Metabolism of Benfuracarb in Young Cotton, Bean, and Corn Plants

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The metabolism of [*ring*-^{14}C]benfuracarb, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-[*N*-[2-(ethoxycarbonyl)ethyl]-*N*-isopropylsulfenamoyl]-*N*-methylcarbamate, was examined in the cotton, bean, and corn plants. The metabolites were characterized by cochromatography with authentic standards using two-dimensional thin-layer chromatography. Except for minor differences, the metabolism of benfuracarb was qualitatively similar in all three plants. The principal first step in the metabolic pathway was cleavage of an N–S linkage, giving rise to carbofuran, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, which was subsequently metabolized to 3-hydroxycarbofuran. The principal hydrolytic products were carbofuran phenol and 3-hydroxy- and 3-ketophenol, all isolated in the form of plant conjugates. Significant amounts of 3-hydroxy- and 3-ketobenfuracarb were also detected. Overall, the major reactions involved in the metabolism of benfuracarb in the cotton, bean, and corn plants were N–S bond cleavage, oxidation, hydrolysis, and conjugation.

INTRODUCTION

Benfuracarb, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-[*N*-[2-(ethoxycarbonyl)ethyl]-*N*-isopropylsulfenamoyl]-*N*-methylcarbamate, is a new carbamate insecticide that controls a wide range of insects such as aphids, wireworms, corn rootworm, loopers, borers, and thrips in many different crops including cotton, corn, and beans. A preceding paper on benfuracarb (Umetsu et al., 1985) described the absorption, translocation and metabolism of a carbonyl-labeled ^{14}C sample of this material in the cotton plant. The absorption and translocation of benfuracarb was also examined in the bean and corn plant. The results revealed that benfuracarb was initially converted in and on the plant into carbofuran, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, which was subsequently oxidized at the 3-position of the ring and the *N*-methyl moiety. In this study, a substantial amount of radioactivity was lost over the 10-day experimental period, presumably by $^{14}CO_2$ evolution following hydrolytic degradation of the carbamyl moiety. This observation indicated the formation of phenolic metabolites, and therefore the study was repeated using ring-labeled benfuracarb to determine the nature and amounts of these metabolites. The metabolic fate of benfuracarb in the bush bean and corn plant was also carried out to determine the effect of difference in plant species on metabolism.

This report summarizes results obtained from a study of the metabolism of [*ring*-^{14}C]benfuracarb in the cotton, bush bean, and corn plant.

MATERIALS AND METHODS

Chemicals. [*Ring*-^{14}C]benfuracarb (sp act. 12.3 mCi/mmol) was synthesized from [*ring*-^{14}C]carbofuran (purchased from Pathfinder Laboratory, Inc., St. Louis, MO) and bis[*N*-[2-(ethoxycarbonyl)ethyl]-*N*-isopropylamino] disulfide by the method described previously (Umetsu et al., 1985).

The structure of benfuracarb and nonradioactive standards and their abbreviations are shown in Table I. CF-S_2-N(ip)(EP), CF-S_n-N(ip)(EP) ($n \geq 3$), CF-SO_2-N(ip)(EP), CF-S_2-CF, CF-S_n-CF ($n \geq 3$), carbofuran (CF), 3-OH-CF, 3-C=O-CF, *N*-CH_2OH-CF, 3-OH-*N*-CH_2OH-CF, 3-C=O-*N*-CH_2OH-CF, NH_2-CF, 3-OH-NH_2-CF, 3-C=O-NH_2-CF, CF-P, 3-OH-P, and 3-C=O-P were available from previous studies (Umetsu et al., 1979, 1980, 1981a, 1981b, 1985).

2,3-Dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl *N*-[*N*-[2-(ethoxycarbonyl)ethyl]-*N*-isopropylsulfenamoyl]-*N*-methylcarbamate (3-C=O-benfuracarb) was synthesized as follows. To a chilled dichloromethane solution of *N*-[2-(ethoxycarbonyl)ethyl]-*N*-isopropylaminosulfenyl chloride (Umetsu et al., 1984), prepared from 1.9 g (5 mmol) of bis[*N*-[2-(ethoxycarbonyl)ethyl]-*N*-isopropylamino] disulfide and 1.18 g (5 mmol) of 2,3-dihydro-2,2-dimethyl-3-oxo-7-benzofuranyl *N*-methylcarbamate (3-C=O-CF) in dichloromethane, was added dropwise 1.0 g (10 mmol) of triethylamine. After stirring for 18 h the reaction mixture was washed with water and dried, and the product was purified by silica gel column chromatography, yielding 1.3 g of an oil. 3-C=O-benfuracarb showed the following NMR signals (δ, $CDCl_3$): 6.9–7.7 (m, 3 H, aromatic protons), 4.09 (q, 2 H, OCH_2CH_3), 3.43 (s, 3 H, *N*-CH_3), 3.3–3.8 (m, 3 H, *N*-CH_2CH_2 + *N*-$CH(CH_3)_2$), 2.77 (t, 2 H, CH_2CO_2), 1.47 (s, 6 H, *gem*-$(CH_3)_2$), 1.22 (d, 6 H, *N*-$CH(CH_3)_2$), 1.19 (t, 3 H, OCH_2CH_3). The EI MS (electron-impact MS) molecular ion peak of 424 provided additional support for the structure.

2,3-Dihydro-3-hydroxy-2,2-dimethyl-7-benzofuranyl *N*-[*N*-[2-(ethoxycarbonyl)ethyl]-*N*-isopropylsulfenamoyl]-*N*-methylcarbamate (3-OH-benfuracarb) was synthesized as follows. To a solution of 100 mg of 3-C=O-benfuracarb in 20 mL of methanol at 0.5 °C was added 100 mg of sodium borohydride, and the mixture was stirred for 30 min. The reaction mixture was extracted with dichloromethane, and the product was purified by silica gel preparative TLC to give 80 mg of an oil. 3-OH-benfuracarb (δ, $CDCl_3$) showed the following NMR signals: 6.7–7.5 (m, 3 H, aromatic protons), 4.63 (br d, 1 H, ph-C*H*OH), 4.06 (q, 2H, OCH_2CH_3), 3.35 (s, 3H, *N*-CH_3), 3.2–3.8 (m, 3 H, *N*-$CH(CH_3)_2$ + *N*-CH_2CH_2), 2.72 (t, 2 H, CH_2CO_2), 1.38 and 1.28 (s, 3 H each, *gem*-$(CH_3)_2$), 1.22 (d, 6 H, *N*-$CH(CH_3)_2$), 1.19 (t, 3 H, OCH_2CH_3). The EI MS molecular ion peak of 426 provided additional support for the structure.

Plant Material. Bush bean (Burpee's stringless, green pod), cotton (Deltapine 61), and corn (Golden cross bantam) seeds were surface sterilized by 10-min immersion in 0.1% mercuric chloride solution. The seeds were germinated and grown in vermiculite for 2 days (bush bean) or for 3 days (cotton and corn). Seedlings of uniform size were then selected, transplanted into pots (14-cm diameter,

Pesticide and Biological Science Research Laboratories, Otsuka Chemical Company, Limited, Satoura-cho, Naruto, Tokushima-ken 772, Japan (A.K.T., N.U.), and Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside, California 92521 (T.R.F.).

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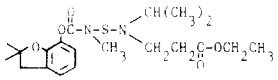
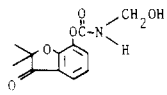
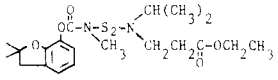
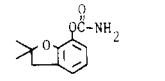
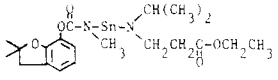
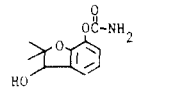
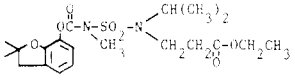
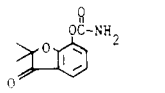
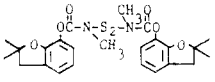
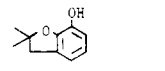
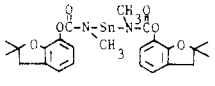
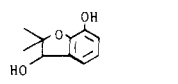
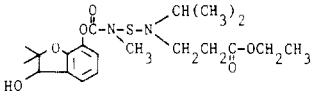
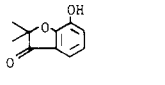
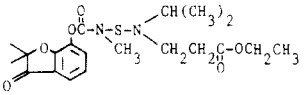
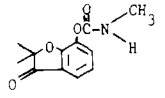
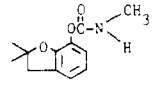
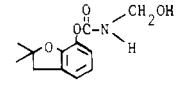
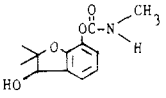
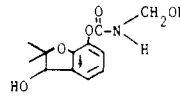
Table I. Structure of Benfuracarb and Authentic Standards

structure	name [abbrev]	structure	name [abbrev]
	Benfuracarb		3-Keto-N-hydroxymehtyl-carbofuran [3-C=O-N-CH_2OH-CF]
	Disulfide derivative of benfuracarb [CF-S_2-N(ip)(EP)]		N-Desmethyl-carbofuran [NH_2-CF]
	Polysulfide derivative of benfuracarb [CF-S_n-N(ip)(EP), n ≥ 3]		3-Hydroxy-N-desmethyl-carbofuran [3-OH-NH_2-CF]
	[Benfuracarb-oxon] [CF-SO_2-N(ip)(EP) or sulfon]		3-Keto-N-desmethyl-carbofuran [3-C=O-NH_2-CF]
	Carbofuran-disulfide [CF-S_2-CF]		Carbofuran-phenol [CF-P]
	Carbofuran-polysulfide [CF-S_n-CF, n ≥ 3]		3-Hydroxy-carbofuran-phenol [3-OH-P]
	3-Hydroxy-benfuracarb [3-OH-benfuracarb]		3-Keto-carbofuran-phenol [3-C=O-P]
	3-Keto-benfuracarb [3-C=O-benfuracarb]		3-Keto-carbofuran [3-C=O-CF]
	Carbofuran [CF]		N-Hydroxymethyl-carbofuran [N-CH_2OH-CF]
	3-Hydroxy-carbofuran [3-OH-CF]		3-Hydroxy-N-hydroxymehtyl-carbofuran [3-OH-N-CH_2OH-CF]

13-cm height) containing soil, and placed in a greenhouse at 25–30 °C (cotton and corn) or 28–35 °C (bush bean). Ten-day-old bush bean (early first trifoliate stage, 2.5–3.6 g in fresh weight), 13-day-old cotton (in the early first true leaf stage, 0.9–1.0 g in fresh weight), or 19-day-old cotton (in the first true leaf stage, 1.5–1.9 g in fresh weight) and corn seedlings (about 16-cm height with three leaves, 1.6–1.7 g in fresh weight) were used for the metabolism studies.

Metabolism following Foliar Application. Two dose levels were used in the metabolism studies. At the low dose, the two primary leaves of two bush bean plants in the early trifoliate stage or two cotyledons of cotton plants in the early first true leaf stage were painted with 40 μL of a 1:1 acetone–water solution containing 8.49 μg (0.223 μCi) or 4.65 μg (0.113 μCi) of [*ring*-^{14}C]benfuracarb, respectively. At predetermined intervals (1, 3, 6, 10 days), the treated leaves were excised at the base of the stem and rinsed with 50 mL of benzene–dichloromethane (1:1) to remove surface residue. The wash, designated as the leaf rinse, was analyzed by two-dimensional TLC for metabolites. The rinsed leaves, stems, and roots were combined and ground with a mortar and pestle in 20 mL of acetonitrile–phosphate buffer (4:1, pH 7.8) containing 3.2×10^{-4} M *N*-ethylmaleimide (NEM) to prevent cleavage of the N–S bond during workup (Umetsu et al., 1979). The homogenates were divided into organic-soluble, water-soluble, and unextractable fractions (internal extracts) and analyzed for metabolites as described in the previous paper (Umetsu et al., 1985). The water-soluble fraction was diluted with an equal volume of 1.0 N hydrochloric acid and heated in a boiling water bath for 20 min. Radioactive material converted into organic-soluble products by the hydrochloric acid treatment was extracted into dichloromethane and analyzed by TLC. In addition, β-glucosidase was used to hydrolyze material in the water-soluble fraction with some samples (Umetsu et al., 1985) in order to determine whether any artifactual products were formed during acid treatment.

Metabolism following Stem Injection. A 1.0-μL sample of a 1:1 acetone–water solution containing [*ring*-^{14}C]benfuracarb was injected into the stem of each bean plant (4.04 μg, 0.099 μCi) and corn seedling (3.75 μg, 0.091 μCi). At each predetermined interval (1, 3, 6, 10 days) duplicate plants were analyzed for metabolites. Each plant was cut into small pieces, ground thoroughly with a mortar and pestle in 20 mL of acetonitrile–phosphate buffer (pH 7.8, 0.05 M) mixture (5:1) containing 3.2×10^{-4} M NEM,

Table II. Thin-Layer Chromatography Properties of Possible Metabolites of Benfuracarb in Four Solvent Systems

compd	solvent[a] A	B	C	D
benfuracarb	0.42	0.58	0.42	0.76
CF-S_2-N(ip)(EP)	0.45	0.66	0.43	0.76
CF-S_n-N(ip)(EP) ($n > 3$)	0.53	0.68	0.50	0.77
CF-SO_2-N(ip)(EP)	0.41	0.58	0.33	0.77
CF-S_2-CF	0.53	0.66	0.42	0.76
CF-S_n-CF ($n > 3$)	0.52	0.65	0.38	0.74
3-OH-benfuracarb	0.06	0.42	0.12	0.63
3-C=O-benfuracarb	0.28	0.61	0.32	0.73
CF	0.18	0.47	0.21	0.68
3-OH-CF	0.02	0.24	0.05	0.49
3-C=O-CF	0.13	0.42	0.16	0.65
N-CH_2OH-CF	0.03	0.21	0.06	0.49
3-OH-*N*-CH_2OH-CF	0.00	0.06	0.01	0.28
3-C=O-*N*-CH_2OH-CF	0.02	0.15	0.03	0.41
NH_2-CF	0.09	0.31	0.11	0.61
3-OH-NH_2-CF	0.01	0.05	0.02	0.48
3-C=O-NH_2-CF	0.06	0.25	0.09	0.60
CF-P	0.45	0.49	0.52	0.72
3-OH-P	0.10	0.17	0.27	0.61
3-C=O-P	0.24	0.36	0.39	0.68

[a] Solvent systems: (A) benzene–ethyl acetate (9:1); (B) benzene–methanol (19:1); (C) hexanes–ether (1:1); (D) dichloromethane–acetonitrile–ether (2:1:1).

and analyzed for metabolites as described above.

Analysis. Leaf rinse and organic extracts were concentrated and analyzed by TLC using precoated silica gel GHLF plates (0.25 mm, Analtech, Inc.). Samples in small volumes of dichloromethane were spotted on the plates and developed with a variety of solvent systems. R_f values of authentic standards are listed in Table II. For metabolite identification, two-dimensional TLC using solvent systems A and B, A and C, or B and D with authentic standards was carried out. For quantification of each metabolite in the leaf rinse and organic-soluble fractions, two-dimensional TLC with solvent systems A and B was used, while solvent systems B and D were used for the hydrolyzed water-soluble conjugate fraction. When separation of metabolites using these solvent systems was not satisfactory, i.e., when the R_f values of metabolites were close to each other, additional two-dimensional TLC using systems A and C were used for the leaf rinse and organic-soluble fractions, and systems A and B were used for the hydrolyzed water-soluble conjugate fraction. Location of the standards on TLC plates was accomplished by ultraviolet detection. Location of radioactive spots on the TLC plates was by autoradiography using Kodak X-ray film (SB-5) exposed for 7–15 days.

Radioactivity was quantified in a Beckman Model LS-3145T liquid scintillation counter using 10 mL of a scintillation cocktail (Scint-A, Packard). Liquid samples were determined by counting 0.2-mL aliquots. Radioactivity in each spot on TLC plates was determined by scraping the spot from the plate and placing the silica gel in counting vials with scintillation cocktail. The radioactivity in the unextractable fraction was determined after oxygen combustion of 60–70-mg samples of the dried plant material using a Model 306 Packard sample oxidizer.

NMR spectra were measured in a Hitachi high-resolution NMR spectrometer R24-B. Mass spectra were measured in a Hitachi M-80 double-focusing mass spectrometer equipped with a Hitachi M-003 data processing system.

RESULTS

Metabolism of Benfuracarb in and on the Bush Bean Plant following Low-Dose Foliar Application.

Table III. Metabolism of [^{14}C]Benfuracarb in and on the Bush Bean Plant after Foliar Application of Two Dosages

metabolite	% total benfuracarb appl (at indicated day) 1: low dose	3: low dose[a]	3: high dose[b]	6: low dose	10: low dose	10: high dose
Leaf Rinse						
CF-S_n-N(ip)(EP) ($n > 3$)	0	0.2	0	0.3	0.2	0.6
CF-S_2-CF	1.0	1.6	0.2	2.3	2.7	2.5
CF-S_n-CF ($n > 3$)	0.2	0.4	0.8	0.6	0.6	0.6
CF-S_2-N(ip)(EP)	0	0.5	0	0.6	0.2	0.6
unknown I	0.4	0.2		0.2	0.1	
benfuracarb	59.2	34.4	75.4	17.6	9.7	32.0
3-C=O-benfuracarb	0.4	0.2	0	0.4	0.3	0.3
carbofuran	4.3	4.5	4.4	2.9	1.3	2.4
3-C=O-CF	0.1	0	0	0	0	0.1
unknown II	0	0.2	0.1	0.6	1.5	1.0
3-OH-benfuracarb[c]	0.4	0.9	0.9	1.1	0.9	1.8
3-OH-CF	0.7	1.5	0.6	1.7	1.0	2.8
N-CH_2OH-CF	0.3	0.7	0.2	0.6	0.3	0.8
unknown III	0	0.5	0	1.5	1.4	1.2
unknown IV	0.8	1.6	0.8	3.6	2.6	3.5
subtotal	67.9	47.4	83.5	34.0	22.8	50.2
Organic-Soluble Portion						
CF-S_2-CF			0			0.2
CF-S_2-N(ip)(EP)			0			0.1
benfuracarb			3.3			2.9
carbofuran			0.5			2.3
3-C=O-CF			0			0.1
unknown II			0			0.1
3-OH-benfuracarb[c]			0.2			0.5
3-OH-CF			0.1			1.6
N-CH_2OH-CF			0			0.2
unknown IV			0.2			0.9
subtotal	0.1	0.1	4.3	0.1	<0.1	8.9
Water-Soluble Conjugate						
CF-P	0.2	0.3	0.1	0.2	0.5	0.3
carbofuran	0.6	0.1	0.1	0.1	0	0.1
unknown V	0	0		0	0.1	
3-C=O-P	0.1	0.2	0	0.4	0.4	0.4
NH_2-CF[d]	0	0.4	0.1	0.9	1.4	0.4
3-OH-P	0.3	0.3	0.1	0.5	0.7	0.2
3-OH-CF	2.6	9.7	1.4	14.9	19.2	6.1
3-OH-NH_2-CF[d]	0	0.3		0.4	0.5	
unknown IV	0.1	0.2	0	0.6	0.5	0.2
other unknowns[e]			0.5			0.3
unextractable	0.3	0.9	0.1	1.8	1.3	1.7
subtotal	4.2	12.4	2.4	19.8	24.6	9.7
residual straw	0.7	2.3	0.4	4.3	7.0	4.9
total recovery	72.9	62.3	90.6	58.2	54.4	73.5

[a] Forty microliters of an acetone–water (1:1) solution containing [ring-^{14}C]benfuracarb (8.5 μg, 0.223 μCi) were painted on the two primary leaves of each bush bean plant (2.5–3.0 g in fresh weight). There were two plants per set. [b] Forty microliters of an acetone–water (3:2) solution containing [ring-^{14}C]benfuracarb (180 μg, 4.75 μCi) were painted on the two primary leaves of each bean plant (2.6–2.9 g in fresh weight). There were two plants per set. [c] Includes an unknown metabolite. [d] NH_2-CF and 3-OH-NH_2-CF are artificial products from the acid hydrolysis treatment, and actual metabolites are *N*-CH_2OH-CF and 3-OH-*N*-CH_2OH-CF, respectively. [e] Includes all unknowns in trace quantities except unknown IV.

The metabolism of benfuracarb in and on the bush bean plant was determined following painting of 8.49 μg of [*ring*-^{14}C]benfuracarb on the two primary leaves in the early first trifoliate stage. Table III summarizes data for the distribution, identity, and relative amounts of the various metabolites isolated. Total recoveries of the applied radioactivity from the 1-, 3-, 6-, and 10-day plants were relatively low with values of 72.9, 62.3, 58.2, and 54.4%, respectively. Analysis for radioactivity following oxygen combustion of the potted soil containing the 10-day

plants revealed only a trace of radioactivity, indicating that loss of radioactivity was not through the soil.

After 1 day, most of the radioactivity (67.9%) was observed as leaf rinse and only 0.1% was found internally as organic-soluble and 4.2% as water-soluble materials. The water-soluble materials, presumably mainly in the form of conjugated metabolites, increased during the experimental period and reached 24.6% after 10 days. The radioactivity in the organic-soluble fraction remained very low during the 10-day test period, indicating rapid conjugation of metabolites as they were formed in the plants.

The identity of the various metabolites was confirmed by two-dimensional TLC using three different combinations of the four solvent systems listed in Table II. Figure 1 (parts a and b) shows autoradiographs of two-dimensional TLC plates of the leaf rinse and hydrolyzed water-soluble conjugate fractions. After 1 day, only 59.2% of the radioactivity was present as the parent carbamate, and this gradually decreased to 9.7% during the 10-day test period (Table III). The principal metabolite observed after 1 day was carbofuran but 3-OH-CF (free plus conjugated form) was by far the major metabolites after 3, 6, and 10 days. The other metabolites were of lesser significance. CF-S_2-CF in the leaf rinse fraction and NH_2-CF and 3-OH-CF in the hydrolyzed conjugate fraction gradually increased during the test period and 3-C=O-benfuracarb, 3-OH-benfuracarb, CF-S_n-CF ($n \geq 3$), CF-S_2-N-(iP)(EP), 3-C=O-CF and N-CH_2OH-CF were also identified as minor metabolites. All of these metabolites except 3-C=O-benfuracarb and 3-OH-benfuracarb were previously observed as cotton metabolites of [*carbonyl*-^{14}C]-benfuracarb (Umetsu et al., 1985). The hydrolysis products CF-P, 3-C=O-P, and 3-OH-P were observed as additional minor metabolites in the water-soluble fraction. Unknowns I–III each consisted of several components of similar TLC properties. Unknown IV represents the radioactive material at or near the origin spot of the TLC plate. Unknown V was a single component. The metabolites indicated as NH_2-CF and 3-OH-NH_2-CF doubtlessly were conversion products from the respective N-hydroxymethyl derivatives formed during acid hydrolysis (Umetsu et al., 1985).

Metabolism of Benfuracarb in and on the Bush Bean Plant following High-Dose Foliar Application. The metabolism of benfuracarb in and on the bush bean plant following foliar application at the higher dose of 180 μg/plant [^{14}C]benfuracarb also was studied. Table III summarizes data for the distribution, identity, and relative amounts of the various metabolites. The metabolites observed were the same as at the lower dose except for the absence of 3-OH-NH_2-CF and unknown I. However, differences in the relative amounts of metabolites were noted. After 3 days, 83.5% and 2.4% of the radioactivity were isolated as leaf rinse and water-soluble conjugate, respectively, and 4.3% as internal organic-soluble material. At 10 days the percentages of organic-soluble and conjugated material reached 8.9 and 9.7%, respectively, and 50.2% of the radioactivity was still present as leaf rinse. At the high-dose treatment, large amounts of residual benfuracarb were observed in the leaf rinse fractions, i.e., as much as 75.4% after 3 days and 32.0% after 10 days. The major metabolites were carbofuran and 3-OH-CF.

The leaf rinse, obtained by washing the leaves with benzene–dichloromethane (1:1), showed no color attributable to plant pigments, and the relative amounts of the metabolites in the leaf rinse suggest that the metabolites in the leaf rinse are actually those present on or near the surface and were not leached from the leaf by the solvent.

Table IV. Metabolism of [^{14}C]Benfuracarb in an on the Cotton Plant after Foliar Application of Two Doses[a]

	% total benfuracarb appl (at indicated day)			
	low dose		high dose	
metabolites	3	10	3	10
Leaf Rinse				
CF-S_n-N(ip)(EP) ($n > 3$)	0	0	0.1	0.3
CF-S_2-CF	0.2	0.3	0.5	1.7
CF-S_n-CF ($n \geq 3$)	0	0	0.1	0.6
CF-S_2-N(ip)(EP)	0	0	0.3	0.2
benfuracarb	21.6	4.3	65.3	27.8
3-C=O-benfuracarb[b]	0	0.2	0	0.3
carbofuran	3.1	0.5	4.2	4.8
3-C=O-CF	1.7	0.5	0.6	0.3
unknown II	0	0.2	0.2	0.7
3-OH-benfuracarb	0.3	0.2	0.5	1.9
3-OH-CF	20.6	5.3	1.5	1.0
N-CH_2OH-CF	0.8	0.3	0.5	0.5
3-C=O-N-CH_2OH-CF	0.1	0.1	0	0
3-OH-N-CH_2OH-CF	0.5	0.5	0	0
unknown III	0	0	0.6	2.0
unknown IV	0.2	0.3	1.2	2.9
subtotal	49.1	12.7	75.6	45.1
Organic-Soluble Portion				
CF-S_2-CF			0.1	0.1
CF-S_2-N(ip)(EP)			0	0.1
benfuracarb			6.6	3.8
carbofuran			5.6	8.8
3-C=O-CF			0.2	0.4
unknown II			0	0.3
3-OH-benfuracarb			0.1	0.4
3-OH-CF			0.3	0.3
N-CH_2OH-CF			0.1	0.2
unknown III			0	0.1
unknown IV			0.3	0.8
subtotal	9.0	6.2	13.3	15.3
Water-Soluble Conjugate				
CF-P	1.3	0.9	0.2	2.0
carbofuran	0	0	0.1	0.8
3-C=O-P	2.7	9.5	0.5	2.9
NH_2-CF[c]	2.7	4.2	0.1	0.5
3-OH-P	9.0	4.5	0.1	0.3
3-OH-CF	17.2	36.4	1.7	5.5
3-OH-NH_2-CF[c]	0.6	2.8	0	0
unknown IV	0.3	1.1	0.2	0.8
other unknowns[d]	0	0	0.2	0.6
unextractable	6.4	6.5	0.5	4.6
subtotal	40.2	65.9	3.6	18.0
residual straw	1.4	4.6	1.2	6.0
total recovery	99.7	89.4	93.7	84.4

[a] In the case of low-dose treatment, 40 μL of an acetone–water (1:1) solution containing [*ring*-^{14}C]benfuracarb (4.7 μg, 0.113 μCi) was painted on the two primary leaves of each cotton plant (0.9–1.0 g in fresh weight). At high-dose treatment 40 μL of an acetone–water (3:2) solution containing [*ring*-^{14}C]benfuracarb (180 μg, 4.75 μCi) were painted on the two primary leaves of each cotton plant (1.5–1.9 g in fresh weight). There were two plants per set. [b] Includes an unknown metabolite. [c] NH_2-CF and 3-OH-NH_2-CF are artificial products from the acid hydrolysis treatment and actual metabolites are N-CH_2OH-CF and 3-OH-N-CH_2OH-CF, respectively. [d] Includes all unknowns in trace quantities except unknown IV.

Metabolism of Benfuracarb in and on the Cotton Plant following Foliar Application. Table IV summarizes data for the distribution, identity, and relative amounts of the various metabolites of benfuracarb in the cotton plant following low- and high-dose foliar treatment with [*ring*-^{14}C]benfuracarb. The same metabolites found in the bean plant were also found in cotton, the major metabolites being 3-OH-CF and carbofuran. Differences between cotton and bush bean were noted in the amounts of 3-OH-CF present in the leaf rinse fraction, especially

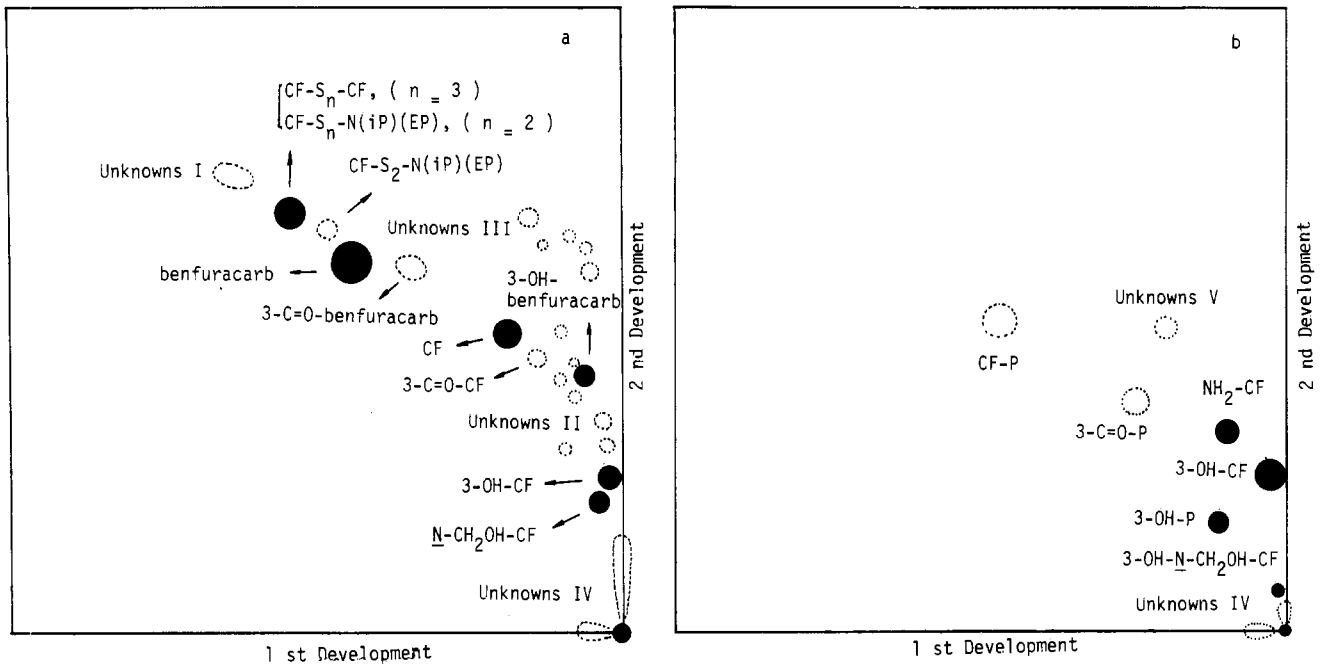


Figure 1. Autoradiographs of two-dimensional thin-layer chromatograms of leaf rinse (a) and hydrolyzed water-soluble conjugate (b) fractions from the bush bean plant 10 days after a low-dose treatment with [^{14}C]benfuracarb. Solvent systems: first development, benzene–ethyl acetate (9:1); second development, benzene–methanol (19:1). Solid spots represent major metabolite; dotted spots represent minor metabolite.

Table V. Metabolism of [^{14}C]Benfuracarb in the Corn Plant after Stem Injection[a]

metabolite	% of total benfuracarb appl (at indicated day) 1	3	6	10
Organic-Soluble Portion				
CF-S_n-CF ($n \geq 2$) / CF-S_n-N(ip)(EP) ($n \geq 2$)	0.8	0.4	0.2	0.1
benfuracarb	57.0	54.6	46.2	4.5
carbofuran	45.6	25.6	15.7	15.6
3-C=O-CF	0.5	1.5	0.6	0.7
3-OH-CF	3.9	11.1	16.2	17.2
N-CH_2OH-CF	0.6	0.5	0.6	1.0
3-C=O-*N*-CH_2OH-CF	0	0.1	<0.1	0.2
3-OH-*N*-CH_2OH-CF	0.4	0.1	0.7	1.3
unknown IV	1.2	0.7	1.7	1.7
subtotal	110.0	94.6	81.9	42.3
Water-Soluble Conjugate				
CF-P	1.0	1.1	2.5	2.0
carbofuran	0.7	1.1	0.4	0.2
3-C=O-P	0.2	1.3	5.3	16.3
NH_2-CF[b]	0.1	0.4	0.9	0.9
3-OH-P	0.2	0.8	1.9	4.0
3-OH-CF	0.1	0.6	3.1	9.3
3-OH-NH_2-CF[b]	0	0.1	0.4	2.7
unknown IV	0.1	0.1	0.6	1.3
unextractable	0.7	1.2	4.6	7.6
subtotal	3.1	6.7	19.7	44.1
residual straw	1.0	2.2	4.5	10.7
total recovery	114.1	103.5	106.1	95.1

[a] One microliter of an acetone–water (1:1) solution containing [*ring*-^{14}C]benfuracarb (3.8 μg, 0.0914 μCi) was injected into the stem of each corn seedling (1.6–1.7 g in fresh weight). There were two plants per set. [b] NH_2-CF and 3-OH-NH_2-CF are artificial products from the acid hydrolysis treatment, and actual metabolites are *N*-CH_2OH-CF and 3-OH-*N*-CH_2OH-CF, respectively.

Table VI. Metabolism of [^{14}C]Benfuracarb in the Bush Bean Plant after Stem Injection[a]

metabolite	% of total benfuracarb appl (at indicated day) 3	10
Organic-Soluble Portion		
benfuracarb	42.7	17.3
carbofuran	26.8	7.0
3-OH-CF	3.3	1.7
N-CH_2OH-CF	0	0.3
3-C=O-*N*-CH_2OH-CF	0	0.3
3-OH-*N*-CH_2OH-Cf	0	
unknown IV	0.5	1.5
subtotal	73.3	28.1
Water-Soluble Conjugate		
CF-P	7.1	8.9
carbofuran	0.7	0
3-C=O-P	0.4	0.9
NH_2-CF[b]	0.4	1.2
3-C=O-NH_2-CF[b]	0.5	0.6
3-OH-P	0.8	2.4
3-OH-CF	8.9	28.6
3-OH-NH_2-CF[b]	0.2	0.5
unknown IV	0.5	4.0
unextractable	1.4	4.6
subtotal	20.9	51.7
residual straw	4.0	8.7
total recovery	98.2	88.5

[a] One microliter of an acetone–water (1:1) solution containing [*ring*-^{14}C]benfuracarb (4.0 μg, 0.0985 μCi) was injected into the stem of each bush bean plant (3.2–3.6 g in fresh weight). There were two plants per set. [b] NH_2-CF and 3-C=O-NH_2-CF are artificial products from the acid hydrolysis treatment, and actual metabolites are *N*-CH_2OH-CF, 3-C=O-*N*-CH_2OH-CF, and 3-OH-*N*-CH_2OH-CF, respectively.

at the lower dose treatment, and in the distribution of radioactivity between the leaf rinse and water-soluble fractions. Compared to the bean plant, benfuracarb was converted more rapidly into carbofuran and 3-OH-CF, which were subsequently converted into different conjugated metabolites. The percentage of radioactivity as water-soluble conjugate at the low-dose treatment reached 40.2 and 65.9% after 3 and 10 days, respectively. 3-C=O-*N*-CH_2OH-CF, which was not observed in the bean plant, was obtained as a minor metabolite.

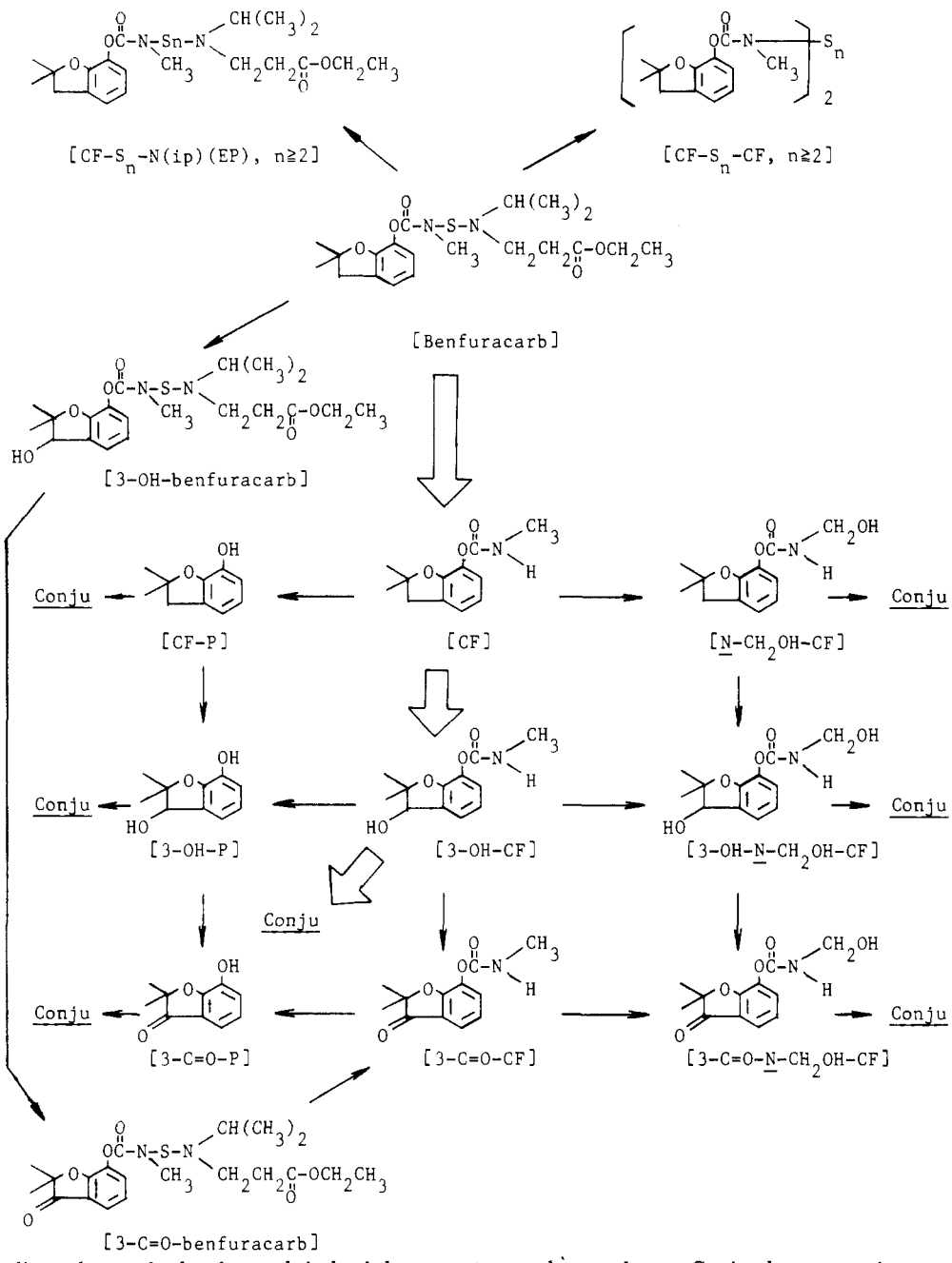


Figure 2. Metabolic pathways for benfuracarb in bush bean, cotton, and corn plants. Conju. denotes conjugate.

Metabolism of Benfuracarb in the Corn and Bush Bean Plant following Stem Injection. An attempt was initially made to examine the metabolism of benfuracarb following application of [*ring*-^{14}C]benfuracarb on the corn leaf. However, because of poor absorption of radioactivity into the corn leaf, a study was made following stem injection.

Table V summarizes data for the distribution, identity, and relative amounts of the various metabolites of benfuracarb in corn plants following stem injection of 3.8 μg of [^{14}C]benfuracarb. The corn plant was very effective in metabolizing benfuracarb. After 1 day, only 57.0% of the radioactivity was present as the parent carbamate, and this gradually decreased during the 10-day test period. The principal metabolite after 1 and 3 days was carbofuran, but 3-OH-CF (free plus conjugated material) was of equal importance after 6 and 10 days. At 10 days, the phenolic product 3-C=O-P was also obtained as a major metabolite (16.3%). Other metabolites were in relatively minor amounts. 3-OH-benfuracarb, 3-C=O-benfuracarb, and the various unknown metabolites I–III, which were observed in the bean and cotton plants following foliar application, were not observed in the corn plant. This may be attributed to the difference in application method than to the difference in plant. This conclusion was supported by a metabolism study in the bean plant following stem injection. Table VI summarizes data for the distribution, identity, and relative amounts of the various metabolites of benfuracarb in the bean plant following stem injection of 4.0 μg of [^{14}C]benfuracarb. The metabolites 3-OH-benfuracarb, 3-C=O-benfuracarb, and various unknowns (II, III), which were observed in the bean plant following foliar application (see Table III), were not observed in the same plant following stem injection.

DISCUSSION

The purpose of this study was to determine the metabolic fate of benfuracarb in the bush bean, cotton, and corn plants following foliar treatment and stem injection of [*ring*-^{14}C]benfuracarb. The metabolic behavior of ben-

furacarb in these plants is summarized by the scheme given in Figure 2. The results clearly indicated N–S bond cleavage as the initial step in the metabolic pathway, giving rise to carbofuran as the first major metabolite. Carbofuran then was further metabolized to 3-OH-CF which, in turn, was conjugated. Overall, carbofuran and 3-OH-CF were the major metabolites, with carbofuran being more significant in the early period after treatment and 3-OH-CF becoming more important in the later periods. These results are in good agreement with those obtained in the metabolism study of [*carbonyl*-^{14}C]benfuracarb in the cotton plant (Umetsu et al., 1985). Except for the identification of 3-C=O-P as one of the major metabolites in the corn plant after stem injection, all other metabolites were of minor significance. Therefore, N–S bond cleavage, oxidation, hydrolysis, and conjugation remain the principal reactions in the metabolism of benfuracarb in the bush bean, cotton, and corn plants.

In the previous study on the metabolism of benfuracarb in the cotton plant following foliar application of [*carbonyl*-^{14}C]benfuracarb (Umetsu et al., 1985), the metabolic fate of the ring moiety following ester hydrolysis of carbamate linkage remained unknown. The present study clearly reveals CF-P, 3-OH-P, and 3-C=O-P, all in the form of conjugates, as the metabolites resulting from hydrolysis of the carbamate moiety.

It is noteworthy that 3-OH-benfuracarb and 3-C=O-benfuracarb, metabolites in which the N-substituted β-alanine ethyl ester moiety remained attached to the carbamate moiety through the sulfur bridge, were detected in significant amounts. *N*-(2-Toluenesulfenyl)-3-ketocarbofuran was previously reported to be a metabolite of *N*-(2-toluenesulfenyl)carbofuran (Black et al., 1973).

Registry No. CF-S_2-N(ip)(EP), 98859-42-8; CF-SO_2-N(ip)(EP), 98874-78-3; CF-S_2-CF, 39995-74-9; 3-OH-benfuracarb, 98859-43-9; 3-C=O-benfuracarb, 98859-44-0; CF, 1563-66-2; 3-OH-CF, 16655-82-6; 3-C=O-CF, 16709-30-1; *N*-CH_2OH-CF, 18999-70-7; 3-OH-*N*-CH_2OH-CF, 17781-14-5; 3-C=O-*N*-CH_2OH-CF, 19019-30-8; NH_2-CF, 4790-87-8; 3-OH-NH_2-CF, 98874-79-4; 3-C=O-NH_2-CF, 98859-45-1; CF-P, 1563-38-8; 3-OH-P, 17781-15-6; 3-C=O-P, 17781-16-7; EtOC(O)$(CH_2)_2$N(*i*-Pr)SCl, 83129-89-9; (EtOC(O)$(CH_2)_2$N(*i*-Pr)S)$_2$, 95255-55-3; benfuracarb, 82560-54-1.

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Mineralization of Chloroaniline/Lignin Conjugates and of Free Chloroanilines by the White Rot Fungus *Phanerochaete chrysosporium*

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Lignin conjugates of [ring-U-^{14}C]-4-chloroaniline and [ring-U-^{14}C]-3,4-dichloroaniline were prepared enzymatically and characterized by gel permeation chromatography and chemical degradation. These samples, as well as the free [ring-U-^{14}C]chloroanilines and a control lignin made from [ring-U-^{14}C]coniferyl alcohol, were fermented with the white rot fungus *Phanerochaete chrysosporium*. The chloroaniline/lignin conjugates were mineralized to carbon dioxide to the same extent as the control lignin, about 60% of the initial radioactivity being trapped as [^{14}C]CO_2 after 30 days. The free [ring-U-^{14}C]-4-chloroaniline and -3,4-dichloroaniline were mineralized to 35% and 50%, respectively. Several fungal chloroaniline-derived metabolites were formed, but the free chloroanilines or their azo and azoxy derivatives could not be detected. It is concluded that lignin incorporation, followed by fungal oxidation, represents a pathway for the complete removal of chloroanilines from the environment.

INTRODUCTION

Chlorinated anilines are widely applied in agriculture as components of acylanilide, *N*-phenylcarbamate, and *N*-phenylurea herbicides. Plant metabolic studies have shown that usually the free chloroanilines are released as primary herbicide metabolites, followed predominantly by incorporation into the plant "insoluble" residue fraction. Lignin has been proposed as a primary binding site in the plant "insoluble" residue on the basis of solubilization and degradation experiments (Balba et al., 1979; Still, 1968; Still et al., 1976; Still et al., 1981; Sutherland, 1976; Yih et al., 1968). Chloroaniline/lignin conjugates have also been prepared enzymatically (Balba et al., 1979; Still et al., 1981; von der Trenck et al., 1981), and the nucleophilic addition of the chloroanilines to the side chain α-carbon atom of lignol quinone methide intermediates has been identified as a major chemical mechanism of copolymerization (Still et al., 1981; von der Trenck et al., 1981). NMR spectral evidence indicated that incorporation via aromatic linkages also occurred (von der Trenck et al., 1981).

In spite of the high amounts of "insoluble" chloroaniline plant residues usually formed, knowledge of their bioavailability is extremely limited. The bioavailability of the total "insoluble" residue formed in rice from propanil labeled in the 3,4-dichloroaniline moiety has been studied in rats and dogs (Sutherland, 1976). Most (76%) of the radioactivity appeared in feces. A small amount (2.4%) of the initial "insoluble" residue appeared in urine and had apparently become bioavailable. The chemical nature of the excreted radioactivities and the fate of the nonrecovered radioactivity (21.5%) were not reported. Lignin itself

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