Comparative Disposition of 3-Hydroxycarbofuran Glucoside and Its Aglycon in Rats

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When ^{14}C-ring-labeled 3-hydroxycarbofuran glucoside (3-OH-C-Glu) and its aglycon (3-OH-C) were administered orally to rats, both compounds were excreted predominantly in the urine. However, the rate of excretion was much faster with 3-OH-C. Urine from the bladder at 1.5 h contained 40% of the 3-OH-C dose but only 7% of the 3-OH-C-Glu treatment; at 6 h, the values were 79 and 43%, respectively. Gastrointestinal absorption of 3-OH-C was complete after 1.5 h, and about half of the dose had been returned to the small intestine via the bile in the form of glucuronide conjugates. Both glucuronide and sulfate conjugates were major components of urinary radiocarbon. With 3-OH-C-Glu, the same process occurred but only after cleavage of the glucosidic linkage in the gut. This cleavage was not complete until the conjugate had moved into the microb-rich cecum, which, in effect, increased the residency time of the toxic metabolite, 3-OH-C, in the body.

With few exceptions, insecticides and most other pesticidal chemicals undergo extensive conjugation metabolism in both plant and animal systems (Dorough, 1979). In most cases, these conjugates are considered toxicologically insignificant when present as residues in crops or animal products intended for human consumption. This is based on the fact that conjugates are usually formed from degradation products of the pesticide that, in themselves, are not toxic and, consequently, the conjugated forms are of even less concern. A potential problem does exits, however, when the conjugate is of a nature that a toxic pesticide moiety could be released when ingested. Such is the case with the carbamate insecticide carbofuran.

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl *N*-methylcarbamate) is oxidatively metabolized, at least in part, to 3-hydroxycarbofuran (3-OH-C) in plants and animals, and this metabolite is of similar toxicity to mammals as the parent compound: acute oral LD50 in rats of 5–20 mg/kg (Metcalf et al., 1968; Dorough, 1968). In animals, 3-OH-C is largely further degraded and eliminated from the body. Contrarily, plants conjugate 3-OH-C with glucose to form 3-hydroxycarbofuran glucoside (3-OH-C-Glu), a major residue in many plants treated with carbofuran and one that is included in analytical methods for this carbamate (Lee and Westcott, 1983; Sonobe et al., 1983). Previous radiotracer studies have shown that 3-OH-C-Glu is absorbed from the gastrointestinal tract of animals as evidenced by the urinary excretion of the compound and its metabolites as glucuronide and sulfate conjugates (Knaak et al., 1970; Marshall and Dorough, 1977). Therefore, there is no question but that 3-OH-C-Glu, in some form, enters the circulatory system and could exert any toxic effects that may be characteristic of the absorbed material. The primary purpose of the present study was to determine if 3-OH-C-Glu was detoxified in the gut prior to absorption or if the glucoside conjugate was cleaved and absorbed as the toxic metabolite, 3-hydroxycarbofuran. Since the latter was observed to be the case, the comparative toxicokinetics of 3-OH-C-Glu and its aglycon, 3-OH-C, were investigated following oral administration to rats.

MATERIALS AND METHODS

Chemicals. Carbofuran-*ring*-^{14}*C* (2,3-dihydro-2,2-dimethyl-7-[*benzo*-^{14}C]benzofuranyl *N*-methylcarbamate; specific activity, 2.85 mCi/mmol) was obtained from Mallinckrodt Nuclear. 1-Naphthyl glucuronide, 1-naphthyl glucoside, and 1-naphthyl sulfate were biosynthesized from 1-naphthol-^{14}*C* (15.2 mCi/mmol) as described previously (Mehendale and Dorough, 1972a,b; Dorough, 1971). Carbofuran, 3-hydroxycarbofuran (2,3-dihydro-2,2-dimethyl-3-hydroxy-7-benzofuranyl *N*-methylcarbamate), 3-ketocarbofuran (2,3-dihydro-2,2-dimethyl-3-keto-7-benzofuranyl *N*-methylcarbamate), carbofuran benzofuran (2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran), 3-hydroxybenzofuran (2,3-dihydro-2,2-dimethyl-3,7-dihydroxybenzofuran), and 3-ketobenzofuran (2,3-dihydro-2,2-dimethyl-3-keto-7-hydroxybenzofuran) were obtained from FMC Corp., Middleport, NY. Saccharo-1,4-lactone was purchased from Calbiochem. NADPH, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, β-glucosidase, β-glucuronidase (Type B-10), and aryl sulfatase (Type V) were purchased from Sigma Chemical Co.

3-Hydroxycarbofuran-*ring*-^{14}*C* was biosynthesized by incubating carbofuran-*ring*-^{14}*C* in a 15000-g rat liver preparation (Dorough, 1968). Livers were obtained from 250-g female rats whose drug metabolizing enzymes had been induced by an ip injection (500 mg/kg) of Aroclor 1254 5 days prior to sacrifice. The glucoside of 3-hydroxycarbofuran was isolated from bean plants treated with 3-hydroxycarbofuran-*ring*-^{14}*C* (Marshall and Dorough, 1977). Acid and glucosidase treatment of the conjugate, followed by TLC analysis of the radiocarbon, showed that the purity of the glucoside metabolite exceeded 98%.

Radioassay. A Beckman Model LS-9000 scintillation spectrometer was used for quantifying radioactivity. Direct scintillation counting of liquid samples was accomplished by using a commercial scintillation cocktail (type 3a70B, Research Products International). Solid samples were combusted in a Packard Model 306 sample oxidizer, and the evolved [^{14}C]carbon dioxide was trapped and radioassayed.

Chromatography. Thin-layer chromatograpy was carried out by using 250 μm thickness Merck silica gel F-254 chromatoplates. Carbofuran metabolites were analyzed by single-dimensional cochromatography using 7:3 benzene–ether and by two dimensional cochromatography with 7:3 benzene–ether as the first solvent system and 4:1 dichloromethane–acetonitrile as the second. Radioactive areas were located by autoradiography, while the nonradioactive standards were visualized by first spraying the plates with 5% KOH in methanol and then spraying with

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a saturated methanolic solution of *p*-nitrobenzenediazonium fluoborate (Marshall andd Dorough, 1977).

Treatment and Sampling. Male Sprague-Dawley rats weighing 250–400 g were used in these studies. Single oral doses of 3-hydroxycarbofuran-*ring*-^{14}C (0.1 mg/kg; 9.5×10^5 dpm) and its glucoside conjugate (0.1 mg/kg; 4.5×10^5 dpm) were prepared in water containing 20% ethanol and administered orally by intubation. For studies involving collection of bile, the cannulation of bile ducts was performed and the rats were partially restrained in plastic metabolism cages as described previously (Marshall and Dorough, 1979). These animals were treated 6 h after surgery. Bile was collected hourly with an Isco Model 1200 fraction collector into tubes containing 1 mL of 0.1 M acetate buffer (pH 5.0) as a precaution against degradation of any metabolites after excretion had occurred.

To investigate the movement of the carbofuran metabolites through the gastrointestinal tract, nonsurgically modified rats were serially sacrificed at 1.5, 3.0, and 6.0 h after treatment. The entire gastrointestinal (GI) tract was removed and sectioned into stomach, small intestine, cecum, and colon. Urine was removed from the bladder and combined with any collected between treatment and sacrifice time. The contents were removed from each segment of the intestine with washes of 10 mL of water and 10 mL of acetone. These washings were mixed, blended in a Virtis homogenizer, and then filtered to remove solid materials. The filtrate was transferred to separatory flasks, saturated with sodium chloride, and extracted 3 times with 30-mL portions of a 1:1 mixture of chloroform and acetonitrile. The organic and aqueous fractions were assayed for radiocarbon content.

In companion studies, segments of the gastrointestinal tract of anesthetized rats were isolated by ligature (Walsh and Levine, 1975) and radioactive carbofuran, 3-hydroxycarbofuran, and 3-hydroxycarbofuran glucoside were injected (0.1 mg/kg in 0.1 mL of 20% ethanol in water) directly into the lumen of an isolated segment with a 30-gauge needle. Segments into which the compounds were individually injected included the stomach, duodenum, ileum, cecum, and colon. Different animals were used for each combination of compound and segment under consideration. After approximately 15 min and then at 6 h, animals were sacrificed, the isolated segment was removed, and the contents were analyzed. As before, urine was removed from the bladder when the rats were sacrificed.

Metabolite Analysis. Ether-extracted bile and urine, as well as the aqueous fractions from gut content extractions, were lyophilized to dryness, and the radiocarbon was recovered from the residue by washing with a 2 to 1 mixture of methanol and chloroform. Only about 3% of the radiocarbon in the bile or urine was extracted with ether, and this was not subjected to further analysis. The polar metabolites were treated with glucosidase, glucuronidase, and sulfatase in 0.1 M acetate buffer (pH 5.0) to determine the nature of the conjugate, and the radiocarbon was converted to ether-extractable metabolites. Saccharo-1,4-lactone (25 mM) was utilized in all sulfatase treatments to inhibit the glucuronidase present in the preparation. The treatments included side-by-side assays of the hydrolytic activity of each enzyme using 1-naphthol-^{14}C conjugates as positive controls. Where sufficient radiocarbon was released, the metabolites were analyzed by TLC.

All data except that pertaining to individual metabolites are presented as the mean obtained from the analysis of a minimum of two different animals. For metabolite analysis, the uring, bile, or GI tract contents from different animals were combined so that sufficient radiocarbon was available to permit examination by enzyme hydrolysis, TLC, and autoradiography.

Table I. Substrate Specificity of Enzyme Preparations Used for Classifying Polar Carbofuran Metabolites as Glucoside, Glucuronide, or Sulfate Conjugates

conjugate standard[a]	% cleavage of conjugate by[b] glucosidase	glucuronidase	sulfatase
1-naphthyl-^{14}C glucoside[c]			
buffer only	82.4	1.0	0.7
+GI tract contents	77.6	1.5	2.2
+bile	59.1	1.0	0.8
+urine	20.4	6.8	3.6
1-naphthyl-^{14}C glucuronide			
buffer only	17.4	97.6	1.8
+GI tract contents	19.0	84.4	1.4
+bile	8.4	90.3	1.3
+urine	5.6	93.4	6.1
1-naphthyl-^{14}C sulfate			
buffer only	5.6	6.5	91.7
+GI tract content	7.7	6.2	91.4
+bile	4.3	5.5	74.9
+urine	7.9	8.8	75.8

[a] Reactions were run in buffer alone and with extracts of control samples (GI contents, bile, urine) prepared in the same manner as those used for analysis of carbofuran conjugates as described in the text. The equivalents of whole GI tract contents, bile, and urine per incubation were 1, 3, and 5 mL, respectively. [b] Each value is the mean of two or more analysis. [c] Similar results were obtained by using 3-hydroxycarbofuran-^{14}C glucoside.

RESULTS AND DISCUSSION

Polar carbofuran metabolites were identified as glucosides, glucuronides, or sulfate conjugates based on conjugate cleavage by the appropriate enzyme preparation. To confirm that the enzymes were active under the conditions of analysis and that they were specific for the intended substrate, conjugates of 1-naphthol were used as positive controls (Table I). Each of the enzyme preparations showed good activity against its substrate when assayed in the standard buffer mixture. Glucosidase enzyme cleaved 82.4% of the 1-naphthyl glucoside, glucuronidase hydrolyzed 97.6% of the 1-naphthyl glucuronide, and sulfatase hydrolyzed 91.7% of the 1-naphthyl sulfate. The specificity was excellent with the exception that the glucosidase preparation did cleave 17.4% of the glucuronide substrate. Its preference for the glucoside, however, was so much greater that this presented no problem in identifying the nature of a conjugate. All of the enzyme preparations were rendered less active when GI tract contents, bile, and urine, were introduced (Table I). Fortunately, the reduced enzyme activity was not of a magnitude that would negate the use of the enzyme preparations as tools for classifying the nature of conjugate metabolites.

The absorption and movement of total radiocarbon in the GI tract of rats treated orally with ^{14}C-labeled 3-hydroxycarbofuran glucoside (3-OH-C-Glu) and its aglycon (3-OH-C) are illustrated in Figure 1. In those samples containing high levels of [^{14}C]carbofuran equivalents, the nature of the conjugate is indicated based on results attained by enzymatic cleavage and TLC analysis (Table II). For these studies, the animals did not have the bile ducts cannulated.

Comparative rates of absorption of the orally administered compounds are best demonstrated by the urinary radiocarbon in animals sacrificed at 1.5, 3, and 6 h after treatment. The most dramatic difference was at 1.5 h

Table II. Glucoside (Glu), Glucuronide (GR), and Sulfate (Sul) Conjugates of Carbofuran Metabolites in the Gastrointestinal Tract and Urine of Rat Treated Orally with ^{14}C-Ring-Labeled 3-Hydroxycarbofuran Glucoside and 3-Hydroxycarbofuran[a]

sample and h after treatment	% of radiocarbon in sample as[b]								
	3-hydroxycarbofuran			3-hydroxybenzofuran			3-ketobenzofuran		
	Glu	GR	Sul	Glu	GR	Sul	Glu	GR	Sul
	3-Hydroxycarbofuran Glucoside[c]								
stomach									
1.5	62.5	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0
3	64.6	0	0	4.4	0	0	0	0	0
small intestine									
1.5	45.6	0	0	7.3	0	0	0	0	0
6	0	31.9	0	0	22.8	0	0	4.2	0
cecum									
3	40.1	0	0	8.7	0	0	0	0	0
urine									
0–6	0	29.9	2.9	0	10.4	6.9	0	3.5	27.9
	3-Hydroxycarbofuran[c]								
small intestine									
1.5	0	46.2	0	0	8.4	0	0	10.6	0
3	0	38.4	0	0	15.6	0	0	14.9	0
urine									
0–6	0	41.9	0.8	0	5.6	9.3	0	1.1	17.1

[a] Analysis of those samples shown in Figure 1 containing the highest levels of radioactive residues. [b] Identification based on the analysis of that radiocarbon converted from water-soluble extractables to organoextractables by glucosidase, glucuronidase, and sulfatase treatments of different aliquots of the same sample. The remainder of radiocarbon in the sample consisted primarily of materials remaining in the water phase after enzyme treatment and solvent extraction. [c] Indicates administered compound.

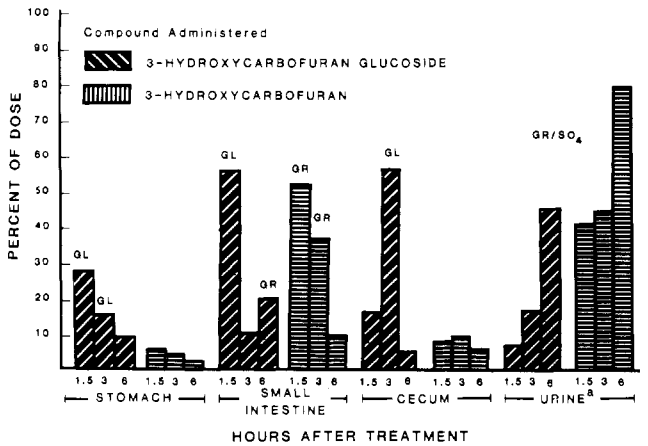

Figure 1. Total radiocarbon and nature of conjugates in the gastrointestinal tract and urine of rats treated orally with ^{14}C-labeled 3-hydroxycarbofuran glucoside and 3-hydroxycarbofuran. GL = glucosides; GR = glucuronides; SO_4 = sulfates. [a] *Includes* urine removed from bladder; for urine only, the data are presented as the cumulative percent of the dose.

where the urine of 3-OH-C-Glu-treated rats contained only 6.9% of the dose, whereas the corresponding value for rats treated with the free metabolite, 3-OH-C, was 40.4% of the dose. By 6 h, urinary excretion was 43.3 and 79.6% of the two doses, respectively (Figure 1). The urinary metabolites were qualitatively the same in both treatment groups and were quite similar quantitatively (Table II). Both glucuronide and sulfate conjugates of 3-OH-C and its hydrolysis metabolites were present in the urine. None of the radiocarbon appeared to be a glucoside since no aglycon was generated by treatment of the urine with glucosidase enzyme. Based solely on the urine data, it appeared that absorption of 3-OH-C-Glu from the GI tract was slower than that for 3-OH-C but that the general metabolic scheme once absorption had occurred was the same for the conjugate and its aglycon.

Absorption was indeed slower for the glucoside metabolite as evidenced by analysis of individual segments of the GI tract (Figure 1). The process of absorption, metabolism, and excretion for the aglycon was quite direct. 3-OH-C was absorbed rapidly from the stomach; only 5% of the administered radiocarbon was in this portion of the GI tract after only 1.5 h, and even less quantities were observed at 3 and 6 h. The small intestine contained 51.4% of the 3-OH-C dose at 1.5 h, 37.2% at 3 h, and 8.6% at 6 h. At no time was free 3-OH-C detected. Rather, the radiocarbon in the small intestine consisted primarily of glucuronide conjugates of 3-OH-C and of its hydrolysis products 3-hydroxybenzofuran and 3-ketobenzofuran (Table II). No sulfate conjugates were detected in the GI tract.

It is possible that 3-OH-C was not absorbed prior to glucuronidation since there is evidence that compounds containing free hydroxy groups may be conjugated as glucuronides by enzymes within the intestinal epithelium (Hietane et al., 1972; Josting et al., 1976). However, this appears unlikely because no glucuronidation of 3-OH-C occurred when the compound was injected directly into various ligated segments of the GI tract (Table III). In fact, the 3-OH-C behaved identically with carbofuran, per se, insofar as absorption from the GI segments and ultimate urinary excretion were concerned. Had the 3-OH-C undergone glucuronidation in the gut, a distinct difference in its absorption and excretion from that of carbofuran would have been expected. It is concluded, therefore, that the 3-OH-C was absorbed and converted primarily to 3-OH-C glucuronide (GR) in the liver and that this compound entered into the process of enterohepatic circulation where other metabolic reactions also came into play until the metabolic products were ultimately eliminated from the body by renal excretion.

The situation was not so direct with 3-OH-C-Glu in rats (Figure 1). At 1.5 h after treatment by gavage, 24.7% of the dose remained in the stomach, and, by 3 h, 15% still remained. Of the radiocarbon in the stomach at these times, between 60 and 65% was as the administered compound (Table II). Unlike the free metabolite, 3-OH-C-Glu moved intact to the small intestine and even into the cecum. At 3 h, 55.7% of the dose was in the cecum; 40% of this was as the administered glucoside and 9% was as 3-hydroxybenzofuran 7-glucuronide (Table II). The remainder was material that could not be cleaved with any of the enzyme treatments. As with the aglycon, less than

Table III. Conjugate Cleavage and Absorption of Radiocarbon after Injection of ^{14}C-Ring-Labeled 3-Hydroxycarbofuran Glucoside into Various Ligated Segments of Rat Gastrointestinal Tract[a]

	% of radiocarbon injected into segment				
	remaining in segment as				
	conjugate		aglycon		urine,
segments	15 min	6 h	15 min	6 h	0–6 h[b]
	3-Hydroxycarbofuran Glucoside				
stomach	73.8	60.8	0.0	1.5	6.3
duodenum	79.3	19.5	5.8	1.4	37.3
ileum	72.2	3.3	8.3	1.5	60.9
cecum	7.3	1.0	71.8	0.7	67.1
colon	69.7	51.8	18.9	4.4	16.7
	3-Hydroxycarbofuran[c]				
all segments, $\bar{X}$	0	0	82.4	2.4	64.4

[a] Abdominal cavity was opened, segments isolated by ligation at both ends, and compound injected into leumen. Animals sacrificed after 15 min and 6 h, and the contents of the segment were analyzed. Other tissues were not analyzed. [b] Includes urine voided and that within bladder at sacrifice. [c] Both 3-hydroxycarbofuran and carbofuran were included in these experiments for comparative purposes. No differences were noted between these compounds or between the various segments. Therefore, the mean values taken from all segments are presented here to demonstrate the rapid absorption and excretion of these apolar compounds.

1% of the 3-OH-C-Glu dose was detected in the colon, suggesting that microbial hydrolysis of the conjugate was occurring in the cecum and that the cleaved 3-OH-C was rapidly absorbed from the cecum.

This was supported by the finding that 3-OH-C-Glu injected directly into the cecum was immediately cleaved to 3-OH-C (Table III). While cleavage and absorption occurred in the ligated duodenum and ileum, very little took place in the stomach and colon. These data indicated that the intact glucoside was not absorbed at any point in the GI tract but that absorption was readily accomplished at any site provided cleavage of the glucoside linkage had taken place. It is also clear from the data in Figure 1 and Table II that the ultimate fate of 3-OH-C in rats is the same whether administered as the free metabolite or as the glucose conjugate. The difference is that the conjugate serves as a slow-release mechanism for 3-OH-C and results in an increased residence time of the toxicant in the body.

In order to document that biliary excretion was of paramount importance in the metabolism of 3-OH-C-Glu just as it was for the aglycon, rats having their bile ducts cannulated were treated with the two carbofuran metabolites. Biliary excretion of radiocarbon after oral treatment of the animals was evident with both chemicals, but the process was slower with 3-OH-C-Glu (Figure 2). After 6 h, 6% of the glucoside dose had been excreted in the bile while 23% biliary excretion had occurred with 3-OH-C. By 24 h, the levels of biliary excretion were similar, being 20 and 29% of the doses, respectively. The nature of the metabolites in the bile was almost identical regardless of whether the rats were treated with 3-OH-C-Glu or its aglycon (Table IV). Only glucuronides were detected in the bile, whereas both glucuronide and sulfate conjugates were voided in the urine. There was no indication that any glucoside metabolite was present in either the bile or urine. The excretion patterns and complement of metabolites observed in these studies with bile duct cannulated rats supported the earlier finding which indicated that the glucoside of 3-OH-C was more slowly absorbed from the GI tract than the aglycone.

Figure 3 summarizes the fate of 3-OH-C-Glu, formed in plants from carbofuran, when this compound is consumed

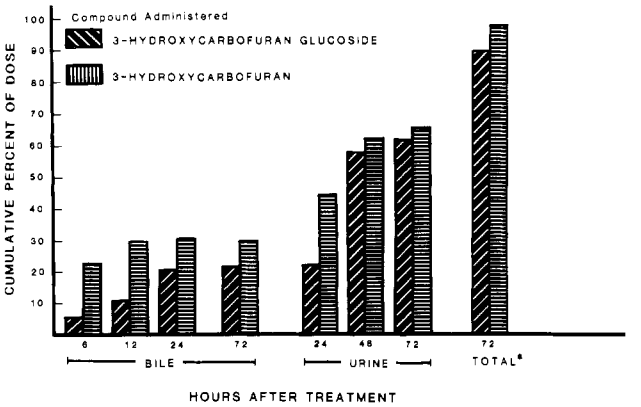

Figure 2. Biliary and urinary elimination of radiocarbon by rats treated orally with ^{14}C-labeled 3-hydroxycarbofuran glucoside and 3-hydroxycarbofuran. [a] *Total* includes fecal excretion of 3.2% and 4.4% of the two doses, respectively.

Table IV. Metabolites in Bile and Urine of Rats Treated Orally with ^{14}C-Ring-Labeled 3-Hydroxycarbofuran (3-OH-C) and 3-Hydroxycarbofuran Glucoside (3-OH-C-Glu)

	% of radiocarbon in sample/treatment			
	bile (0–24 h)		urine (0–48 h)	
metabolite and conjugate[a]	3-OH-C	3-OH-C-Glu	3-OH-C	3-OH-C-Glu
3-hydroxycarbofuran				
glucoside	0.0	0.0	0.0	0.0
glucuronide	62.7	61.2	36.5	23.2
sulfate	0	0	6.5	1.5
total	62.7	61.2	43.0	24.7
3-hydroxybenzofuran				
glucoside	0	0	0	0
glucuronide	2.6	1.7	6.4	4.4
sulfate	0	0	7.3	16.1
total	2.6	1.7	13.7	20.5
3-ketobenzofuran				
glucoside	0	0	0	0
glucuronide	11.8	9.6	6.8	9.6
sulfate	0	0	20.8	31.2
total	11.8	9.6	27.6	40.8
unknowns[b]	22.9	27.5	15.7	14.0

[a] Identification based on the analysis of that radiocarbon converted from water-soluble extractables to organoextractables by glucosidase, glucuronidase, and sulfatase treatments of different aliquots of the same sample. [b] Includes radiocarbon (~3%) extracted prior to enzyme treatment, plus that which remained in the aqueous phase following enzyme treatment and solvent extraction.

in the diet of mammals, in this case rats. On the basis of the present data and assuming the rat is an acceptable model for humans, individuals consuming 3-OH-C-Glu as a plant residue would be exposed to the toxic metabolite, 3-OH-C, in much the same manner as if the aglycon per se was the pesticide residue. The major differnce is that the glucose conjugate resides in the stomach much longer than the aglycon and that conjugate cleavage must occur prior to absorption. Since cleavage is not complete until the 3-OH-C-Glu reaches the cecum, the process of "cleavage followed by absorption" constitutes a mechanism whereby the toxic aglycon, 3-OH-C, becomes bioavailable at a slower rate than if the 3-OH-C was ingested directly.

The toxicological significance of the slow release of 3-OH-C is difficult to predict. On one hand, it might be advantageous since the dose would be in effect fractionated, thereby preventing saturation of biochemical defense mechanisms. On the other, it could be a disadvantage toxicologically because 3-OH-C is a potent anticholin-

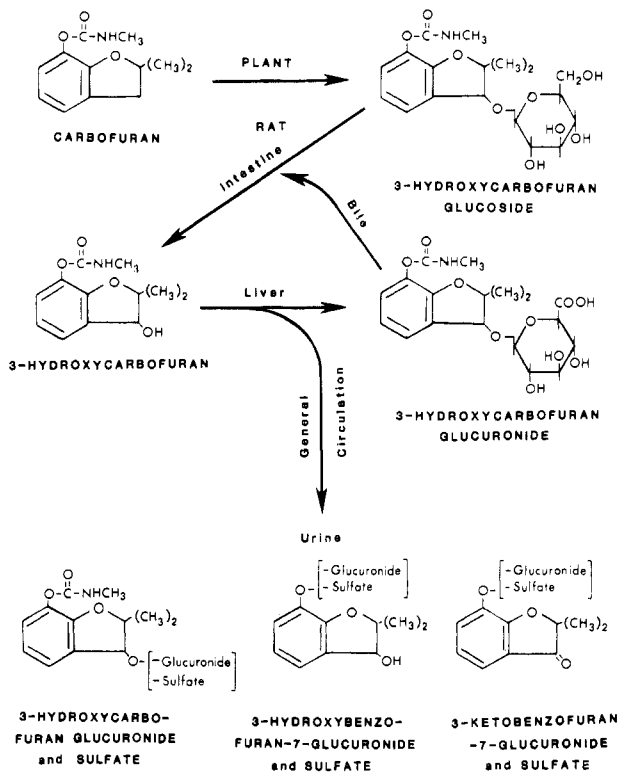

Figure 3. Major metabolic pathway of 3-hydroxycarbofuran glucoside (a plant metabolite of carbofuran) in rats.

esterase agent and any process that increases its residency time in the body could prove detrimental. Fortunately, 3-OH-C-Glu is included in the analysis used to monitor carbofuran residues in plants and is included in the acceptable level of residues established for this insecticide by the regulatory authorities. However, it is not known for certain whether this or any other pesticide conjugate is as safe as the parent compound when consumed in the diet on a chronic basis.

Registry No. 3-OH-C, 16655-82-6; 3-OH-C-Glu, 30368-38-8; 3-hydroxycarbofuran glucuronide, 53305-32-1; 3-hydroxycarbofuran sulfate, 70988-90-8; 3-hydroxybenzofuran 7-glucuronide, 90433-37-7; 3-hydroxybenzofuran 7-sulfate, 90433-38-8; 3-ketobenzofuran 7-glucuronide, 70988-93-1; 3-ketobenzofuran 7-sulfate, 70988-94-2; 3-hydroxybenzofuran glucoside, 30368-38-8.

LITERATURE CITED

Dorough, H. W. *J. Agric. Food Chem.* **1968**, *16*, 319–325.
Dorough, H. W. In "Pesticide Terminal Residues"; Tahori, A., Ed.; Butterworth: New York, 1971; pp 173–183.
Dorough, H. W. *Pharmacol. Ther.* **1979**, *4*, 433–471.
Hietanen, E.; Aitio, A.; Hanninen, O. *Acta Pharmacol. Toxicol.* **1972**, *31*, 186–192.
Josting, D.; Winne, D.; Brock, K. W. *Biochem. Pharmacol.* **1976**, *25*, 613–616.
Knaak, J. B.; Munger, D. M.; McCarthy, J. F. *J. Agric. Food Chem.* **1970**, *18*, 827–832.
Lee, Y. W.; Westcott, N. D. *J. Agric. Food Chem.* **1983**, *31*, 92–96.
Marshall, T. C.; Dorough, H. W. *J. Agric. Food Chem.* **1977**, *25*, 1003–1009.
Marshall, T. C.; Dorough, H. W. *Pestic. Biochem. Physiol.* **1979**, *11*, 56–63.
Mehendale, H. M.; Dorough, H. W. *Pestic. Biochem. Physiol.* **1972a**, *1*, 307–318.
Mehendale, H. M.; Dorough, H. W. *J. Insect Physiol.* **1972b**, *18*, 981–990.
Metcalf, R. L.; Fukuto, T. R.; Collins, C.; Borck, K.; Abd El-Aziz, S.; Munoz, R.; Cassil, C. C. *J. Agric. Food Chem.* **1968**, *16*, 301–311.
Sonobe, H.; Carver, R. A.; Krause, R. T.; Kamps, L. R. *J. Agric. Food Chem.* **1983**, *31*, 96–104.
Walsh, C. T.; Levine, R. R. *J. Pharmacol. Exp. Ther.* **1975**, *197*, 303–310.

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Structural Systematics for 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane-Type Compounds: Crystal Structures of Five Analogues and Comments on Mode of Action

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The crystal structures of five 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) analogues are reported. They are 1,1,1-trichloro-2,2-bis(phenyl)ethane, bis(4-chlorophenyl)methane, 9-(trichloromethyl)-9,10-dihydroanthracene, 1-chloro-2,2-bis(4-chlorophenyl)ethene, and 1,1,1-trichloro-2-(4-chlorophenyl)-2-(2,4-dichlorophenyl)ethane. Holan's DDT mode of action theory is analyzed in terms of available crystallographic data.

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) has been the most widely used and effective insecticide so far developed. The structural criteria among the various DDT analogues for optimum activity are well documented (Riemschneider and Otto, 1954; Metcalf and Fukuto, 1968; Fahmy et al., 1973; Metcalf, 1973). The majority of the theories proposed, regardless of their generation, attempt to relate toxicity with molecular shape and size (derived from structural models) in order to get a hypothetical molecular site model (Gunther et al., 1954; Mullins, 1956; Rogers et al., 1953; Holan, 1969). More recently, Fahmy et al. (1973) reviewed the available theories on structure–activity relationships for the DDT analogues and introduced a more precise theoretical model based on Taft steric substituent parameters (Taft, 1956).

By analyzing data collected from several hundred DDT analogues, it is possible to determine specifications for an active DDT-type insecticide. They are a diphenylmethane

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