{-4} M [*carboxy*-^{14}C]chloramben (1.1 μCi/μmol) for 30 h at 25 °C. (*b*) Figures in parentheses represent the percent ^{14}C recovered in each fraction.

scraped from the chromatograms, and eluted from the silica gel with 95% EtOH. Ethanol eluates were concentrated in vacuo at 30 °C and rechromatographed with solvent system 2 (Table I). The ethanol eluate from the second TLC separation was concentrated almost to dryness, taken up in a small volume (0.5–1.0 mL) of distilled water, placed on a 1 × 50 cm column of Bio-Gel P-2, and eluted with distilled water at 0.75 mL/min. The column eluate was monitored at 254 nm and collected in 4-mL fractions. The purified metabolite was eluted as a broad symmetrical peak (80–100 mL), lyophilized, and stored at 4 °C.

Enzyme Hydrolysis. The isolated metabolite was partially hydrolyzed with either α- or β-glucosidase (Sigma Chemical Co.). Isolated samples of the metabolite (3.8 μg; 5 × 10^4 dpm) were incubated at 30 °C with 1 mL of either α-glucosidase [2 mg (2 EU)/mL of 0.05 M potassium phosphate buffer, pH 6.8, with 0.003 M NaCl] or β-glucosidase [2 mg (≃10 EU)/mL of 0.05 M sodium acetate buffer, pH 5.0]. Control samples were incubated in 1 mL of appropriate buffer. After 20 h, reaction mixtures were frozen with dry ice, lyophilized, and extracted with 1.5 mL of MeOH. TLC separated (solvent system 5, Table I) methanol-soluble ^{14}C products (chloramben and unreacted metabolite) were determined quantitatively. Metabolite hydrolysis by α- and β-glucosidase was 26 and 22%, respectively.

Glucose Determinations. Qualitative and quantitative glucose determinations (Table II) were made as reported previously (Frear and Swanson, 1972) after the purified metabolite (10–25 μg) was hydrolyzed (90%) with 1 N HCl at 100 °C for 2 h.

Metabolite Acetylation. Purified ^{14}C metabolite (4 mg) was reacted with 0.5 mL of acetic anhydride at 35 °C for 20 h. Excess acetic anhydride was removed with a stream of nitrogen and the acetylated metabolite was purified by LC on a 4 mm × 30 cm μ Porasil column (5–10 μm). A 15-min linear solvent program from 75% $CHCl_3$ in CH_2Cl_2 to 100% $CHCl_3$ was used. With a flow rate of 1 mL/min, the metabolite had a retention time of 13 min.

Table II. Quantitative Analysis of Metabolite Hydrolysis Products

determination	sample no.	glucose or glucose equiv, nmol	chloramben, nmol	ratio glucose/chloramben
reducing sugar	1	14.2	14.0	1.01
	2	14.6	14.0	1.04
	3	21.3	20.3	1.05
glucose	1	28.1	27.2	1.03
	2	29.0	27.2	1.07

Instrumentation. Mass spectra were obtained with a Varian MAT CH-5 spectrometer at 70 eV either by direct insertion of solid samples or by GC–MS with an on line Varian Aerograph series 200 gas chromatograph interfaced with a two-stage Watson-Biemann separator. The glass column (300 cm × 2 mm i.d.) was packed with a 3.5% OV-101 on 100/120 mesh Gas-Chrom Q. Injector and detector temperatures were 250 and 350 °C, respectively. The oven temperature was programmed from 175 to 225 °C at 5 °C/min and the helium carrier gas flow was 15 mL/min.

Proton magnetic resonance spectra were measured in $CDCl_3$ at 100 or 90 mHz with a Varian XL-100 or a Bruker WH-90 spectrometer. Me_4Si was used as the internal standard.

Quantitative ^{14}C measurements were made with a Packard 3375 liquid scintillation spectrometer. Methanol-insoluble ^{14}C residues were determined by combustion analysis with a Packard 306 sample oxidizer.

Preparation of 1-*O*-(3-Acetamido-2,5-dichlorobenzoyl-β-D-glucose Tetraacetate. Reaction conditions and procedures for the preparation of 1-*O*-(3-amino-2,5-dichlorobenzyl)-β-D-glucose tetraacetate were according to the method of Koenigs and Knorr (1901) and similar to those reported by Hiraga et al. (1974) for the synthesis of giberellin β-D-glucosyl esters. Chloramben (300 mg) was dissolved in dry dioxane (20 mL). A few beads of molecular sieve, tetra-*O*-acetyl-β-D-glucopyranosyl bromide (380 mg) and Ag_2O (500 mg) were added. The mixture was stirred at room temperature in darkness. After 24 h the solids were filtered off, the filter cake was washed with EtOAc, and the combined filtrates were extracted with aqueous $NaHCO_3$. The organic phase was dried with anhydrous $MgSO_4$ and the solvent removed. The crude 1-*O*-(3-amino-2,5-dichlorobenzoyl)-β-D-glucose tetraacetate was purified by preparative TLC (silica gel HF, 500 μm). Chromatograms were developed successively in ether (R_f 0.75) and in ether–hexane (90:5) (R_f 0.50). The separated β-D-glucose tetraacetate ester (≃60% yield) was eluted from the silica gel and recrystallized three times from ether–hexane. A portion (15–20 mg) of the crystallized product was purified further by LC on a 4 mm × 30 cm μ Porasil column (5–10 μm) developed with 20% $CHCl_3$ in CH_2Cl_2 at 1 mL/min. The purified product had a retention time of 8 min and was detected by UV absorption at 254 nm.

The isolated 1-*O*-(3-amino-2,5-dichlorobenzoyl)-β-D-glucose tetraacetate was acetylated by reaction overnight with an excess of acetic anhydride at 39 °C. Excess acetic anhydride was removed by a stream of nitrogen. The resultant N-acetylated product, 1-*O*-(3-acetamido-2,5-dichlorobenzoyl)-β-D-glucose tetraacetate, was purified by LC as described for the acetylated chloramben metabolite.

RESULTS AND DISCUSSION

Chloramben-X Identification. A partially purified sample of [*carboxy*-^{14}C]chloramben-X was provided by Dr.

Stoller. It chromatographed as a single ^{14}C spot on TLC (solvent system 1; Table I), but contained substantial quantities of residual silica gel and other minor impurities from previous TLC separations. The impurities were removed by gel filtration on Sephadex LH-20. Mass spectral analysis of the purified chloramben-X established that it was methyl 3-amino-2,5-dichlorobenzoate. An intense molecular ion was observed at m/e 219 together with a base peak at m/e 188 (M – OCH_3). Two additional major ion fragments were detected at m/e 160 (M – $COOCH_3$) and m/e 124 (M – $COOCH_3$, – Cl). The mass spectrum was identical with that of the known chloramben methyl ester. Additional data that confirmed the mass spectral identification of chloramben-X as the methyl ester included: (1) acid hydrolysis and TLC separation of chloramben as the only ^{14}C hydrolysis product; (2) the absence of a free carboxy group as evidenced by the failure of the purified chloramben-X to undergo anion exchange on DEAE cellulose; and (3) cochromatography of chloramben-X with a known sample of chloramben methyl ester in five TLC solvent systems (Table I).

Chloramben-X Formation. In an effort to confirm the presence of chloramben-X as a metabolite in chloramben-treated giant foxtail seedling tissues, an attempt was made to reproduce the earlier studies reported by Stoller (1968, 1969). Initial results showed little, if any, chloramben-X in methanol extracts of treated seedling tissues. However, a closer examination of previous isolation procedures showed that methanol extracts were not cleaned up on a Florisil column before TLC separation of metabolic products. When this was done, chloramben-X was present as reported (Stoller and Wax, 1968; Stoller, 1968). At that point it was felt that chloramben-X probably was an artifact of sample clean up on Florisil. Indeed, an ethyl ester of 2,4-D has been identified recently as an artifact of metabolite isolation from rice root callus tissues (Feung et al., 1976).

When methanol extracts of untreated giant foxtail tissues were spiked with [^{14}C]chloramben and examined by TLC before and after chromatography on Florisil, chloramben was not esterified. Acetone extracts of chloramben-treated seedling tissues did not contain chloramben-X, either before or after chromatography on Florisil. Apparently, a reactive metabolite was present in methanol extracts of [^{14}C]chloramben-treated tissues that was transesterified when chromatographed on Florisil. This was demonstrated when methanol extracts from [^{14}C]chloramben-treated giant foxtail tissues were examined by TLC before and after Florisil chromatography. An unknown metabolite was detected in methanol extracts before they were chromatographed on Florisil. The chromatographic behavior of the metabolite was determined in five TLC solvent systems (Table I). When this metabolite was separated by TLC, eluted with methanol, and chromatographed on Florisil, it was transesterified and converted to the methyl ester. However, similar studies with ethanol yielded only small quantities of the corresponding ethyl ester.

Isolation and Identification of the Unknown Chloramben Metabolite. The unknown methanol-soluble metabolite was purified by adsorption chromatography on Amberlite XAD-2, ion-exchange chromatography on DEAE cellulose, thin-layer chromatography in solvent systems 2 and 4 (Table I), and chromatography on Bio-Gel P-2 as illustrated in Figure 1. In later studies, more active etiolated barley shoot tissues (4- to 5-day-old seedlings) were used for metabolite biosynthesis, isolation, and identification. The same treatment, isolation, and purification procedures were used with a three–four-fold increase in the yield of purified metabolite.

Table III. Chemical Properties of Chloramben Metabolites

property	*N*-glucoside	unknown
acid stability	–	+
base stability	+	–
ammonolysis	–	+
anion exchange	+	–
ehrlich reagent	slow	fast
phosphomolybdic acid reagent		–
β-glucosidase hydrolysis	–	partial
α-glucosidase hydrolysis	–	partial
acid hydrolysis products	chloramben and glucose	chloramben and glucose

The chemical properties of the purified metabolite are compared with those previously reported for the *N*-glucoside metabolite in Table III. The unknown metabolite was quite stable to acid treatment but base labile and underwent ammonolysis when chromatographed in solvent systems containing ammonium hydroxide or when exposed to saturated ammonia vapors. Similar properties have been reported for the glucose ester of indole-3-acetic acid (Zenk, 1961). Ammonolysis products included both chloramben and chloramben amide. Amide formation was confirmed by TLC and GC–mass spectral analysis. TLC R_f values, GC retention times, and mass spectra of the isolated ammonolysis product were identical with a known sample of chloramben amide. A molecular ion at m/e 204 was the base peak. Other major ion fragments at m/e 188 (M – NH_2), 160 (M – $CONH_2$), and 124 (M $CONH_2$ – Cl) were the same as those observed for the chloramben methyl ester. Failure of the unknown metabolite to undergo anion exchange suggested that the carboxy group was blocked. The rapid reaction with Ehrlich reagent (Stoller, 1968), however, suggested that the amino group was free. TLC separation of acid hydrolysis products showed that the purified unknown metabolite, like the *N*-glucoside metabolite (Swanson et al., 1966b), contained only [^{14}C]chloramben and glucose. The partial hydrolysis of the purified unknown with either α- or β-glucosidase and the negative test with the phosphomolybdic acid reagent for reducing groups (Waldi, 1965) suggested that the glucose was linked to chloramben through the anomeric carbon. The purified metabolite was tentatively identified as the glucose ester of chloramben from these chemical properties.

Metabolite identification was established unequivocally by quantitative analysis of acid hydrolysis products, mass spectroscopy (MS), and proton magnetic resonance (^1H NMR) analysis of an acetylated derivative. Quantitative analysis of acid hydrolysis products (Table II) demonstrated that the purified metabolite contained glucose and chloramben in a 1:1 molar ratio. Mass spectral analysis of the peracetylated metabolite confirmed the quantitative analysis data. A small molecular ion, corresponding to the pentaacetate derivative, was observed at m/e 577. Ion fragments at m/e 542 (M – Cl), 535 (M – CH_2CO), 482 (M – Cl, – CH_3COOH), 440 (M – Cl, – CH_2CO, – CH_3COOH), and 380 (M – Cl, – CH_2CO, – $2CH_3COOH$) also supported the identification of the unknown chloramben metabolite as the glucose ester. Characteristic glucose acetate ions at m/e 331, 289, 271, 169, and 109 were also observed together with chloramben acetate and chloramben ions at m/e 247, 230, and 194 and m/e 205, 188, and 160, respectively. Mass spectra of the synthetic 1-*O*-(3-acet-

Table IV. ^{1}H NMR Analysis of Chloramben Glucosyl Ester Acetates[a]

compound	aromatic protons H-4 (d)	aromatic protons H-6 (d)	anomeric proton H-1′ (d)
1-*O*-(3-amino-2,5-dichlorobenzoyl)-β-D-glucose tetraacetate	6.91	7.19	5.95 (8)
1-*O*-(3-acetamido-2,5-dichlorobenzoyl)-β-D-glucose tetraacetate	8.66	7.56	5.97 (8)
acetylated glucose ester metabolite	8.68	7.55	6.34 (3.7)

[a] Chemical shifts and coupling constants (J) are expressed in δ values and Hz, respectively. Solvent = $CDCl_3$ and d = doublet.

amido-2,5-dichlorobenzoyl)-β-D-glucose tetraacetate showed a similar ion fragmentation pattern and a molecular ion at m/e 577.

^{1}H NMR data established the anomeric configuration of the glucose ester metabolite. Chemical shifts for the aromatic and anomeric protons of reference chloramben β-D-glucosyl ester acetates and the acetylated glucosyl ester metabolite are shown in Table IV. Mass spectral evidence for the acetylation of the chloramben amino group in the synthetic pentaacetate derivative [1-*O*-(3-acetamido-2,5-dichlorobenzoyl)-β-D-glucose tetraacetate] and the acetylated glucose ester metabolite was confirmed by identical large downfield shifts of the adjacent protons (H-4) and smaller shifts in the protons at the 6 position (H-6). The doublet (1 H) at δ 5.97 (J = 8 Hz) in the spectrum of the synthetic pentaacetate derivative collapsed to a singlet when the tentative position of the H-2′ proton at δ 5.39 was irradiated. This doublet was assigned to the anomeric proton (Angyal, 1972; Stoddart, 1971). The chemical shifts and coupling constants of the doublet anomeric proton signals from both of the synthetic reference compounds (tetra- and pentaacetates) were identical and characteristic of a β-configuration (Angyal, 1972; Stoddart, 1971). In contrast, the sharp anomeric proton doublet signal from the acetylated glucosyl ester metabolite was shifted downfield slightly (δ 6.34) and had a much smaller coupling constant (J = 3.7 Hz). These differences are characteristic of an α configuration (Angyal, 1972; Stoddart, 1971) and showed that the chloramben metabolite was the α-D-glucosyl ester rather than the tentatively identified β anomer suggested previously (Frear and Swanson, 1977). The present study suggests that tentative identification of auxin β-D-glucosyl esters (Shindy et al., 1973; Zenk, 1961) based primarily on β-glucosidase hydrolysis should be reexamined.

The significance of the chloramben glucose ester α-anomer is unknown. However, from the standpoint of possible enzyme stereospecificity, the α-anomer might behave differently from the β-anomer in different plant species and tissues. Indeed, recent biological activity studies with synthetic α- or β-anomers of gibberellin (GA_3) glucosyl esters (Sembdner et al., 1976) have shown that the biological activities of the anomers vary significantly in four bioassay systems with tissues from different species. In bioassays where gibberellin activity was observed, the GA_3 β-D-glucosyl ester showed much greater biological activity than the corresponding α-anomer.

Significance of Chloramben Glucose Ester. Stoller (1969) suggested that the formation of chloramben-X (the methyl ester) was correlated with chloramben phytotoxicity and selectivity. However, since chloramben-X was derived from a reactive glucose ester metabolite, it was important to determine if the formation of this new metabolite was also correlated with increased chloramben phytotoxicity and selectivity. Tissue sections from resistant and susceptible plant species were examined with respect to their ability to form the glucose ester metabolite (Table V). Chloramben glucose ester levels were generally highest in susceptible species; particularly the grasses. High levels of unreacted chloramben and low levels of the *N*-glucoside also appeared to be associated with susceptible species. Methanol-insoluble residues were present in all tissues but the highest levels were in the roots. These data supported the earlier conclusions of Stoller (1968, 1969) except that the glucose ester rather than the methyl ester, together with the *N*-glucoside, may be significant metabolic factors in chloramben phytotoxicity and selectivity.

Several reports (Hiraga et al., 1974; Kopcewicz et al., 1974; Leshem et al., 1974; Sembdner et al., 1976) have

Table V. Metabolism of [^{14}C]Chloramben by Tissue Sections from Resistant and Susceptible Plants

species[a]	tissue[b]	methanol-soluble ^{14}C: chloramben, %	*N*-glucoside, %	glucose ester, %	chloramben + glucose ester, %	methanol-insoluble ^{14}C, %
morningglory (R)	root[c]	3	76	2	5	19
	hypocotyl	15	61	15	30	9
squash (R)	root	5	84	3	8	9
	hypocotyl	29	61	3	32	7
snapbean (R)	root	3	67	1	4	29
	hypocotyl	24	70	2	26	4
soybean (R)	root	11	62	5	16	23
	hypocotyl	24	66	2	26	9
corn (I)	root	28	28	13	41	31
	shoot	58	16	21	79	4
cucumber (I)	root	22	33	10	32	36
	hypocotyl	83	8	3	86	6
velvet leaf (S)	root[c]	50	23	2	52	24
	hypocotyl	63	23	3	69	11
barley (S)	root	21	19	21	42	39
	shoot[c]	56	17	17	73	10
giant foxtail (S)	shoot[c]	51	15	12	63	22
barnyardgrass (S)	shoot[c]	58	12	23	81	7

[a] R = resistant; I = intermediate; S = susceptible. [b] Excised tissue sections from 3- to 4-day-old etiolated seedlings were placed in 50-mL beakers, wet with 0.5 mL of 8.3×10^{-5} M [^{14}C]chloramben (2.19 μCi/μmol) and incubated in the dark for 7 h at 25 °C. Treated tissues were rinsed with distilled water and extracted with MeOH. [c] Fresh weight of tissue sections = 0.5 g. All others = 1.0 g.

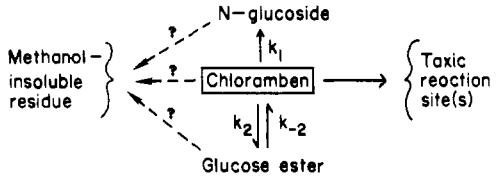

Figure 2. Bioregulation of chloramben phytotoxicity.

suggested that glucose ester conjugates of plant growth regulators may serve as growth regulator reserves and provide a possible mechanism for the bioregulation of growth regulator activity. If the chloramben glucose ester metabolite functions in a similar manner, both the unreacted chloramben and the glucose ester metabolite could be considered as phytotoxic or potentially phytotoxic compounds. Indeed, intermediate and susceptible tissue sections did contain higher total percentages of unreacted chloramben and the glucose ester metabolite than resistant tissues (Table V).

Excised tissues and seedlings of susceptible (barley) and resistant (soybean) plants were treated with isolated [^{14}C]chloramben glucose ester or [^{14}C]*N*-glucoside metabolites to assess the possible role of the glucose ester in chloramben phytotoxicity and selectivity. The *N*-glucoside metabolite remained essentially unchanged in tissues from both species. In glucose ester-treated tissues, however, both [^{14}C]chloramben and [^{14}C]*N*-glucosylchloramben were formed. Apparently, the glucose ester was hydrolyzed to chloramben and conjugated primarily as the *N*-glucoside. Subsequent studies with crude cell-free homogenates from etiolated barley, soybean, corn, and cucumber seedling tissues showed that the isolated glucose ester metabolite was hydrolyzed to chloramben.

The possible roles of the glucose ester and *N*-glucoside conjugates and the methanol-insoluble or bound residues in chloramben metabolism and selectivity are summarized in Figure 2. *N*-Glucoside biosynthesis was a significant chloramben detoxication pathway in all species examined. Several studies (Baker and Warren, 1962; Swanson et al., 1966a; Taylor and Warren, 1970a,b) have suggested that the *N*-glucoside is immobile and unreactive and remains as a nonphytotoxic terminal residue. In susceptible species, *N*-glucoside biosynthesis apparently is reduced and glucose ester biosynthesis effectively competes for the available chloramben. However, the glucose ester conjugate is apparently quite reactive and readily hydrolyzed to phytotoxic chloramben. The net effect is to prolong the residence time of phytotoxic chloramben levels at key target sites in susceptible plant species. Critical biochemical differences in the rate and extent of *N*-glucoside biosynthesis (k_1), glucose ester formation (k_2), and hydrolysis (k_{-2}) appear to determine chloramben phytotoxicity in different plant species.

Methanol-insoluble or bound chloramben residues also may provide an effective means for the reduction of chloramben concentrations in vivo. Several reports (Baker and Warren, 1962; Colby, 1966; Stoller, 1969; Taylor and Warren, 1970a,b) have suggested that chloramben is bound directly in plant tissues. Unfortunately, the chemical nature and biological significance of bound chloramben has not been investigated.

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