Metabolism of Dimethyl *p*-(Methylthio)phenyl Phosphate in Animals and Plants

Don L. Bull* and Richard A. Stokes

The metabolism of ^{32}P- and S-methyl-^{14}C-labeled dimethyl *p*-(methylthio)phenyl phosphate (Allied Chemical GC-6506) in white rats, tobacco budworms, *Heliothis virescens* (F.), and cotton plants was investigated. In all biological forms, GC-6506 was oxidized to the toxic sulfoxide and sulfone derivatives. Initial inactivation of toxicants was achieved by cleavage of the O-methyl or P-O-phenyl linkages; however, the rates of these two reactions differed considerably depending on the test species. The substituted phenols were detoxified primarily by conjugation with glucuronic and sulfuric acid in rats and with glucose in insects and plants. Conjugates were excreted rapidly by the animals, but in plants they remained in the treated foliage without translocation and were metabolized slowly to secondary metabolites, apparently through alteration of the glycone moiety.

A previous report described the systemic activity of ^{32}P-labeled dimethyl *p*-(methylthio)phenyl phosphate (Allied Chemical compound GC-6506) in field-grown cotton plants and its metabolism to different products containing phosphorus (Wendel and Bull, 1970). The experimental evidence indicated that GC-6506 and its two toxic oxidation products were detoxified rapidly in plants by cleavage of the O-methyl and P-O-phenyl molecular linkages. Since the O-demethyl derivatives also were shown to be unstable in the plants owing to decomposition by cleavage at the P-O-phenyl linkage, it was apparent that one ultimate end result of the metabolism of GC-6506 would be complete liberation of the substituted phenols into the plant system. The present paper reports the results of subsequent studies of the metabolism of GC-6506 in rats, insects, and plants with emphasis on the fate of the substituted phenolic moiety of the insecticide.

EXPERIMENTAL

Chemicals. The technical, ^{32}P-labeled (initial specific activity 10 mc per mmole, Amersham/Searle Corp., Des Plaines, Ill.), and S-methyl-^{14}C-labeled (5 mc per mmole, Mallinckrodt Nuclear, St. Louis, Mo.) GC-6506 were provided by Allied Chemical Co., New York, N.Y. The oxidation products, dimethyl *p*-(methylsulfinyl)phenyl phosphate (GC-6506-SO) and dimethyl *p*-(methylsulfonyl)phenyl phosphate (GC-6506-SO_2), and the O-monodemethylated derivatives, methyl *p*-(methylthio)phenyl phosphate (O-demethyl GC-6506), methyl *p*-(methylsulfinyl)phenyl phosphate (O-demethyl GC-6506-SO), and methyl *p*-(methylsulfonyl)phenyl phosphate (O-demethyl GC-6506-SO_2), were synthesized as described by Wendel and Bull (1970).

A pure preparation of *p*-(methylthio)phenol (phenol-S, Crown Zellerbach, Camas, Wash.) was used to synthesize: *p*-(methylsulfinyl)-phenol(phenol-SO), *p*-(methylsulfonyl)phenol(phenol-SO_2), and the acetates of the three substituted phenols (Zincke and Ebel, 1914). The substituted phenols were used to prepare the β-[*p*-(methylthio)phenyl]-D-glucoside (phenol-S glucoside), m.p. 179–180° C (Montgomery *et al.*, 1942). Calcd.: C, 51.64; H, 6.00%. Found: C, 51.39; H, 6.19%. The β-[*p*-(methylthio)phenyl]-D-glucuronide (phenol-S-glucuronide), m.p. 261–263° C decomp. (Bollenback *et al.*, 1955). Calcd.: C, 49.36; H, 5.10%. Found: C, 47.13; H, 5.07% and the *p*-(methylthio)phenyl sulfate (phenol-S sulfate) m.p. 215–218° C (Feigenbaum and Neuberg, 1941). Calcd.: C, 32.78; H, 2.87%. Found: C, 32.55; H, 2.71%. Radiolabeled samples of certain of the compounds were prepared similarly from ^{14}C-labeled phenol-S that was obtained by hydrolyzing ^{14}C-labeled GC-6506 with a slight excess of aqueous sodium hydroxide. Unchanged GC-6506 was removed from this reaction mixture by extraction with chloroform, the aqueous fraction was acidified (pH 3), and the phenol-S recovered by extraction with chloroform. Small amounts of the ^{14}C-labeled or the unlabeled sulfoxide and sulfone derivatives of the phenol-S conjugates were prepared by oxidation of the appropriate compound with hydrogen peroxide in methanol.

Biological Materials and Their Treatment. Female white rats (175 to 200 g, Sprague-Dawley Inc., Madison, Wis.), fifth-instar tobacco budworms, *Heliothis virescens* (F.), and cotton plants of the Deltapine Smoothleaf variety that were grown in a greenhouse were treated with the different radioactive chemicals and then held as described previously (Bull and Ridgway, 1969). In addition, special tests were conducted with the rats and tobacco budworms to determine whether any $^{14}CO_2$ was evolved during the metabolism of ^{14}C-labeled GC-6506 (Bull, 1968).

Analytical Procedures. The urine of rats treated with different chemicals was collected for 16 hr and then adjusted to a convenient volume for further analysis by different methods; radioactive products in feces were not characterized. Procedures used for the preparation of extracts of treated tobacco budworms (Bull and Ridgway, 1969) and cotton leaves (Wendel and Bull, 1970) have been described.

Column chromatography was done with DEAE-cellulose (Cellex D-high capacity, Calbiochem, Los Angeles, Calif.) with glass columns ((2 × 55 cm) and gradient elution (400 ml of each concentration, 0.01*M* to 0.05*M*; 0.05*M* to 0.1*M*) with Tris-HCl buffer (pH 7.5, Sigma Chemical Co., St. Louis, Mo.). A flow rate of 50 ml per hr was established with a buffer pump (The Holter Co., Bridgeport, Pa.). Then 5-ml fractions were collected automatically (Gilson Medical Electronics, Madison, Wis.) and 200-μl aliquots were analyzed for ^{14}C by liquid scintillation (Nuclear-Chicago Corp., Des Plaines, Ill.).

Paper chromatography (pc) done with uncoated Whatman 3 MM paper and a solvent mixture of 12:8:6 butanol, pyridine and water required 16 hr for the completion of ascending development. The thin-layer chromatographic (tlc) procedures and the methods used for detection, quantitation, and identification of radioactive materials were the same as described previously (Wendel and Bull, 1970) except that an

Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas 77840

* To whom correspondence should be addressed.

Table I. Chromatography of GC-6506 and Its Derivatives

Compound	R_f Value in Tlc System[a]			Glc Retention Time (min)[b]	
	A	B	C	160° C	185° C
GC-6506	0.88	0.50	...	...	...
GC-6506-SO	0.10	0.18	...	...	...
GC-6506-SO_2	0.75	0.32	...	...	...
Phenol-S	0.96	0.62	0.86	4.0	1.8
Phenol-S acetate	0.98	0.86	0.96	1.2	0.7
Phenol-SO	0.19	0.15	0.18	...	...
Phenol-SO acetate	0.30	0.21	0.33	15.0	5.3
Phenol-SO_2	0.86	0.26	0.38	...	78.0
Phenol-SO_2 acetate	0.89	0.48	0.77	30.0	10.0

[a] Tlc was done with silica gel G and the following mixtures: A, 4 to 1 ethyl ether and acetonitrile; B, 90:10:3 benzene, ethanol, and ethyl ether; C, 6:3:2 chloroform, hexane, and acetic acid. [b] Where indicated, the free phenols did not elute from the column.

Table II. Relative Concentrations of Radioactive Compounds Resolved by Paper Chromatography of the Urine of Rats After Intraperitoneal Injection of 100 μg of ^{32}P-labeled GC-6506

Compound	R_f	% of Radioactivity Collected 16 Hr Post-treatment[a]
H_3PO_4	0.00	1.6
Methyl phosphate	0.04	7.7
Dimethyl phosphate	0.16	72.6
O-Demethyl GC-6506-SO	0.26	4.1
O-Demethyl GC-6506-SO_2	0.37	0.8
O-Demethyl GC-6506	0.56	6.9
GC-6506-SO	...	4.6
GC-6506-SO_2	...	1.5
GC-6506	...	0.2

[a] After 16 hr, urine contained 85% of administered radioactivity and the feces 5.5%.

additional solvent mixture (6:3:2 chloroform, hexane, and acetic acid) was included to provide better resolution of the substituted phenols.

Certain procedures used for identifications were facilitated by hydrolyzing different conjugated materials, either with enzyme preparations or with 10*N* sulfuric acid; the liberated substituted phenols were recovered by extraction with chloroform. The enzymes (Sigma Chemical Co., St. Louis, Mo.) used included β-glucosidase (emulsin, from almonds), β-glucuronidase (Type B-10, from bovine liver), and phenolsulfatase (Type III, from limpets). All were prepared at a concentration of 5 mg per ml in 0.1*M* phosphate buffer, pH 5.0. Radioactive samples were incubated with the enzyme preparations in open flasks for 2 hr at 37° C in a water-bath shaker (Research Specialties Co., Richmond, Calif.). When acid was used for hydrolyses, samples were heated for 1 hr at 100° C in an oil bath (Fisher Scientific Co., Houston, Tex.). (However, enzyme treatment proved most satisfactory and was used for the major portion of the work.)

Gas chromatography (glc) was done with an F. & M. 402 instrument (F. & M. Scientific, Avondale, Pa.) equipped with a flame photometric detector (Tracor Inc., Austin, Tex.) operated in the sulfur mode (394-mμ filter). A U-shaped glass column (4 ft × 3 mm i.d.) packed with 2% diethylene glycol succinate on 60/80 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.) was used at oven temperatures of 160 or 185° C, injection port temperatures of 175 to 195° C, and a detector temperature of 150° C (external). Nitrogen was used as the carrier gas at a flow rate of 185 ml per minute.

Table III. Relative Concentrations of Radioactive Compounds Resolved By DEAE-Cellulose Column Chromatography of Urine Collected From Rats After Intraperitoneal Injection With 100 μg of ^{14}C-Labeled GC-6506 or Its Derivatives

Fraction	% of Chromatographed Radioactivity in Indicated Fraction After Treatment With					
	GC-6506	GC-6506 SO	GC-6506 SO_2	Phenol S	Phenol SO	Phenol SO_2
A	7.8	6.8	0.7	...	...	...
B	0.4	0.5	...	0.2	0.3	...
C	0.6	1.0	...	0.7	3.5	...
D	8.8	9.2	...	10.1	14.0	...
E	10.8	10.0	31.9	...	...	43.4
F	18.9	7.3	...	22.3	1.9	...
G	27.2	43.2	...	21.1	58.1	...
H	15.2	13.0	67.4	5.6	7.0	56.6
I	10.3	9.0	...	40.0	15.2	...
% of dose excreted in urine after 16 hr	82.0	86.5	90.0	89.0	92.6	93.5

Table IV. Relative Concentrations of Radioactive Compounds Resolved by Paper Chromatography of the Urine of Rats After Intraperitoneal Injection with 100 μg of ^{14}C-Labeled GC-6506 or Its Derivatives

R_f and Spot Number	% of Chromatographed Radioactivity in Indicated Spot After Treatment With					
	GC-6506	GC-6506 SO	GC-6506 SO_2	Phenol S	Phenol SO	Phenol SO_2
0.10 I	5.2	6.7	0.0	0.0	0.0	0.0
0.15 II	8.3	0.0	0.0	3.7	8.5	0.0
0.23 III	2.1	5.4	26.8	0.0	0.0	20.5
0.30 IV	1.3	3.0	0.0	0.0	0.0	1.3
0.37 V	8.3	4.5	0.0	13.4	1.5	0.0
0.52 VI	32.7	47.0	0.0	11.8	66.5	0.0
0.60 VII	17.8	20.2	69.3	1.4	1.7	61.8
0.70 VIII	17.6	11.2	0.0	58.8	15.7	0.0
0.82 IX	2.1	2.0	3.9	2.6	4.2	16.4
0.93 X	2.4	0.0	0.0	0.0	0.0	0.0
0.97 XI	2.2	0.0	0.0	8.3	1.9	0.0

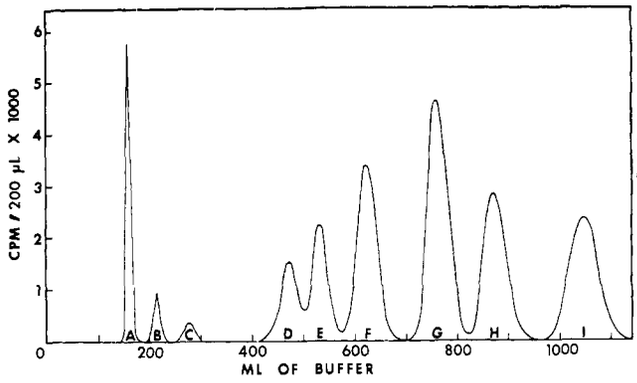

Figure 1. Chromatography of the rat urinary metabolites of ^{14}C-labeled GC-6506 with a DEAE-cellulose column

RESULTS AND DISCUSSION

Chromatography. The R_f values for GC-6506 and its chloroform-soluble derivatives in the tlc systems used and the glc retention times for the substituted phenols are listed in Table I. Table II includes R_f values in the pc system for the three O-demethyl derivatives, dimethyl phosphate, methyl phosphate, and H_3PO_4; the values in the same system for the phenolic conjugates are listed in Tables IV and VI.

Metabolism in Rats. After an intraperitoneal injection of 100 µg of ^{32}P-labeled GC-6506, more than 90% of the dose was excreted, primarily in the urine (Table II). Only small amounts (6.3%) of the toxic compounds were excreted; most of the radioactivity (81.9%) was produced by cleavage of the P-O-phenyl linkage, with dimethyl phosphate the predominant product (72.6%). The O-demethylated products represented 11.8% of the recovered radioactivity.

When rats were treated with a similar dose of ^{14}C-labeled GC-6506, 82% was excreted in urine after 16 hr (Table III). Fractionation of this urine on DEAE-cellulose revealed nine distinct peaks of radioactivity (Figure 1). Subsequently, rats were treated with similar doses (100 µg) of ^{14}C-labeled GC-6506-SO, GC-6506-SO_2, and the three substituted phenols, and urine was collected through 16 hr and fractionated as before on DEAE-cellulose columns. Amounts of the administered dose excreted and the relative concentrations of radioactive material appearing in each DEAE fraction after the treatment with different compounds are shown in Table III. The pc behavior of the same urine samples is shown in Table IV. These data show that treatment with each derivative of GC-6506 led to the formation of radioactive compounds that coincided with certain of those formed from the parent compound.

Table V. Apparent Identity of Radioactive Compounds Recovered From the Urine of Rats After Treatment With ^{14}C-Labeled GC-6506 or Its Derivatives

PC Spot No.	DEAE Fraction	Components of Conjugate: Phenolic Moiety	Components of Conjugate: Nonphenolic Moiety	Unconjugated Compounds
I	D	phenol-SO	glucuronic	
II		phenol-SO	unknown	
III	E	phenol-SO_2	glucuronic	
IV		phenol-SO_2	unknown	
V	F	phenol-S	glucuronic	
VI	G	phenol-SO	sulfuric	
VII	H	phenol-SO_2	sulfuric	
VIII	I	phenol-S	sulfuric	
IX	E	...	...	phenol-SO_2
X	C	...	...	phenol-SO
XI	A, B	...	...	(A) = GC-6506, GC-6506-SO, and GC-6506-SO_2 (B) = phenol-S

Special tests with rats treated with ^{14}C-labeled GC-6506 or phenol-S revealed that no $^{14}CO_2$ was evolved during 24 hr posttreatment. In addition, exhaustive two-dimensional tlc and glc analyses of the substituted phenols (either free or after acetylation) liberated by acid hydrolysis of whole urine samples from the different treatments indicated that only the three substituted phenols listed were present. Thus, the *C-S*-phenyl linkage apparently was not ruptured during metabolism, nor was there any alteration of the phenyl ring, such as hydroxylation, that could be detected with the procedures used.

Since previous studies of phenols in animals had indicated that the principal metabolic fate of monohydric substituted phenols involved enzymatic conversion to monoesters of sulfuric or glucuronic acid (Williams, 1964), we anticipated that the substituted phenols included in this study would encounter a similar fate. Each DEAE fraction was therefore cochromatographed on pc with the conjugates that were synthesized. The information obtained indicated that each substituted

Table VI. Relative Concentrations of Radioactive Compounds in Individual Cotton Leaves After Treatment by Petiole Injection of 100 µg of ^{14}C-Labeled GC-6506

Nature of Radioactivity	R_f [a]	% of Dose at Indicated Days After Treatment: 0	1	2	4	8
GC-6506	1.00	100.0	25.2	5.9	0.0	0.0
GC-6506-SO	0.98	...	48.8	34.5	16.2	5.0
GC-6506-SO_2	1.00	...	1.8	9.0	5.9	2.7
Phenol-SO	0.90	...	4.4	4.5	2.7	1.5
Phenol-SO_2	0.96	...	0.0	0.8	1.2	1.4
Phenol-S glucoside	0.85	...	3.9	8.0	6.1	2.6
Phenol-SO glucoside	0.55	...	6.5	12.2	23.4	21.2
Phenol-SO_2 glucoside	0.69	...	1.4	11.5	22.9	29.0
Unknown A	0.27	...	4.9	5.5	10.1	18.2
Unknown B	0.38	...	0.0	2.6	4.1	10.3
Unknown C	0.46	...	0.0	1.4	2.3	2.4
Lost		0.0	3.1	4.1	5.1	5.7

[a] The first five compounds were resolved by combined pc and tlc procedures; the remainder were resolved by pc.

Table VII. Relative Concentration of Radioactive Compounds in Cotton Leaves After Petiole Injection With 50 μg of ^{14}C-Labeled Phenol-S, Phenol-SO, or Phenol-SO_2

Nature of Radioactivity	% of Dose at Indicated Day After Treatment With								
	Phenol-S			Phenol-SO			Phenol-SO_2		
	0	1	2	0	1	2	0	1	2
Unconjugated phenols	97.5	47.4	14.3	80.5	31.1	12.8	75.6	14.2	2.6
Phenol-S glucoside	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phenol-SO glucoside	2.5	27.8	30.5	19.5	44.8	44.9	0.0	0.0	0.0
Phenol-SO_2 glucoside	0.0	0.0	8.3	0.0	8.1	6.9	24.4	68.4	68.0
Unknown(s)	0.0	10.4	32.0	0.0	10.5	25.8	0.0	15.4	25.4
Lost	0.0	14.4	14.9	0.0	5.5	9.6	0.0	2.0	4.0

Table VIII. Relative Concentrations of Radioactive Compounds in Fifth-Instar Tobacco Budworms After Oral Treatment With 2 μg ^{32}P-Labeled GC-6506 or Its Oxidation Products

Nature of Radioactivity	% of Dose at Indicated Hours After Treatment With								
	GC-6506			GC-6506-SO			GC-6506-S_2		
	0	1	2	0	1	2	0	1	2
					Internal				
H_3PO_4	0.1	0.2	0.2	0.0	0.0	0.1	0.0	0.1	0.1
Dimethyl phosphate	0.2	2.8	3.5	0.4	3.5	4.1	0.2	4.9	6.1
O-Demethyl GC-6506-SO	0.1	2.0	1.8	0.8	2.9	3.5	0.0	0.0	0.0
O-Demethyl GC-6506-SO_2	0.0	2.2	2.1	0.0	1.2	1.5	0.2	5.0	7.2
O-Demethyl GC-6506	0.2	0.5	0.6	0.0	0.0	0.0	0.0	0.0	0.0
GC-6506	79.2	15.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0
GC-6506-SO	19.6	44.5	33.0	93.2	56.4	37.5	0.0	0.0	0.0
GC-6506-SO_2	0.0	9.4	15.7	5.0	15.6	15.2	99.6	68.9	48.4
					Excreta				
H_3PO_4	...	0.0	0.2	...	0.0	0.2	...	0.1	0.2
Dimethyl phosphate	...	0.2	1.4	...	0.7	2.1	...	0.9	2.3
O-Demethyl GC-6506-SO	...	0.2	1.4	...	0.7	3.1	...	0.0	0.0
O-Demethyl GC-6506-SO_2	...	0.1	1.7	...	0.6	1.1	...	2.4	3.9
O-Demethyl GC-6506	...	0.4	0.3	...	0.0	0.0	...	0.0	0.0
GC-6506	...	6.9	6.1	...	0.0	0.0	...	0.0	0.0
GC-6506-SO	...	4.6	11.6	...	9.6	16.1	...	0.0	0.0
GC-6506-SO_2	...	0.4	2.0	...	1.5	2.2	...	9.5	15.6
Unextractable	0.6	3.6	3.9	0.6	4.7	5.7	0.7	4.6	7.0

phenol was conjugated with both sulfuric and glucuronic acid. These initial identifications were checked by incubating samples from each DEAE fraction with β-glucuronidase and phenolsulfatase and identifying the liberated phenols by tlc and glc as described. (The β-glucuronidase caused no changes in the sulfate fractions; phenolsulfatase contained small amounts of β-glucuronidase and thus caused some hydrolysis of glucuronide fractions.) The apparent identities of the radioactive fractions are described in Table V. In general, it was shown that small concentrations of GC-6506, GC-6506-SO, and GC-6506-SO_2 were excreted unchanged, and even smaller amounts of the free phenols were excreted (Table III); these compounds eluted with or shortly after the void volume of DEAE columns. With all treatments, the concentrations of ethereal sulfates excreted exceeded those of the glucuronides. The recovery of significant levels of phenol-S conjugated materials in urine when rats were treated with GC-6506-SO or phenol-SO was of special interest. It indicated that there was a substantial reduction of the sulfoxide group to the sulfide; no comparable reduction of the sulfone group was detected. It was apparent that the O-demethyl derivatives cochromatographed with certain of the conjugate fractions; however, the identities and relative concentrations of these products were established in the ^{32}P studies, so no further attempt at their resolution was made in the ^{14}C work.

Metabolism in Plants. In individual cotton leaves treated by petiole injection, ^{14}C-labeled GC-6506 was converted rapidly to its toxic oxidation products, and all toxicants were detoxified readily (Table VI) much as shown in previous field studies with ^{32}P-labeled GC-6506 (Wendel and Bull, 1970). Cochromatographic studies with synthetic conjugate standards and the identification of substituted phenols liberated from certain plant metabolites (purified by preparative pc) by hydrolysis with β-glucosidase were used to establish that the major portion of the water-soluble radioactivity extracted after 8 days was in the form of glucosylated phenols. The predominant products were the glucosides of phenol-SO and phenol-SO_2. The area of radioactivity designated as unknown A probably included some O-demethyl GC-6506-SO, and unknown B included some O-demethyl GC-6506-SO_2 because their R_f values were identical; also, these metabolites were demonstrated in the ^{32}P studies (Wendel and Bull, 1970). However, these were probably minor metabolites because their instability in cotton plants would tend to preclude accumulation. The information obtained from subsequent studies of the metabolism of the individual ^{14}C-labeled substituted phenols suggested that the initial reaction involved glucosylation (Table VII). Then the phenolic glucoside, in turn, was changed slowly to an unidentified metabolite(s), possibly by alteration of the glucosyl moiety of the molecule. Thus, for special tests of this hypothesis, some of the phenol-SO_2 glucoside was extracted from leaves that had been treated with ^{14}C-labeled phenol-SO_2, purified with preparative pc, and then reinjected (20 μg) into a cotton leaf. After 4 days, it was found that 31% of the dose had been converted to the same unknown metabolite (R_f 0.38) reported in Table VII. When this metabolite was hydrolyzed with β-glucosidase, phenol-SO_2 was the only radioactive compound liberated. This

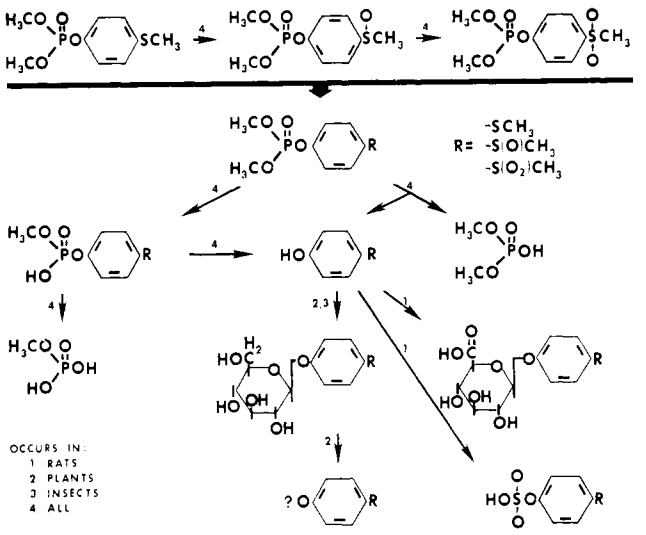

Figure 2. Composite pathway for the formation of certain metabolites of GC-6506 in animals and plants

evidence supports speculation that the glycone portion of the phenolic conjugates is indeed altered in plants, possibly by the formation of a β-gentiobioside comparable to those reported from studies of other phenols in plants (Towers, 1964). The high efficiency of extraction of applied radioactivity after treatments with ^{14}C-labeled GC-6506 or with the substituted phenols and the fact that much of this was conjugated material support the findings of Miller (1940) that glucosides are not translocated from the tissues in which they are synthesized.

In the previous study (Wendel and Bull, 1970) it was reported that after 8 days 26.2% of the injected dose of ^{32}P-labeled GC-6506 was bound in leaf tissues in unextractable form. Since the extraction procedures were identical, results of the ^{14}C studies represent additional evidence for the conclusion that the unextractable ^{32}P was probably a result of the incorporation of inorganic phosphate, formed during metabolism, into the insoluble structural components of plants. However, no direct attempt was made to identify the bound radioactivity.

Metabolism of Insects. The results of studies of the metabolism of ^{32}P-labeled GC-6506 and its two oxidation products after oral administration (2 μg) to fifth-instar tobacco budworms are shown in Table VIII. Qualitatively, the metabolism followed the same route as in plants; however, the rates of certain reactions were somewhat different and significant amounts of toxicants were eliminated directly by excretion. The initial detoxification of GC-6506 and its oxidation products was achieved by approximately equivalent cleavage of the O-methyl and P-O-phenyl linkages of the molecules.

Current *in vitro* studies of partially purified enzymes from tobacco budworms suggest that the O-demethylation is a glutathione-dependent reaction and that P-O-phenyl cleavage is effected by at least two enzymes, one associated with the soluble fraction and the other with the insoluble fraction (Bull and Whitten, 1970). From special tests with larvae that were treated with ^{14}C-labeled phenol-SO_2, it was found by analysis on pc that 24.8% of the dose was excreted unchanged through a 48-hr experimental period, 60.5% was the phenol-SO_2 glucoside, and the remainder was in the form of two unidentified compounds (10.6% at R_f 0.07, and 4.2% at R_f 0.18). The phenol-SO_2 glucoside from insects appeared undistinguishable from that found in plants. (In fact, when the purified phenol-SO_2 glucoside from insects was injected into cotton leaves, it was converted to the same metabolite that was formed when the leaves were treated with the plant-produced glucoside.) The unknown metabolites of phenol-SO_2 were not fully characterized; however, acid hydrolysis liberated only the phenol-SO_2. This evidence agrees with previous reports that glucosylation is a major mechanism for the detoxification of phenolic compounds in insects (Dutton, 1966; Smith, 1962).

Thus, the metabolism of GC-6506 in the animals and plants studied follows a complicated route; much of this was elucidated and is summarized in the composite pathway depicted in Figure 2.

LITERATURE CITED

Bollenback, G. N., Long, J. W., Benjamin, D. G., Lindquist, J. A., *J. Amer. Chem. Soc.* **77,** 3310 (1955).
Bull, D. L., J. AGR. FOOD CHEM. **16,** 610 (1968).
Bull, D. L., Ridgway, R. L., J. AGR. FOOD CHEM. **17,** 837 (1969).
Bull, D. L., Whitten, C. J., unpublished data (1970).
Dutton, G. J., "Glucuronic Acid, Free and Combined," pp. 185–299, Academic Press, New York and London (1966).
Feigenbaum, J., Neuberg, C. A., *J. Amer. Chem. Soc.* **63,** 3529 (1941).
Miller, L. P., *Contr. Boyce Thompson Inst.* **11,** 271 (1940).
Montgomery, E. M., Richtmyer, N. K., Hudson, C. S., *J. Amer. Chem. Soc.* **64,** 690 (1942).
Smith, J. N., *Ann. Rev. Entomol.* **7,** 465 (1962).
Towers, G. H. N., "Biochemistry of Phenolic Compounds," pp. 249–294, Academic Press, New York and London (1964).
Wendel, L. E., Bull, D. L., J. AGR. FOOD CHEM. **18,** 420 (1970).
Williams, R. T., "Biochemistry of Phenolic Compounds," pp. 205–248, Academic Press, New York and London (1964).
Zincke, T., Ebel, C., *Ber.* **47,** 1100 (1914).

Received for review June 1, 1970. Accepted August 17, 1970. This work was done in cooperation with Texas A&M University, Texas Agricultural Experiment Station, College Station, Texas, and supported in part by Allied Chemical Co., New York, N.Y. Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement by the U.S. Department of Agriculture.