Metabolism of *O,O*-Dimethyl Phosphorodithioate *S*-Ester with 4-(Mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one (Geigy GS-13005) in Plants and Animals

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Relatively small amounts of Geigy GS-13005-[*O,O*-dimethyl phosphorodithioate *S*-ester with 4 - (mercaptomethyl) - 2 - methoxy - Δ^2 - 1,3,4-thiadiazolin-5-one] were absorbed by cotton plants after foliar, seed, or stem treatments. Only small concentrations of GS-13005 were translocated to new plant growth and none was detected in the fruit. The highly toxic oxygen analog of GS-13005 was found in extracts of treated plants and insects but not detected in the urine of white rats.

Geigy GS-13005 [*O,O*-dimethyl phosphorodithioate *S*-ester with 4-(mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one] is an experimental insecticide-acaricide that has shown promise of controlling certain arthropod pests of alfalfa, cotton, citrus, and tobacco. The metabolism of the compound in plants and animals has been described briefly in preliminary reports by Bull (1965) and Esser and Müller (1966). The present paper presents a more complete report of the fate of the compound in plants and animals.

EXPERIMENTAL

Chemicals. Several radioactive preparations of GS-13005 were provided by the Geigy Chemical Corp., Ardsley, N. Y. They included ^{14}C labels in the 2-methoxy (3.67 μc. per mg.), carbonyl (3.43 μc. per mg.), and methylene (3.42 μc. per mg.) positions, and a ^{32}P-labeled preparation (initial specific activity 61 μc. per mg). Also supplied were carbonyl-^{14}C-labeled (5.16 μc. per mg.) 2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one (MTDO), and carbonyl-^{14}C- (6.42 μc. per mg.) and methylene-^{14}C- (5.5 μc. per mg.) labeled 4-(hydroxymethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one (hydroxymethyl MTDO). A radioactive sample of GS-13007 [*O,O*-dimethyl phosphorothiolate *S*-ester with 4-(mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one], the oxygen analog of GS-13005, was prepared from GS-13005-2-methoxy-^{14}C by chemical oxidation with one molar equivalent of *m*-chloroperoxybenzoic acid in chloroform solution and isolated by thin-layer chromatography (TLC).

Nonradioactive chemicals used included GS-13005, GS-13007, MTDO, and hydroxymethyl MTDO (supplied by Geigy Chemical Corp.), methyl phosphate and dimethyl phosphate (obtained from Shell Development Co., Modesto, Calif.), and *O,O*-dimethyl phosphorothioate and *O,O*-dimethyl phosphorodithioate (obtained from American Cyanamid Co., Princeton, N. J.). The desmethyl derivative of GS-13005 [*O*-methyl, *O*-hydrogen phosphorodithioate *S*-ester with 4-(mercaptomethyl)-2-methoxy-Δ^2-1,3,4-thiadiazolin-5-one] was prepared as the sodium salt by reaction of equimolar amounts of GS-13005 and sodium thiophenoxide in 95% ethanol (Hollingworth *et al.*, 1967; Miller, 1962). Desmethyl GS-13005 was recrystallized from chloroform–*n*-hexane (m.p. 103–5° C.) and analyzed: Calculated for $C_5H_8N_2O_4PNaS_3$, C 19.35%, H 2.60%; found, C 19.22%, H 2.87%. A radiolabeled preparation of desmethyl GS-13005 also was prepared using GS-13005-2-methoxy-^{14}C.

Radioactive compounds and the chemicals used for toxicity studies were free of impurities as indicated by two-dimensional TLC using the detection methods described below. Other chemicals had a purity of approximately 95% or greater as indicated by two-dimensional TLC or one-dimensional paper chromatography.

Plants and Their Treatment. Cotton plants of the Deltapine Smoothleaf variety were grown either normally in the field or in a greenhouse. For bioassay studies, three types of treatment with GS-13005 were used.

PETIOLE INJECTION. Fully expanded, individual leaves of mature plants were treated in situ by injecting the petiole (Bull *et al.*, 1967a) with 100 μg. of GS-13005 in 5 μl. of 75% ethanol.

STEM TREATMENT. Stems of cotton plants with four true leaves were treated with a 5% mixture of GS-13005 in lanolin by applying 100 mg. of the mixture to the main stem in a 1.5-inch band just below the cotyledons.

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FOLIAR TREATMENT. Plants with four true leaves were sprayed with an emulsion of GS-13005 at a rate of 1 pound per acre.

The four principal methods of treatment used for studies of the systemic activity and metabolism of radiolabeled GS-13005 in cotton included:

FOLIAR TREATMENT. 100 μg. of GS-13005 in 50 μl. of 75% ethanol spread as uniformly as possible over the upper surface of each leaf.

PETIOLE INJECTION. Described above.

STEM TREATMENT. 2 mg. of GS-13005 in an acetone corn oil mixture (9 to 1 v./v.) applied near the base of the main stem of each plant (the treated portion of the plant was immediately enclosed with an inverted paper cup to minimize contamination of foliage by volatilized insecticide and was not analyzed).

SEED TREATMENT. Flame-delinted cotton seeds each treated with 1 mg. of GS-13005 in 50 μl. of ethanol and then planted after the solvent had evaporated.

In addition, some metabolism studies were done with excised cotton leaves prepared and treated as described by Bull *et al.* (1967a).

The metabolism of GS-13005-^{32}P in alfalfa plants of the Oklahoma Common variety was investigated by treating greenhouse-grown plants at the base of each stem with 100 μg. of the radiolabeled compound in 25 μl. of ethanol. The site of treatment was included in the analysis when plants were harvested immediately after application; however, plants collected later were cut off above the site.

Bean plants of the Kentucky Wonder variety were used for certain studies of the metabolism of ^{14}C-labeled GS-13005. These plants were grown in the greenhouse and treated by injecting a solution of the radiolabeled compound in ethanol directly into the main stem with a microsyringe.

Rats and Their Treatment. White rats used for metabolism studies were males of the Wistar strain and had an average weight of 250 grams. The animals were treated with different radiolabeled preparations of GS-13005 (10 mg. per kg. in propylene glycol) or MTDO-^{14}C (6 mg. per animal) by intraperitoneal injection. After treatment, the rats were held at 27° C. either in ventilated metabolism cages that allowed separation of urine and feces or in glass metabolism cages (Delmar, Maywood, Ill.) equipped for the collection of expired CO_2 as well as for separation of urine and feces. Food and water were provided throughout each experimental period.

Insects and Their Treatment. Adult boll weevils [*Anthonomus grandis* (Boheman)], boll worms [*Heliothis zea* (Boddie)], tobacco budworms [*Heliothis virescens* (F.)], and adult houseflies (*Musca domestica* L.) were selected at random from insecticide-susceptible laboratory colonies for use in different experiments. All insects were reared and tested under continuous light at 27° C.

Before treatment, the insects were anesthetized lightly with CO_2. Solutions of insecticide for studies of both toxicity and metabolism were administered with a calibrated, micrometer-driven syringe. For toxicity studies, 1 μl. of acetone containing the desired concentration of insecticide was applied dorsally on each insect. The fifth instar tobacco budworm larvae used for metabolism studies were treated by a ventral injection of 1 μl. of a propylene glycol and insecticide solution into the abdominal cavity. Insects used for toxicity studies were allowed to feed during test periods; those used for metabolism studies were confined without food in individual glass containers. For certain studies with C^{14}-labeled compounds, fifth instar tobacco budworms were confined without food in small individual screen cages within a cylindrical glass chamber that allowed recovery of expired $^{14}CO_2$. All $^{14}CO_2$ trapping studies were done according to the procedure of Jeffay and Alvarez (1961).

Bioassays with adult boll weevils were made as described by Bull *et al.* (1964). With bollworms, first instar larvae were caged directly on treated cotton leaves in ventilated $^1/_2$-pint ice cream cartons. Each cage contained 10 larvae, and tests were done in triplicate at each specified time after treatment. Similar numbers of insects were caged on untreated plants. Mortality was recorded daily for 3 days and data were corrected for check mortality. Each complete experiment was replicated three times.

Preparation and Analysis of Extracts. When surface applications were used on leaves, unabsorbed radioactivity was recovered, by rinsing them briefly with methanol, and analyzed separately. Internal extracts were prepared by homogenizing treated plant material in a semimicroblender (Eberbach Corp., Ann Arbor, Mich.) with a 1 to 1 methanol-water mixture (1 gram fresh weight per 25 ml.). Homogenates were centrifuged and the precipitated solids were extracted twice more with similar volumes of the same solvent mixture. After the supernatants were combined, methanol was removed under vacuum and the remaining aqueous portion of the extract was partitioned with 2 parts of chloroform. This procedure was repeated twice. Then the chloroform fractions were dried with anhydrous sodium sulfate and combined.

The treated insects were extracted in a similar manner, except that a Teflon tissue homogenizer (Tri-R-Instruments, Jamaica, N. Y.) was used and the initial homogenate was made with water. In addition, excreta were recovered by scrubbing the containers first with water and then with methanol. Urine of treated rats also was extracted with chloroform as described.

Compounds that partitioned into chloroform fractions (GS-13005, GS-13007, MTDO, and hydroxymethyl MTDO) were resolved by TLC using silica gel G (Brinkman Instruments, Inc., Westbury, N. Y.) with solvent mixture A (1 to 1 *n*-hexane–anhydrous diethyl ether) or B (17 to 3 toluene-acetone). Products that remained in the aqueous fractions were resolved by TLC or paper chromatography (PC) using uncoated Whatman 3-mm. chromatography paper and solvent mixtures C (40:9:1, acetonitrile–water–ammonium hydroxide) or D (12:5:31–butanol–water–acetic acid).

Aliquots of liquid fractions and radioactive areas from chromatograms were radioassayed by liquid scintillation or with a thin-window, gas-flow Geiger-Müller counter. Radioactivity that was not extracted from tissues by the procedures used was determined by counting the material in planchets after it had been dried and pulverized. When necessary, appropriate corrections were made for quenching, self-absorption, or radioactive decay.

Identifications were based primarily on cochromatography of radioactive metabolites (located by autoradiography on x-ray film) with authentic compounds that were located colorimetrically with 0.2% *N*-2,6-trichloro-*p*-benzoquinoneimine in ethanol or with the phosphorus-

detection reagent of Hanes and Isherwood (1949). The R_f values of GS-13005 and certain of its metabolites in four chromatographic systems are listed in Table I. The data are averages of 10 or more analyses.

Anticholinesterase Studies. The anticholinesterase (anti-ChE) properties of GS-13005 and GS-13007 were determined with the manometric technique of Metcalf *et al.* (1955) using a differential respirometer (Gilson Medical Electronics, Middleton, Wis.). The enzyme preparations used for this method included homogenates of housefly heads (final concentration of 1 per ml.) and whole boll weevils (2 per ml.), and purified bovine erythrocyte acetylcholinesterase (2.5 units per ml., Sigma Chemical Co., St. Louis, Mo.). After preincubation for 1 hour at 37° C. with the desired concentrations of inhibitor, the residual enzyme activity was measured and compared with that of untreated controls.

RESULTS AND DISCUSSION

Biological Activity. The results of manometric anti-ChE and topical toxicity studies are shown in Table II. GS-13007 severely inhibited the activity of all enzyme preparations. GS-13005 did not inhibit bovine AChE, even at high concentrations ($10^{-3}M$), but it did inhibit activity in the insect extracts. In all probability, GS-13005 *per se* is a poor inhibitor, as is the case with many phosphorodithioate-type insecticides (Heath, 1961; O'Brien, 1967), and requires oxidation to become insecticidally active. Thus, the in vitro anticholinesterase activity of GS-13005 in insect extracts probably was caused by small concentrations of the highly potent oxygen analog that formed during the incubation. Topical applications of GS-13005 were highly toxic to houseflies and boll weevils but only moderately effective against third instar bollworms and tobacco budworms (Table II). As has been observed with many of the organophosphorus or carbamate insecticides studied (Bull and Lindquist, 1966; Bull *et al.*, 1967b), tobacco budworms were substantially (>5×) more tolerant of GS-13005 than bollworms. The contact toxicity of GS-13007 did not correlate closely with anti-ChE activity. This may be due to rapid decomposition and perhaps to poor penetration. The compound was only slightly more toxic to boll weevils than GS-13005, and the results with bollworms and tobacco budworm larvae were so erratic that no LD_{50} was established.

Results of bioassay tests with adult boll weevils and first instar bollworms indicated that petiole injection and foliar treatments provided effective control of both species for about 16 days after application (Table III). Stem treatments provided similar control of bollworms but were relatively ineffective against boll weevils.

Metabolism of GS-13005 in Plants. Most of the quantitative studies of metabolism were done with GS-13005-^{32}P; the different ^{14}C-preparations were used primarily for qualitative information.

Only three chloroform-soluble products were detected in different extracts of treated plants. These compounds were recovered after treatment with each of the different radiolabeled preparations of GS-13005. They included the parent compound, its oxygen analog (GS-13007), and unknown A. The unidentified material always remained at the origins of TLC plates and may simply represent radioactivity that was trapped by impurities (the amounts of unknown A seemed directly related to the quantity of plant material extracted). Although MTDO and its hydroxymethyl or mercaptomethyl derivatives would partition entirely into chloroform via the procedure used, they were not detected per se as metabolites.

Five phosphorus-containing metabolites were detected in aqueous fractions of different plant extracts. Four of these were formed only in plants treated with GS-13005-^{32}P and included dimethyl phosphorothioate, dimethyl phosphate, methyl phosphate, and phosphoric acid. Measurements of the latter two products were combined because they were not always completely resolved by the

Table I. Chromatographic Properties of GS-13005 and Some of Its Derivatives

Compound	Average R_f TLC A	TLC B	TLC C	PC
H_3PO_4	0.00	0.00	0.00	0.00
Methyl phosphate	0.00	0.00	0.04	0.04
Dimethyl phosphate	0.00	0.00	0.28	0.19
Dimethyl phosphorothioate	0.00	0.00	0.58	0.43
Dimethyl phosphorodithioate	0.00	0.00	0.82	0.50
MTDO	0.58	0.38	0.78	0.43
Hydroxymethyl MTDO	0.29	0.27	0.78	0.43
Desmethyl GS-13005	0.00	0.00	0.67	0.71
Unknown A	0.00	0.00	...	...
GS-13007	0.07	0.20	0.86	0.95
GS-13005	0.70	0.75	0.95	0.95

Table II. Anticholinesterase Activity and Toxicity of GS-13005 and GS-13007 to Insects

Compound	I_{50} (Molar)[a] Housefly	Boll weevil	Bovine
GS-13005	1.7×10^{-6}	6.1×10^{-6}	$>10^{-3}$
GS-13007	6.0×10^{-10}	2.2×10^{-8}	8.3×10^{-8}

	Topical LD_{50}, μg. per Insect[b] Housefly	Weevil	Bollworm	Budworm
GS-13005	0.16	0.20	1.2	6.5
GS-13007		0.14		

[a] Final homogenate concentrations 1 fly head per ml., 2 whole weevils per ml., 1.5 units AChE per ml.
[b] Mortality determined after 24 hours with adult houseflies and boll weevils and after 72 hours with third instar (30- to 45-mg. range) bollworm and tobacco budworm larvae.

Table III. Bioassay of Cotton Plants Treated in Greenhouse with GS-13005

Species and Type of Application	% Mortality after 72 Hours at Indicated Days after Treatment 1	4	8	16	30
First instar bollworms					
Petiole injection	100	90	90	71	47
Stem treatment	97	71	63	69	33
Foliar spray	100	100	96	57	11
Adult boll weevils					
Petiole injection	92	88	84	47	43
Stem treatment	60	60	50	40	33
Foliar spray	100	90	76	42	13

methods used. The fifth water-soluble metabolite, detected after treatments with each of the different radiolabeled preparations of GS-13005, was identified as desmethyl GS-13005. The identification of this metabolite was further supported by the observation that it was not formed in biological materials after treatments with GS-13007-2-methoxy-^{14}C or any of the ^{14}C-labeled preparations of MTDO and hydroxymethyl MTDO.

Major experiments on cotton were conducted in the field to obtain a more realistic evaluation of the fate of the compound. The first three experiments described below were made with young plants that had four to eight fully expanded leaves. At each specified time after treatment, duplicate samples of leaves or whole plants were collected and analyzed. Each complete experiment was repeated at least twice.

After foliar application, essentially all radioactivity absorbed by the leaves (24 to 25%) penetrated during the first 24 hours (Table IV). That not absorbed volatilized rapidly and was depleted after 2 days. Absorbed radioactivity remained at a constant level for 4 days and then declined as hydrolytic products were translocated to other parts of the plant.

The oxygen analog (GS-13007) was not formed on the leaf surfaces, but small concentrations were detected in internal extracts. The peak level of GS-13007 (1.3% of the dose) was found 1 day after treatment, but the compound was completely metabolized after 32 days. The parent compound was relatively stable inside the leaves and had a biological half life of almost 2 weeks. Of the hydrolytic metabolites detected, desmethyl GS-13005 was the major product at most time intervals.

After petiole injections, the concentration of GS-13005-^{32}P in treated leaves declined rapidly during the first 2 days and then gradually decreased during the remainder of the experimental period (Table IV). This rapid initial decline was the result of metabolic conversion and probably loss of volatile compounds from within the leaf. The dominant metabolic product, desmethyl GS-13005, reached a maximum concentration (14.2% of the dose) after 2 days and then declined slowly to 3.4% after 32 days. Again, the detected concentrations of GS-13007 were highest (2.6%) the first day after treatment and the compound was absent after 32 days. Since this was a consistent occurrence in plants, the oxidative mechanism responsible for biosynthesis of GS-13007 may have been inhibited by one or more of the compounds present. When cotton leaves were treated by petiole injection with 100 μg. of GS-13007-2-methoxy-^{14}C, the compound was completely metabolized in 2 days.

Results of studies of the uptake and metabolism of GS-13005-^{32}P after stem treatments are summarized in Table V. Of the applied dose, slightly more than 14% was absorbed and translocated during the 16-day experimental period. As in the preceding tests, the highest level of GS-13007 (9.2 μg. GS-13005-^{32}P equivalents) was detected early in the experiment, and the compound was absent after 16 days. GS-13007 was not found either in new growth or in the fruit of treated plants, and small concentrations of GS-13005 were recovered from new growth but none from fruit. Again, desmethyl GS-13005 was the most important hydrolytic product, particularly in foliage that was present at the time of treatment. The only radioactive products identified in the fruit were methyl and dimethyl phosphate and inorganic phosphate.

After treatment of seeds with GS-13005-^{32}P, 11.7% of

Table IV. Relative Concentrations of GS-13005 and Its Metabolites in Leaves of Field-Grown Cotton after Treatment with 100 μg. of GS-13005-^{32}P

Days after Treatment	% of Applied Dose Present as							
	H_3PO_4 and methyl phosphate	Dimethyl phosphate	Dimethyl phosphoro-thioate	Desmethyl GS-13005	Unknown A	GS-13007	GS-13005	Unextracted radioactivity
				After Foliar Treatment, External Rinse				
0	0.0	0.0	0.0	0.0	0.0	0.0	96.5	...
1	0.0	0.3	0.4	0.9	0.0	0.0	8.9	...
2	0.0	0.4	0.4	0.4	0.0	0.0	1.7	...
4	0.0	0.1	0.1	0.0	0.0	0.0	0.0	...
				After Foliar Treatment, Internal Extract				
0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	0.0
1	1.3	1.8	1.9	3.4	0.0	1.3	14.4	0.9
2	1.1	2.2	2.5	3.9	0.1	0.7	14.2	1.2
4	1.2	2.1	2.6	5.9	0.1	0.2	11.9	1.5
8	0.8	1.4	2.0	4.0	0.0	0.1	10.7	1.1
16	1.6	2.0	1.5	2.2	0.6	0.1	5.8	0.9
32	1.8	2.1	0.6	0.6	0.1	0.0	3.2	0.9
				After Petiole Injection, Internal Extract				
0	0.0	0.0	0.1	0.1	0.0	0.0	99.8	0.0
1	2.8	3.7	4.7	11.3	0.1	2.6	59.7	1.6
2	3.1	3.8	6.0	14.2	0.2	1.4	37.1	3.7
4	2.4	4.1	6.0	13.3	0.1	1.3	38.8	3.6
8	2.5	4.6	6.5	12.7	0.0	0.3	31.3	3.5
16	4.0	4.6	3.7	6.2	1.7	0.2	26.3	3.6
32	9.4	4.4	1.6	3.4	0.3	0.0	16.9	3.3

Table V. Relative Concentrations of GS-13005 and Its Metabolites in Field-Grown Cotton Plants after Treatment of Each Plant with 2 Mg. of GS-13005-^{32}P at the Base of Stem

Days after Treatment[a]	Wet Wt. of Plant Part, G.	GS-13005-^{32}P μg.-Equivalents Present as: H_3PO_4 and methyl phosphate	Dimethyl phosphate	Dimethyl phosphoro-thioate	Desmethyl GS-13005	Unknown A	GS-13007	GS-13005	Unextracted radioactivity
2 W	16.4	2.9	19.5	25.5	35.5	4.4	9.2	57.6	10.9
4 W-T	31.5	3.0	11.7	27.9	37.6	4.6	2.1	64.5	15.7
4 T	1.7	1.1	1.7	0.6	0.4	1.0	0.0	0.2	2.0
8 W-T	45.8	2.6	23.0	38.9	37.9	5.1	1.6	70.9	43.4
8 T	3.6	0.5	2.1	2.9	0.6	1.1	0.0	0.2	4.5
8 S	1.0	0.3	1.0	0.0	0.0	0.0	0.0	0.0	1.6
16 LL	39.3	4.9	12.3	16.2	26.7	4.8	0.0	51.7	107.5
16 UL	25.1	0.8	4.9	1.9	1.9	2.9	0.0	1.2	32.0
16 T	2.5	2.6	3.5	0.0	0.0	1.1	0.0	0.0	7.6
16 S	1.9	0.1	0.8	0.0	0.0	0.2	0.0	0.0	2.7

[a] W = whole plant, W-T = whole plant less terminal, T = terminal, S = square, LL = lower leaves, UL = upper leaves.

the applied dose was found in seedlings 2 days after their emergence and 17.8% in plants that were 24 days old. The results (Table VI) were somewhat similar to those reported for the other treatments. Again, GS-13005 was relatively stable within the plant (half life about 2 weeks). Also, relatively large concentrations of hydrolytic products were detected in 2- and 4-day-old seedlings, the major portion of which was inorganic phosphate. Comparable results have been obtained during studies of other organophosphorus insecticides (Bull and Lindquist, 1964; Lindquist and Bull, 1967), and may be related to the vigorous metabolic activity that accompanies germination.

The large concentrations of unextracted radioactivity reported in tissues from the stem (Table V) and seed treatments (Table VI) appeared to be directly related to the wet weight of plant material processed. Since multiple extractions did not improve recovery, the radioactivity probably could be attributed to the incorporation of inorganic ^{32}P formed during metabolsim of GS-13005 into natural plant constituents.

An experiment was designed to evaluate the systemic mobility of GS-13005 and its metabolites within greenhouse-grown cotton plants that had eight fully expanded leaves at the time of treatment. The third, fourth, and fifth leaves above the cotyledons were each treated with 100 μg. of GS-13005-^{32}P by petiole injection. Then at specified times, plants were harvested, separated into several parts, weighed, dried 24 hours at 50° C., pulverized, and radioassayed. In addition, terminal growth and fruit were analyzed separately to determine the nature of any radioactivity that was detected. The results (Table VII) indicated that relatively small percentages of the injected dose were translocated from treated leaves to other parts of the plant and most of the translocated radioactivity was found in growth above the treated leaves and in the stem. The small amounts found in terminals and fruit included only inorganic phosphate and methyl and dimethyl phosphate.

In alfalfa that was treated topically with 100 μg. of the GS-13005-^{32}P, about 21% of the applied dose was absorbed and translocated above the site of treatment (Table VIII). Absorbed GS-13005 had a shorter half life in alfalfa (2 to 3 days) than in cotton. The amounts of GS-13007

Table VI. Relative Concentrations of GS-13005 and Its Metabolites in Cotton Seedlings Grown from Seeds Treated with 1 Mg. of GS-13005-^{32}P

Compounds	GS-13005-^{32}P μg.-Equivalents at Indicated Days after Treatment: 2	4	8	12	16	24
H_3PO_4 and methyl phosphate	27.9	33.8	8.0	7.8	13.4	11.4
Dimethyl phosphate	8.6	8.7	8.0	8.4	8.2	11.0
Dimethyl phosphorothioate	4.9	7.4	15.0	16.7	22.3	24.2
Desmethyl GS-13005	9.9	10.4	33.9	48.6	44.1	39.4
Unknown A	2.1	2.4	6.0	2.8	2.8	2.0
GS-13007	0.7	2.8	2.5	1.5	0.5	0.3
GS-13005	54.0	51.2	46.7	34.6	24.5	21.9
Unextractable	9.0	10.6	13.6	31.0	58.8	67.5
Wet weight of seedlings, grams	0.7	0.8	1.6	2.5	4.4	7.5

Table VII. Distribution of Radioactivity in Greenhouse-Grown Cotton Plants after Injection of 100 μg. of GS-13005-^{32}P in Each of Three Leaves on Each Plant

Plant Part	% of Applied Dose Recovered at Indicated Days after Treatment: 2	4	8	16
Terminal	0.4	1.7	0.7	1.0
Fruit	...	...	0.0	0.4
Upper leaves	0.6	0.8	4.2	5.6
Treated leaves	81.6	75.3	74.0	70.3
Lower leaves	0.2	0.3	0.4	0.7
Stem	0.9	1.9	3.0	4.3

detected were highest (1.2%) in samples harvested the first day after treatment, and both GS-13005 and GS-13007 were essentially depleted after 16 days.

Metabolism of ^{32}P-Labeled GS-13005 in Animals. Analyses of the urine of treated rats indicated that only the water-soluble metabolites of GS-13005-^{32}P were excreted (Table IX). The quantities of desmethyl GS-13005 detected were fairly high (11.1% of the dose through 40 hours) but were still lower than the amount of dimethyl phosphate (33.6%) or dimethyl phosphorothioate (24.1%) recovered during the same time. Almost 80% of the ad-

Table VIII. Relative Concentrations of GS-13005 and Its Metabolites in Alfalfa Plants after Stem Treatment with 100 μg. of GS-13005-^{32}P

Compounds	% of Applied Dose Recovered at Indicated Days after Treatment					
	0[a]	1	2	4	8	16
H_3PO_4 and methyl phosphate	0.3	0.4	0.5	0.8	0.6	0.3
Dimethyl phosphate	0.5	2.2	4.8	5.7	6.4	4.9
Dimethyl phosphorothioate	1.2	2.9	5.6	7.5	7.0	7.9
Desmethyl GS-13005	0.0	1.6	1.8	3.0	3.7	3.2
Unknown A	0.0	0.3	0.2	2.1	1.5	0.8
GS-13007	0.0	1.2	0.7	0.7	0.2	0.0
GS-13005	93.0	12.4	6.0	1.6	0.5	0.2
Unextractable	0.0	1.3	2.1	4.0	7.2	11.4
Wet weight, grams	2.5	2.5	3.2	4.1	4.3	5.5

[a] 0-hour harvest includes treatment site, others do not.

Table IX. Relative Concentrations of Metabolites of GS-13005 in Urine of Male Rats after Intraperitoneal Injection with 1.5 Mg. of GS-13005-^{32}P[a]

Compounds	% of Applied Dose at Indicated Hours after Treatment			
	4	8	24	48
H_3PO_4 and methyl phosphate	0.5	0.8	1.3	1.5
Dimethyl phosphate	12.1	17.5	31.6	33.6
Dimethyl phosphorothioate	11.4	15.1	23.2	24.2
Desmethyl GS-13005	4.7	6.4	10.7	11.1

[a] 7.1% excreted in feces through 48 hours.

ministered dose was excreted in 48 hours, including 7.1% in the feces.

Studies of the metabolism of GS-13005-^{32}P in fifth instar tobacco budworms demonstrated that the administered GS-13005 was completely metabolized during the first 4 hours (Table X). Small concentrations of GS-13007 were formed, but this compound also was depleted after 4 hours. Dimethyl phosphorothioate and dimethyl phosphate were the major hydrolytic products detected in internal extracts and excreta throughout the experimental period.

Little is known of the chemistry of substituted 1,3,4-thiadiazolinones or their fate in biological systems. At least a partial understanding of the metabolism of the heterocyclic moiety of the GS-13005 molecule could contribute to the elucidation of the over-all metabolic pathway.

$^{14}CO_2$ Trapping Studies. Rats that were treated with GS-13005-2-methoxy-^{14}C and GS-13005-carbonyl-^{14}C expired 22.4 and 25.8% of the dose, respectively, as $^{14}CO_2$ and excreted 57.4 and 52.0% respectively, within 48 hours. Rats treated with GS-13005-methylene-^{14}C expired 17.7% and excreted 59.0% during the same time interval. Those treated with 6 mg. of MTDO-carbonyl-^{14}C expired 70.6% as $^{14}CO_2$ and excreted 20.3% of the dose in a comparable time.

Fifth instar tobacco budworm larvae that were treated with GS-13005-2-methoxy-^{14}C and GS-13005-carbonyl-^{14}C or MTDO carbonyl-^{14}C expired less than 0.1% of the

Table X. Relative Concentrations of GS-13005 and Its Metabolites in Fifth Instar Tobacco Budworms Following Injection with 5 μg. of GS-13005-^{32}P

Compounds	% of Applied Dose Recovered at Indicated Hours after Treatment					
	0	1	2	4	8	24
	Internal					
Dimethyl phosphate	5.9	19.3	21.3	27.1	25.1	16.7
Dimethyl phosphorothioate	12.8	43.8	34.2	21.7	19.0	7.4
Desmethyl GS-13005	0.0	3.2	7.5	2.0	2.1	1.0
GS-13007	0.0	0.9	1.5	0.5	0.0	0.0
GS-13005	81.3	21.9	10.6	2.3	0.0	0.0
	Excreta					
H_3PO_4 and methyl phosphate		0.0	0.1	0.5	0.8	1.3
Dimethyl phosphate		0.4	1.9	6.2	8.2	21.1
Dimethyl phosphorothioate		5.0	16.0	31.1	35.5	43.1
Desmethyl GS-13005		0.3	1.2	2.5	2.9	3.3
GS-13007		0.1	0.2	0.3	0.3	0.3
GS-13005		1.3	2.0	2.3	2.3	2.3

dose as $^{14}CO_2$ during a 48-hour period. Essentially all the administered radioactivity could be accounted for in excreta and internal extracts. Insects treated with GS-13005-methylene-^{14}C expired only 4.8% of the dose as $^{14}CO_2$ during the same time.

After treatment of isolated cotton leaves with 100 μg. of MTDO-carbonyl-^{14}C, 1.2% of the dose was recovered as $^{14}CO_2$ within 2 weeks. From individual leaves that were treated by petiole injection with 100 μg. of the same compound, 2.0% of the dose was recovered as $^{14}CO_2$ within 8 days; but after similar treatment with GS-13005-carbonyl-^{14}C less than 1.0% of the dose was converted to $^{14}CO_2$ within 1 week. Bean plants treated with 3 mg. of GS-13005-carbonyl-^{14}C metabolized only 1.7% to $^{14}CO_2$ within 6 days.

Thus the $^{14}CO_2$ trapping studies indicated that the heterocyclic moiety of GS-13005 in rats was readily ruptured and metabolized to simpler fragments. In insects and plants, however, $^{14}CO_2$ was clearly not a major end product of the metabolism of the heterocyclic moiety. This evidence is not definite proof that the ring is not ruptured in these biological systems, but if it is, it apparently is not metabolized to such an extent that $^{14}CO_2$ is released. However, if the ring is broken it probably would be easily degraded to simple fragments.

The results obtained with rats were in general agreement with those of Esser and Müller (1966), who demonstrated that 35.6% of the applied GS-13005-carbonyl-^{14}C was converted to $^{14}CO_2$ within 48 hours. However, the same authors reported that 27.4% of the recovered radioactivity could be attributed to $^{14}CO_2$ after foliar treatment of bean plants (0.464 μc. per plant) with GS-13005-carbonyl-^{14}C. The results with beans appear to differ substantially from our findings. Unfortunately, a direct comparison is not possible because the authors did not report the actual amounts of the applied dose that were recovered.

Since the total recovery of applied radioactivity was good

in the present test (90% or more), the insects and plants were probably converting substantial portions of the heterocyclic moiety of GS-13005 to unidentified water-soluble products. Therefore, a partial qualitative comparison of the water-soluble products formed by plants and insects after treatment with different ^{14}C-labeled compounds was done by paper chromatography with solvent mixtures C and D. Fifth instar tobacco budworms converted MTDO-carbonyl-^{14}C rapidly and quantitatively to a single water-soluble metabolite that had an R_f of 0.49 in system C. The chromatographic behavior of this metabolite in system D distinctly demonstrated that it was different from MTDO (R_f values 0.55 and 0.88, respectively). In cotton leaves, MTDO-carbonyl-^{14}C was metabolized rapidly (biological half life $<$ 1 day) to at least seven water-soluble products; only the parent compound was recovered in the chloroform fractions. One water-soluble metabolite corresponded to the one detected in insects but was only a minor product in plants.

The metabolites detected in tobacco budworms following treatment with GS-13005-2-methoxy-^{14}C and GS-13005-carbonyl-^{14}C were identical. At least eight polar metabolites were formed, including desmethyl GS-13005 and a major metabolite that corresponded to the water-soluble metabolite of MTDO. After treatment with GS-13005-methylene-^{14}C, six water-soluble metabolites were detected. Five of these apparently were the same as those formed from GS-13005-carbonyl-^{14}C. In plants, eight water-soluble metabolites of GS-13005-carbonyl-^{14}C were detected, three of which were identical to those formed from MTDO alone. All five water-soluble metabolites detected in cotton leaves treated with GS-13005-methylene-^{14}C apparently were chromatographically identical to products formed from GS-13005-carbonyl-^{14}C, but did not correspond to any produced from MTDO.

These results, coupled with the evidence that little $^{14}CO_2$ was released by insects or plants during metabolism of GS-13005, suggested that either the mercaptomethyl or hydroxymethyl derivatives of MTDO, or perhaps both compounds, were incorporated into conjugates as rapidly as they were liberated by hydrolytic cleavage of the P-S bond of the GS-13005 molecule. In addition, the large number of metabolites found in plants suggests, but by no means proves, that the ring may be split and the fragments rapidly incorporated into conjugates, thus precluding evolution of $^{14}CO_2$.

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