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METABOLISM IN PLANTS

Fate of C^{14}-Carbonyl-Labeled Aryl Methylcarbamate Insecticide Chemicals in and on Bean Plants

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Studies with eight C^{14}-carbonyl-labeled aryl methylcarbamates demonstrate that such insecticide chemicals are degraded with the carbamate moiety intact when applied to glass or silica gel surfaces or leaves of growing bean plants, or injected into the stems of such plants. Methylcarbamates-carbonyl-C^{14} of the following phenols were examined: 1-naphthol (carbaryl), 2-isopropoxyphenol (Baygon), 3-isopropylphenol (UC 10854), 3,5-diisopropylphenol (HRS-1422), 2-chloro-4,5-xylenol (Banol), 4-methylthio-3,5-xylenol (Mesurol), 4-dimethylamino-3,5-xylenol (Zectran), and 4-dimethylamino-3-cresol (Matacil). Rates of loss vary considerably with the nature of the surface (inert or plant), the light, and the compound. Oxidative changes occur on and/or in the plant: Mesurol degrades to sulfoxide and sulfone analogs; Zectran and Matacil form several methylcarbamate derivatives, including the 4-methylamino, 4-amino, 4-methylformamido, and 4-formamido analogs. Little or no formation of organoextractable degradation products, containing an intact methylcarbamate moiety, occurs with the other compounds. The fate of the radiocarbon, 6 days after injection into bean plants, varies considerably: With Baygon and UC 10854 the majority of the radiocarbon is in the water phase; with Zectran and Matacil it appears in the unextractable portion; with Mesurol, it shows up as loss, possibly as the result of expiration as $C^{14}O_2$. Loss from the plant surface is not directly related to the volatility of the compounds, nor is degradation in the plant related to the rate of nonenzymatic hydrolysis. It appears that the relative stability of the methylcarbamate grouping to photooxidation and metabolism, in certain cases, allows the formation of degradation products involving only alteration of the ring or a ring substituent.

CERTAIN aryl methylcarbamates are commercial or experimental insecticide chemicals for the control of insects on a variety of agricultural crops, including food crops. To evaluate the potential hazard from their use, it is necessary to know their fate in and on plants, especially if they have a systemic action or if their use leaves a persistent residue, because the methods used to determine residues must measure products formed by metabolism or degradation in or on the plant, when these are also toxic, as well as the original chemical used.

Carbaryl (1-naphthyl methylcarbamate), in the formulated state, slowly degrades when irradiated by ultraviolet light and sunlight to unidentified products as analyzed by ultraviolet absorption at 280 mμ (*19*). Under exposure to ultraviolet light (2537 A.) on paper chromatograms, isolan (1-isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate) and pyrolan (1-phenyl-3-methyl-5-pyrazolyl dimethylcarbamate) give rise to several unidentified products as detected by chromogenic agents (*17*). Recently, Crosby, Leitis, and Winterlin stated that methylcarbamates produce a variety of unidentified cholinesterease-inhibiting derivatives when exposed as ethanol or hexane solutions to ultraviolet light at 254 mμ or to sunlight (*2*). Matacil (4-dimethylamino-3-cresyl methylcarbamate) and Zectran (4-dimethylamino-3,5-xylyl methylcarbamate) extensively degrade to five or more inhibitory products. Mesurol (4-methylthio-3,5-xylyl methylcarbamate) gives at least two in-

hibitory products other than the corresponding sulfoxide and sulfone, which are also formed or were present originally as contaminants. Carbaryl, UC 10854 (3-isopropylphenyl methylcarbamate), and particularly Baygon (2-isopropoxyphenyl methylcarbamate) are more stable under these conditions, although even carbaryl forms several cholinesterase inhibitors on exposure to intense ultraviolet light. Kenaga, Doty, and Hardy find that the insecticidal activity of Zectran deteriorates under simulated sunlight (ultraviolet light) (*12*).

Methylcarbamate insecticide chemicals are susceptible to biodegradation in plants. Two principal types of initial metabolic attack are possibly involved: hydrolysis of the carbamate ester group; and oxidation or hydroxylation of the *N*-methyl group, a ring substituent, or even the ring itself, with subsequent hydrolysis or conjugation of the metabolites. 3-Isopropylphenyl methylcarbamate is unstable in lima bean plants, as shown by anticholinesterase assay (*3*). Carbonyl-C^{14}-labeled carbaryl in bean plants and Baygon in bean and cotton plants yield first-order degradation curves, and the C^{14}-bearing compounds remain largely in the plants; while the latter are not fully characterized, they are stable (in the plant), and not extractable by organic solvents (*4, 5*). Thus, in the case of these two methylcarbamates, reactions other than hydrolysis seem to be the major degradation mechanism and the products formed are probably, in part, derivatives such as conjugates with the carbamate ester group intact. Williams, Meikle, and Redemann state that Zectran partially degrades in broccoli flowers within 10 days to 4-dimethylamino-3,5-xylenol, 2,6-dimethylhydroquinone, 2,6-dimethyl-*p*-benzoquinone, and 4-dimethylamino-3,5-dimethyl-*o*-benzoquinone, that part of the 4-dimethylamino-3,5-xylenol and all of the 2,6-dimethylhydroquinone are present as water-soluble conjugates, and that some of the metabolites find their way into the lignin fraction (*22*). There is evidence that Mesurol is converted by plants into its sulfoxide and sulfone derivatives (*18*).

Compounds containing *N*-methyl- or *N,N*-dimethylamino groupings frequently undergo *N*-demethylation in biological systems. Chemical oxidation is also known to effect such *N*-demethylation reactions. Intermediates identified or postulated with various compounds include *N*-oxide, *N*-hydroxymethyl, and *N*-formamido derivatives (*1, 4–10, 16, 21*). All of the compounds considered in the present study have *N*-methylcarbamate groupings, and two (Zectran and Matacil) also have *N,N*-dimethylamino groupings. It appeared possible that photooxidation or metabolism in the plants might attack these sites.

The object of this study was to determine the fate of eight methylcarbamate insecticides, labeled with carbon-14 in the carbonyl position, on the leaves of young bean plants, after injection into the stems of such plants, on glass surfaces, and in a basic aqueous solution. Also, the stability of their nonradioactive forms under room light and ultraviolet light was of interest. The study did not include determination of the penetration rates of the compounds into plants from the leaves, or characterization of all possible degradation products formed.

Materials, Apparatus, and Test Conditions

Table I lists the pure radioactive and nonradioactive compounds used, along with their sources. The radiochemical purity of the radioactive compounds was 99% or better and all had an adjusted specific activity of 1.0 mc. per mmole.

Radioactivity was counted with a Packard Tri-Carb liquid scintillation spectrometer (Model 3003) using 10 ml. of 0.55% 2,5-diphenyloxazole in toluene-2-methoxyethanol (2 to 1) in each vial. Medical x-ray film ("no screen" type, Eastman Kodak Co., Rochester, N. Y.) was used for radioautography. Thin-layer chromatography was accomplished with Silica Gel G obtained from the Kensington Scientific Corp., Berkeley, Calif., and 20 × 20 cm. chromatoplates. Two chromogenic reagents were used for detection of compounds on the chromatoplates. Following a spray with 10% aqueous sodium hydroxide, the plates were heated for 3 minutes at 70° C., cooled, oversprayed with ninhydrin reagent (2% ninhydrin in 65% ethanol), and reheated for 30 minutes at 70° C. to detect as red spots the amines released on degradation of the carbamates. (Red spots were obtained with all of the carbamates but none of the phenolic hydrolysis products of the known compounds studied.) Ferric chloride–potassium ferricyanide reagent was also used, as described by Krishna, Dorough, and Casida (*15*). Ether, used for extractions and chromatography, was anesthetic ether containing 3.5% ethanol. The Florisil was 60- to 100-mesh, used without activation, and came from the Floridin Co., Tallahassee, Fla. The charcoal was a mixture of common animal "bone-black" charcoal. Veronal buffer solution (pH 9.3) was made up as directed by Kolbezen, Metcalf, and Fukuto (*13*). The ultraviolet light sources were the short-wavelength (2537 A.) and long-wavelength (3660 A.) units provided with a Chromato-Vue Model C-3 (Ultra-Violet Products, Inc., San Gabriel, Calif.). A Hamilton Microliter syringe (No. 705-N) was used for injection into the plants. The plants were homogenized in a Virtis 45 mixer (Research Equipment, Gardiner, N. Y.).

Garden snapbean seeds (Contender variety) were planted in mid-June 1964, and allowed to grow, in individual pots, under greenhouse conditions prevailing in Berkeley, Calif., and under continuous fluorescent lighting of normal intensity and quality. For use in a follow-up experiment in the plant injection study, another batch of beans was planted and raised as before in the last half of March 1965: Injections of this group were made after the actively growing plants had been held one day in the laboratory; the plants remained in the laboratory until sacrificed. At the time of treatment, the stem plus leaves weighed approximately 2 grams and, after 6 days, their weight was approximately 3 grams. The weight of the leaf at the time of treatment was approximately 0.4 gram (*1*).

Methods

Loss from Glass Surface. Each of the eight radiolabeled methylcarbamates was applied, as a chloroform solution, to the upper surface of 2-cm. round glass cover slips, yielding on evaporation a deposit of approximately 0.5 μg. per sq. cm. and about 2000 counts per minute per cover slip. The treated cover slips were held at 25° C. under fluorescent illumination until only approximately 10% of the radioactivity remained on them, as determined by scintillation counting at five or more evenly spaced intervals. The entire experiment was done in duplicate and three or more replicates were made for each interval. In the case of Matacil, Mesurol, and Zectran, an attempt was made to determine the degradation products present, at the end of each interval, by TLC and radioautography. The residual radioactivity was plotted against time on semilog paper and the half life determined.

Stability in Presence of Light. One hundred micrograms of each of the eight nonradiolabeled methylcarbamates were dissolved in chloroform and spotted on each of four 20 × 20 cm. chromatoplates, coated with Silica Gel G at a thickness of 0.5 mm., to yield a series of spots at the origin approximately 0.5 sq. cm. in area. One plate was kept in the dark, one was exposed at bench-top level to normal fluorescent light used for room illumination, a third was exposed to short-wavelength ultraviolet light (2537 A.) at a distance of approximately 10 cm., and a fourth was exposed to long-wavelength ultraviolet light (3660 A.) at the same distance. After an exposure of 3 hours, the chromatograms were developed and carbamate decomposition products were detected, using ninhydrin as the chromogenic reagent.

Foliage Treatment and Method of Analysis. Each of the eight radioactive compounds was individually applied, as uniformly as possible, to the upper surface of the two primary leaves of 10- to 12-day-old bean seedlings at a time when the leaves were approximately 5 days old and had an area of approximately 10 sq. cm. on one side. Eighty micrograms (equivalent to approximately 160,000 counts per minute, or 190 p.p.m., or 8.3 μg. per sq. cm.) were applied to each leaf in three 10-μl. portions of an alcoholic solution, the solvent being allowed to evaporate after each application. Immediately after the

Table I. Name, Chemical Name, and Source of Compounds Used

Compound	Chemical Name	Source
Carbaryl[a] (Sevin)[b]	1-Naphthyl methylcarbamate	Union Carbide Corp., New York, N. Y.
Carbaryl carbonyl-C^{14}		J. G. Krishna (*14, 15*)
Baygon[b]	2-Isopropoxyphenyl methylcarbamate	Chemagro Corp., Kansas City, Mo.
Baygon carbonyl-C^{14}		J. G. Krishna (*14, 15*)
UC 10854[b]	3-Isopropylphenyl methylcarbamate	Hercules Powder Co., Wilmington, Del.
UC 10854 carbonyl-C^{14}		J. G. Krishna (*14, 15*)
HRS-1422[b]	3,5-Diisopropylphenyl methylcarbamate	Hooker Chemical Corp., Niagara Falls, N. Y.
HRS-1422 carbonyl-C^{14}		J. G. Krishna (*14, 15*)
Banol[b]	2-Chloro-4,5-xylyl methylcarbamate	The Upjohn Co., Kalamazoo, Mich.
Banol carbonyl-C^{14}		J. G. Krishna (*14, 15*)
Mesurol[b]	4-Methylthio-3,5-xylyl methylcarbamate	Chemagro Corp., Kansas City, Mo.
Mesurol carbonyl-C^{14}		J. G. Krishna (*14, 15*)
Mesurol sulfoxide	4-Methylsulfinyl-3,5-xylyl methylcarbamate	Chemagro Corp., Kansas City, Mo.
Mesurol sulfone	4-Methylsulfonyl-3,5-xylyl methylcarbamate	Chemagro Corp., Kansas City, Mo.
Zectran[b]	4-Dimethylamino-3,5-xylyl methylcarbamate	Dow Chemical Co., Midland, Mich.
Zectran carbonyl-C^{14}		J. G. Krishna (*14, 15*)
Zectran oxidation product	4-Methylamino-3,5-xylyl methylcarbamate	Oxidation of Zectran by neutral $KMnO_4$ (*1*)
Zectran oxidation product	4-Amino-3,5-xylyl methylcarbamate	Dow Chemical Co., Midland, Mich.
Zectran oxidation product	4-Methylformamido-3,5-xylyl methylcarbamate	Oxidation of Zectran by neutral $KMnO_4$ (*1*)
Zectran oxidation product	4-Formamido-3,5-xylyl methylcarbamate	Dow Chemical Co., Midland, Mich.
Matacil[b]	4-Dimethylamino-3-cresyl methylcarbamate	Chemagro Corp., Kansas City, Mo.
Matacil carbonyl-C^{14}		J. G. Krishna (*14, 15*)
Matacil oxidation product	4-Methylamino-3-cresyl methylcarbamate	Dow Chemical Co., Midland, Mich.
Matacil oxidation product	4-Amino-3-cresyl methylcarbamate	Dow Chemical Co., Midland, Mich.
Matacil oxidation product	4-Methylformamido-3-cresyl methylcarbamate	Reaction of corresponding amino compound with formic acid (*1, 20*)
Matacil oxidation product	4-Formamido-3-cresyl methylcarbamate	Reaction of corresponding amino compound with formic acid (*1, 20*)
Sodium carbonate-C^{14}		Nuclear-Chicago Corp., Des Plaines, Ill.

[a] Common name. [b] Trade name.

treatment the plants were placed outside in an unshaded, unprotected area. (This treatment did not harm the leaves and each leaf was treated as a replicate.)

After the treated, growing plants had stood 0, 8, 24, 48, or 72 hours, respectively, each of a set of three treated leaves was cut at the petiole and placed in a small, separate beaker containing 7 ml. of chloroform (reagent grade). In the case of Mesurol, Zectran, and Matacil, additional samples were taken at 2 and 4 hours. The beaker, with the leaf partially immersed, was shaken for 10 minutes and the liquid decanted into a graduated tube. The beaker and leaf were rinsed with 3 ml. of chloroform and the liquid was added to the graduated tube, the volume was adjusted to 10 ml. with chloroform, and the contents were well mixed.

A 2-ml. aliquot of each chloroform extract was transferred to a scintillation vial, the solvent removed by means of a stream of dry air at room temperature, and the radioactivity measured. The remainder of the chloroform extract (8 ml.) was carefully evaporated to dryness in the tube by means of a stream of dry air, with precautions to minimize loss. The residue was taken up with 0.1 ml. of chloroform and the entire amount spotted on a TLC plate coated with Silica Gel G 0.5 mm. thick. The plate was subjected to one-dimensional chromatographic development in the case of compounds I to V and to two-dimensional development in the case of compounds VI to VIII, using a 2 to 1 mixture of ethyl acetate and toluene as the solvent for all compounds except compound VII, for which a 1 to 1 acetonitrile-toluene mixture was used. Radioactive materials were detected and located by radioautography, and the R_f value and radioactivity of each spot were measured. The three replicate results obtained for each treatment—i.e., each exposure time—were averaged.

Recovery tests were made by extracting treated leaves immediately after treatment and measuring the total radioactivity recovered. All results were corrected for the recovery obtained, as follows:

Compound	Recovery, %
Carbaryl and Banol	92
Baygon and Zectran	85
UC 10854	79
HRS-1422	103
Mesurol and Matacil	95

Radioactivity remaining on or in the leaves after washing was not determined.

The amount of radioactive, unaltered methylcarbamate recovered was plotted against time on semilog paper and the 50% recovery time determined. A similar plot and determination were made of the total amount of radioactivity recovered.

Tentative identification of certain degradation products of Mesurol, Zectran, and Matacil was attempted by two-dimensional co-chromatography with pure nonradioactive compounds. The extract from bean leaves, taken 48 hours after treatment with the respective radiolabeled compound, was mixed with approximately 30 μg. of the pure, nonradiolabeled material, evaporated to dryness, dissolved in chloroform, and subjected to thin-layer chromatography, using a 2 to 1 mixture of ethyl acetate and toluene, for Mesurol and Matacil, and a 1 to 1 mixture of acetonitrile and toluene, for Zectran, in both directions. The chromatography experiments were repeated, using a 2 to 1 ethyl acetate–toluene mixture followed by a 4 to 1 ether-hexane mixture for Mesurol- and Matacil-degradation products, and a 1 to 1 acetonitrile-toluene mixture followed by a 4 to 1 ether-hexane mixture for Zectran products. The position and shape of the spot on the radioautogram, representing the product in question, were compared with those for the pure material visualized by the ninhydrin reagent (*1*).

Stem Injection and Method of Analysis. Each of the eight radioactive compounds was individually injected into the stem of 11-day-old bean seedlings. Each injection consisted of 25 μg. of the chemical (about 50,000 counts per minute) dissolved in 25 μl. of acetone-water solution (2 to 3), a dose equivalent to 12 p.p.m. in fresh weight of the portion of the plant above the soil. In each injection, a small hole was made at the base of the stem with a needle and then the injection was carefully made, from a 50-μl. syringe, into the hollow of the stem at a point just below the primary leaves and at a rate not producing any seepage from the hole at the base of the stem. The injection was made in the greenhouse and the treated plants remained there until sampled for analysis. Three plants were treated for each sampling time.

After the injected, growing plants had been held for 0, 0.5, 1, 2, 3, 4, and 6 days, respectively, each of a set of three plants was cut just below the soil surface, weighed, placed in an individual plastic bag (without cleaning), immediately frozen, and kept in frozen storage (−5° C.) until analyzed 1 to 4 weeks later. (For the 0-day sample, the labeled methylcarbamate was actually in the plant for about 20 minutes before sampling and freezing.)

At the start of the analysis, each plant

was removed from its plastic bag, cut into small pieces while still cold, and homogenized for 5 minutes with 30 ml. of acetone; the brei was filtered through filter paper (Whatman No. 1) into a 125-ml. separatory funnel. The residue was homogenized twice for 3 to 4 minutes with 30-ml. portions of chloroform (each time), and filtered through the same paper into the same separatory funnel. The residue, or unextracted fraction, after evaporation of the solvent content, was transferred to a small vial and stored at 5° C. until analyzed subsequently. After addition of 10 ml. of water the filtrate was shaken for 5 minutes and allowed to stand for about one hour.

The water layer was transferred to a small vial and the total radioactivity of a 0.2-ml. aliquot was determined. The remainder of the water solution (12 to 13 ml. in volume) was stored at 5° C. for possible use. The residue or unextractable fraction (a white to light green mixture of dry powder and fiber) was allowed to dry out under room conditions and the resulting powder weighed. A 50-mg. sample was burned in an atmosphere of oxygen, using the method described by Kalberer and Rutschmann as modified by Krishna and Casida (*11*, *14*). The combustion products were absorbed in 6 ml. of a mixture of monoethanolamine and 2-methoxyethanol (1 to 2) and the radioactivity of a 3-ml. aliquot was determined.

The organic phase was passed (by gravity) through a small, dry chromatographic column, approximately 13 cm. long and 2.5 cm. in i.d., containing successive layers (downward) made up to 10 ml. of granular anhydrous sodium sulfate, 5 ml. of animal charcoal, 5 ml. of Florisil, 5 ml. of charcoal, and 5 ml. of Florisil. The organic phase was allowed to drain completely through the column; the column was then washed with 15 ml. of chloroform-acetone mixture, and the combined effluent volume was measured. (The influent to the column was dark green in color and the effluent was colorless to light yellow.) The total radioactivity of a 2-ml. aliquot of the effluent (which varied from 90 to 100 ml. in volume) was determined. (The effluent did not show appreciable quench when a 2-ml. aliquot was evaporated in a vial and subjected to scintillation counting.) The remainder of the effluent was evaporated to dryness, under reduced pressure, and the residue transferred to a small vial with hexane. The resulting solution was stored at 5° C. until analyzed by TLC.

The hexane solution, representing the original chloroform-soluble fraction, was adjusted to 1 ml. in volume. A 0.1-ml. aliquot from each of the three replicates (for each time interval) was mixed with approximately 30 μg. of the respective pure, nonradioactive methylcarbamate and an equal amount of certain pure nonradioactive materials (analogs of Matacil, Zectran, and Mesurol, for product identification purposes). Three such mixtures were made and the entire amount of the mixture, in each case, was spotted on a TLC plate coated with Silica Gel G 0.5 mm. thick. The plate was subjected to two-dimensional chromatographic development, using a 4 to 1 mixture of ether and hexane as the solvent in each direction. Radioactive materials were detected and located by radioautography and the R_f value for each spot was measured. The radioactivity of the spots from two of the three replicated plates was determined. The two replicate results obtained for each treatment were averaged, and the percentage of the total radioactivity in each spot was calculated.

The third developed TLC plate was sprayed with ninhydrin reagent, in the case of HRS-1422 and Mesurol, and with alkali solution followed by ferric chloride–potassium ferricyanide reagent in the case of the other compounds (*1*, *15*). The colored spots were matched and compared with the radioautograph of the plate.

Recovery tests were made by extracting treated plants immediately after treatment and measuring the total radioactivity recovered after cleanup. All results were corrected for the recovery obtained, as follows:

Compound	Recovery, %
Carbaryl	86
Baygon and Banol	98
UC 10854 and Zectran	99
HRS-1422 and Matacil	96
Mesurol	95

The amount of radioactive, unaltered methylcarbamate recovered was plotted against time on semilog paper and the 50% recovery time determined. A similar plot and determination were made of the total amount of radioactivity recovered in the chloroform-soluble fraction.

In an attempt to elucidate the apparent rapid fixation of some of the radioactive compounds by the plants, a second, identical experiment was made, with Zectran and Matacil, in March and April 1965, in which samples were taken at very short intervals after injection of the compounds into the bean plants. In addition, one set of plants was injected with 50,000 counts per minute of sodium carbonate-C^{14}, in aqueous solution. After the injected, growing plants had been held for 0, 0.33, 1, 3, 9, and 27 hours, respectively, each of a set of three plants was cut and immediately analyzed, as before, without freezing. In this experiment, there was a 99% recovery of Zectran and 96% of Matacil, and these values were used to correct the results. A set of plants, simulating the 0-day samples, was frozen about 20 minutes after injection and sampling, and held in frozen storage for 3 weeks, under the conditions used in the 1964 experiment.

Hydrolysis Rates in Basic Aqueous Solution. Each of the eight radioactive methylcarbamates was incubated at approximately 1 p.p.m. in 0.1*M* veronal buffer (pH 9.3) in a series of test tubes. At the end of various intervals at 25° C., two extractions were made with 1 ml. of ether; the extracts were combined, the solvent was evaporated, and the radioactivity determined. At 0 time, all of the radioactivity was recovered in the ether. Sampling times were selected for each compound such that eight evenly spaced intervals were analyzed until approximately 10% of the radioactivity was recovered in the ether—i.e., approximately 90% of the compound was hydrolyzed. The 50% recovery time was determined as before. The entire experiment was repeated, and the results were averaged.

Results

Loss from Glass and Plant Surfaces. As shown in Table II, the labeled methylcarbamates exhibit considerable variation in rate of loss from glass surfaces upon exposure under ambient laboratory conditions. UC 10854 and Banol are the least persistent compounds and carbaryl and Mesurol are the most persistent, the latter especially so. As expected, the extra isopropyl group in HRS-1422 appreciably reduced the rate of loss (relative to the related compound UC 10854). The rate of loss curves plotted on semilog paper are approximately linear for the first 80 or 90% of loss in the case of compounds I to V (inclusive), indicating that they are stable (not converted to compounds differing in volatility from their precursors) on glass surfaces. However, those for Mesurol, Zectran, and Matacil are approximately linear for the first few hours only and, in the period of 4 to 12 hours after application, they develop a considerably lower slope (*1*). This indicates that the latter three compounds change rapidly to less volatile products on exposure to air and light. In fact,

Table II. Loss of C^{14}-Carbonyl-Labeled Methylcarbamates from Glass and Growing Bean Plant Surfaces

		50% Loss Times, Hours		
		Glass,	Plant Foliage	
Designation	Compound	all compounds	All compounds	Original compound
I	Carbaryl	14	68	68
II	Baygon	1.8	8	8
III	UC 10854	0.3	2–3	2–3
IV	HRS-1422	5.2	15	15
V	Banol	0.7	13–18	13–18
VI	Mesurol	18	>72	>72
VII	Zectran	1.2	61	2–3
VIII	Matacil	1.6	8	4

TLC analysis of the residues from these three compounds remaining on the glass cover slips reveals degradation products having the following R_f values:

Compound	Solvent	R_f Value of Products
Mesurol	Ethyl acetate–toluene (1 to 1)	0.13 (Mesurol sulfoxide)
Zectran	Acetonitrile-toluene (1 to 1)	0.38; 0.51; 0.60
Matacil	Ethyl acetate–toluene (1 to 1)	0.25; 0.30; 0.64

In the case of Mesurol, the change in slope came after 40 to 45% of the radioactivity had disappeared and in the case of Zectran and Matacil, after 70 to 80% had been lost.

All of the radioactive methylcarbamates shown in Table II are much more persistent on the plant surface than on the glass surface, differing by a factor of 3 or more. As with glass surfaces, the least rate of loss of radioactivity is found for carbaryl and Mesurol but, in contrast, Zectran is also very persistent, when all residual compounds are considered. On the plant, the greatest rate of loss is found for UC 10854, a finding expected from the results obtained on glass surfaces. From Table II, it is evident that compounds I to V, inclusive, suffer very little degradation to form other carbamates on the plant surface but that Zectran and Matacil are changed to products more residual than the original compound, markedly so for Zectran.

Stability in Presence of Light. Exposure of the eight nonradioactive methylcarbamates, on silica gel–coated plates, did not produce any degradation products (as detected by the ninhydrin reagent) when held in the dark or under fluorescent light or long-wavelength ultraviolet light. However, as shown in Table III, all of these compounds, except carbaryl, produce two or more degradation products when exposed to short-wavelength (2537 A.) ultraviolet light. Each of the eight compounds gives a ninhydrin-positive material that does not move from the origin with the TLC solvent—i.e., R_f = 0.00. Short-wavelength ultraviolet light produces a variety of products with Zectran and Matacil, most of which involve modifications only in the 4-dimethylamino group, as discussed below. Mesurol produces the sulfoxide (R_f 0.13) and sulfone (R_f 0.52) analogs, and four other products (R_f = 0.30, 0.45, 0.55, and 0.59). On separate irradiation studies, the sulfoxide yielded products of R_f = 0.45, 0.52, and 0.55, while the sulfone yielded products of R_f = 0.30 and 0.55. The nature of these additional Mesurol products was not examined.

Recovery from Surface-Treated Growing Bean Leaves. It is apparent from the data in Table IV that the fate of the eight radioactive methylcarbamates is very different when they are individually applied to growing bean leaves, but the results are about as expected. Noteworthy is the rapid loss, with little or no degradation to form other carbamate structures, of Baygon and UC 10854. Although not importantly degraded to other carbamate structures, carbaryl, HRS-1422, and Banol are residual and persistent. The identity of the small quantity of degradation products found for these five methylcarbamates [I-A, III-A, IV-A, IV-B, and V-(A + B)] was not established. Attempts to synthesize them by permanganate oxidation failed (*7*).

Mesurol was the most residual material on the bean leaves, even though it suffered considerable degradation to yield a maximum total level of degradation products in 24 hours (Table IV). As expected, one product (the major one) was the sulfoxide and the other was the sulfone, both tentatively identified by co-chromatography. In the case of Zectran, the original compound disappeared almost completely in 2 days, degrading into eight or more products, the sum of which reached a maximum in 24 to 48 hours. The recovery of Matacil dropped off at a moderately fast rate, degrading into eight or more products, the sum of which reached a maximum in 24 hours. The probable identity of the four major decomposition products from Zectran and Matacil is considered below.

In addition to the degradation products listed in Table IV, there is some evidence of the following radioactive products (chromatographic solvents as indicated in Table IV):

Parent Compound	R_f Value	Maximum %
Carbaryl	0.00	0.6
	0.29	0.5
Baygon	0.44	0.7
UC 10854	0.00	0.5
	0.11	0.7
	0.48	0.6 (mixture)
	0.62	0.6
HRS-1422	0.00	0.7
	0.15	0.2
Banol	0.00	0.4
	0.42	0.3
	0.62	0.3
Mesurol	0.00	0.3
	0.05	0.4
	0.59	0.5
Zectran	0.24	0.8
	0.30	0.8
Matacil	None	

The identities of these minor products, some of which might have resulted from trace impurities in the labeled compounds, are not known.

Recovery from Growing Bean Plants after Injection into Stem. Table V gives the recovery results obtained in the experiments made in June 1964, in which the eight radioactive methylcarbamates were individually injected into the stems of young, growing bean plants. While the involvement of biochemical processes probably differs considerably, the degradation product patterns shown in Table V are in line with those obtained after surface treatment of bean leaves (Table IV); however, some results are strikingly unexpected. All of the chemicals, in varying degrees, suffer degradation with time. This happens, for all practical purposes, in one day in the case of Zectran and Matacil. On the other hand, approximately 35% of HRS-1422 is recovered 6 days after its injection into the plant; the other compounds last for intermediate periods, being present in low amounts only in the chloroform-acetone extract at the end of the sixth day. Approximately one half of the radioactivity is unextractable with acetone 12 hours after treatment with Zectran and Matacil, and more than a third is found in the water phase one day after treatment with Baygon, UC-10854, and Banol. Six days after injection, the loss of radioactivity (probably by expiration and/or volatilization) ranged from about 64% for Mesurol to 14% for Zectran.

The carbamate moiety of the eight methylcarbamates remains intact for varying lengths of time in the plant. While the amounts of carbaryl and Baygon recoverable as such become less with time, these compounds do not give rise to chloroform-soluble degradation products of consequence in the plant. Both UC 10854 and Banol form one or two degradation products in small amounts, but their identity is not known. In line with the results on surface-treated plants, Mesurol rapidly degrades partially to its sulfoxide and, to a lesser extent, to its sulfone, both of which disappear rapidly and have been tentatively identified by co-chromatography. In the case of Zectran, the original compound disappears rapidly, partially degrading to three major products (VII-D, VII-E, and VII-F), whose identity is considered below. Matacil disappears very rapidly in the plant to yield, as major organosoluble products, compounds VIII-D and VIII-H, the identity of which is considered.

In addition to the degradation products listed in Table V, there is some evidence of the following radioactive products (4 to 1 ether-hexane as the chromatographic solvent):

Parent Comp.	R_f Value	Max. %
Carbaryl, Banol, Mesurol, Zectran	None	
Baygon	0.39	0.9
UC 10854	0.08	0.5
HRS-1422	0.52	0.9
Matacil	0.00	0.8

Table III. Number and Chromatographic Location of Degradation Products Formed from Methylcarbamates Exposed to Short-Wavelength Ultraviolet Light[a] on Silica Gel G Chromatoplates

Designation	Compound	Degradation Products[b] No.	Location, R_f value
I	Carbaryl	1	0.00
II	Baygon	3	0.00, 0.24, 0.45
III	UC 10854	2	0.00, 0.43
IV	HRS-1422	3	0.00, 0.52, 0.70
V	Banol	4	0.00, 0.38, 0.53, 0.65
VI	Mesurol	7	0.00, 0.13, 0.30, 0.45, 0.52, 0.55, 0.59
VII	Zectran	6	0.00, 0.14, 0.31, 0.38 0.51, 0.60
VIII	Matacil	4	0.00, 0.30, 0.45, 0.64

[a] Wavelength of light = 2537 A.
[b] One-direction chromatography: chromatographic solvents; 2 to 1 ethyl acetate–toluene mixture for compounds I to VI, inclusive, and for compound VIII; 1 to 1 acetonitrile-toluene mixture for compound VII: chromogenic reagent; ninhydrin.

Table IV. Relative Amounts of Products Recovered from Growing Bean Leaves Surface-Treated with C^{14}-Carbonyl-Labeled Methylcarbamates and Sampled at Various Intervals after Treatment

Designation	R_f[a] Value	Amounts,[b] % of Radioactivity Applied for Indicated Time after Treatment, Hours 0	2	4	8	24	48	72
	I. 1-Naphthyl Methylcarbamate (Carbaryl)							
I	0.76	99.0	...	...	71.1	71.0	58.1	47.7
I-A	0.48	0.5	...	...	1.0	1.0	0.7	0.5
	II. 2-Isopropoxyphenyl Methylcarbamate (Baygon)							
II	0.76	99.8	...	...	49.4	26.3	22.0	8.1
	III. 3-Isopropylphenyl Methylcarbamate (UC 10854)							
III	0.72	99.3	...	...	21.6	9.6	3.5	1.8
III-A	0.42	0.2	...	...	2.4	2.9	2.3	1.1
	IV. 3,5-Diisopropylphenyl Methylcarbamate (HRS-1422)							
IV	0.85	99.8	...	...	63.9	39.1	30.2	24.4
IV-A	0.50	0.2	...	...	0.9	1.3	1.8	2.9
IV-B[c]	0.63	<0.1	...	...	1.3	0.9	1.7	1.8
	V. 2-Chloro-4,5-xylyl Methylcarbamate (Banol)							
V	0.81	99.2	...	...	54.4	44.2	35.2	31.5
V-A + B	0.50–0.53	<0.5	...	...	2.5	0.8	0.6	0.5
	VI. 4-Methylthio-3,5-xylyl Methylcarbamate (Mesurol)							
VI	0.81	99.3	93.8	84.9	70.8	68.8	60.0	55.4
VI-sulfoxide	0.13	0.4	4.6	8.7	9.3	9.9	8.3	6.7
VI-sulfone	0.52	0.1	0.2	0.5	0.8	1.1	2.2	1.9
	VII. 4-Dimethylamino-3,5-xylyl Methylcarbamate (Zectran)							
VII	0.80	98.0	57.5	20.3	12.4	7.8	1.4	1.2
VII-A	0.00	<0.1	0.5	0.8	0.9	0.8	0.9	1.1
VII-B	0.10	<0.1	<0.1	0.7	1.0	1.2	1.3	1.0
VII-C	0.20	<0.1	<0.1	0.4	0.9	1.3	1.3	1.4
VII-D	0.38	0.2	3.2	5.1	6.6	6.9	8.3	6.9
VII-E	0.51	0.3	6.2	11.5	13.2	14.3	15.6	11.9
VII-F[c]	0.60	1.5	25.1	35.7	32.8	32.1	25.1	17.8
VII-G[c]	0.64	<0.1	<0.1	1.2	2.4	3.7	1.5	1.7
VII-H	0.70	<0.1	<0.1	1.0	1.5	2.6	1.1	0.9
	VIII. 4-Dimethylamino-3-cresyl Methylcarbamate (Matacil)							
VIII	0.75	99.2	82.5	51.2	27.5	13.9	7.8	4.8
VIII-A	0.00	<0.1	0.9	1.4	2.1	2.7	3.2	2.7
VIII-B	0.06	<0.1	<0.1	0.4	0.7	1.2	1.6	2.0
VIII-C[c]	0.10–0.13	<0.1	<0.1	0.5	1.3	2.5	1.8	1.2
VIII-D	0.25	<0.1	0.4	1.1	2.3	2.6	2.8	2.3
VIII-E	0.30	<0.1	2.7	2.8	5.4	6.0	4.9	4.2
VIII-F	0.45	<0.1	0.4	0.5	1.7	1.9	1.4	1.1
VIII-G	0.50	<0.1	0.4	0.6	1.2	0.9	0.5	0.4
VIII-H[c]	0.64	0.7	7.1	9.8	8.6	6.4	5.1	2.9

[a] Chromatographic solvents; 2 to 1 ethyl acetate–toluene mixture except in experiments with compound VII when 1 to 1 acetonitrile–toluene mixture was used: one dimension chromatography for compounds I to V and two dimensions for compounds VI to VIII.
[b] Corrected to 100% recovery, based on experiments with fortified samples; average of three replicate results.
[c] Mixture of components.

The identities of these minor products or impurities are not known or being studied.

In Table VI are given the results of the repeat experiments made in March 1965, in which Zectran and Matacil were injected into growing bean plants, as before, but samples were taken at short intervals after the injection and, for the most part, were processed immediately. The results in Table VI are quantitatively, but not qualitatively, in variance with those in Table V, even though the experimental conditions were generally the same, but are of the same order of magnitude and show the same trends. It is apparent (Table VI) that the mere process of freezing the 0-time samples and storing them (at freezing temperature) results in degradation of both Zectran and Matacil, along with production of certain degradation products, fixation of some of the radioactivity in the unextractable residue, and loss of some of the radioactivity (by expiration or volatility). The initial major product from Zectran is VII-F and from Matacil is VIII-H, and the formation of these compounds is accompanied by a rapid buildup of unextractable residue after the first hour or two. The greenhouse conditions in June 1964 (Table V) were more favorable for the plants to degrade Zectran and Matacil than the laboratory conditions in March 1965 (Table VI), possibly because of light, temperature, and humidity differences.

The 1965 experiment also includes sodium carbonate-C^{14}. In the freezing and storage process, approximately one half of the radiocarbon becomes unextractable and a little less than one half of it is lost, very little being extractable by acetone. Within 20 minutes after injection, a large proportion of the sodium carbonate-C^{14} appears in the unextractable fraction, and this high proportion of incorporation persists for at least several hours.

Identity of Carbamate Degradation Products of Zectran and Matacil. The major degradation products of Zectran and Matacil recovered from the plant surface and/or from the plant injection experiments were tentatively identified by co-chromatography:

Product	Name
VII-D	4-Formamido-3,5-xylyl methylcarbamate
VII-E	4-Methylformamido-3,5-xylyl methylcarbamate
VII-F	Mixture of 4-methylamino-3,5-xylyl methylcarbamate and 4-amino-3,5-xylyl methylcarbamate (the former predominant)
VIII-D	4-Formamido-3-cresyl methylcarbamate
VIII-E	4-Methylformamido-3-cresyl methylcarbamate
VIII-F	4-Amino-3-cresyl methylcarbamate
VIII-H	4-Methylamino-3-cresyl methylcarbamate

The identity of the other degradation products of Zectran and Matacil is not known, but work is in progress to identify them and establish the sequence for formation of the various degradation products.

Rate of Degradation in Growing Bean Plants and in Basic Solution. In the growing bean plant (June 1964 experiment), the fastest degradation was found with Matacil and the slowest with HRS-1422, while in a pH 9.3 buffered solution, the fastest degradation occurred with Banol and the slowest with HRS-1422, Matacil being next to slowest in this regard (Table VII). The results for Matacil are anomalous, as are those for carbaryl, which is readily degraded in basic solution but is the next to the least degradable in the bean plant. Apparently, factors other than nonenzymatic hydrolysis play a determining role in degrading the methylcarbamates in bean plants.

Discussion

This study utilized substituted-phenyl methylcarbamates containing C^{14} in the carbonyl position of the molecules. Therefore, since radioactivity counting was the basis of the qualitative and quantitative analytical measurements, only products containing this carbon atom in their structure were found. Thus, certain hydrolysis products, such as the respective phenols (and their degradation products), were not detected. No work was done to identify, in the leaf-application experiments, degradation products, if any, that were retained by the leaf, were present in the rest of the plant, or were volatilized from the leaf (before or after degradation). In the investigations with injected plants, data were not obtained in regard to degradation products, if any, in the roots and the identity of the products in the water phase, the unextracted residue, and the expired or volatilized products. It is apparent that many of the methylcarbamates disappear rapidly from the leaf; volatilization or loss as $C^{14}O_2$ is probably involved. It is not likely that systemic action and incomplete extraction of the products (from the leaf surface) are important considerations. However, the present study did not include work on possible leaf penetration by the chemical and the effect of the adjuvants used in formulations on such penetration, if any.

Noteworthy are the rapid degradation of several of the injected methylcarbamates and the very large amount of unextractable residue (Table V). The most likely mechanism for this rapid breakdown relates to the enzyme(s) within the plant, but some breakdown cannot be ruled out during the freezing operation, frozen storage period, and/or the extraction and chromatography cleanup steps.

Table V. Products Recovered from Growing Bean Plants Injected into the Stem with C^{14}-Carbonyl-Labeled Methylcarbamates and Sampled at Intervals after Treatment in June 1964

Designation	R_f[a] Value	Amounts,[b] % of Radioactivity Injected for Indicated Time after Treatment, Days 0	0.5	1	2	3	4	6
	I.	1-Naphthyl Methylcarbamate (Carbaryl)						
I	0.58	99.0	74.7	58.2	42.2	24.0	15.0	5.3
Water phase	...	0.2	9.1	12.7	25.0	35.2	35.8	38.7
Unextracted	...	0.8	12.0	13.2	23.3	26.6	31.9	34.9
Loss	...	...	4.2	15.9	9.5	14.2	17.3	21.1
	II.	2-Isopropoxyphenyl Methylcarbamate (Baygon)						
II	0.68	99.4	69.4	48.7	21.9	12.1	8.4	2.0
Water phase	...	0.2	17.5	33.6	53.7	59.0	58.5	68.1
Unextracted	...	0.4	4.6	5.7	9.9	11.3	13.4	11.3
Loss	...	...	8.1	11.2	13.6	16.9	19.4	18.3
	III.	3-Isopropylphenyl Methylcarbamate (UC 10854)						
III	0.62	99.4	42.5	22.4	5.8			
III-A'[c]	0.28	<0.1	4.5	3.4	1.1	3.0[d]	2.4[d]	1.2[d]
III-B	0.38	<0.1	2.8	1.9	1.8			
Water phase	...	0.3	24.0	47.0	59.6	64.4	55.9	61.0
Unextracted	...	0.3	5.5	9.2	10.0	12.4	9.6	10.3
Loss	...	...	20.5	15.6	21.6	20.2	32.1	27.5
	IV.	3,5-Diisopropylphenyl Methylcarbamate (HRS-1422)						
IV	0.73	93.5	88.7	80.7	66.4	56.8	46.0	34.9
IV-A'[c]	0.44	0.9	1.5	0.6	0.4	0.3	0.2	0.1
Water phase	...	0.1	2.4	4.3	9.5	11.7	15.4	23.1
Unextracted	...	0.5	2.4	2.6	3.9	5.9	5.8	8.2
Loss	...	...	4.1	11.2	19.3	24.8	32.3	33.6
	V.	2-Chloro-4,5-xylyl Methylcarbamate (Banol)						
V	0.68	99.4	23.4	10.3	6.8			
V-A'[c]	0.36	<0.1	1.3	1.2	1.3	6.1[d]	4.1[d]	4.4[d]
V-B'[c]	0.43	<0.1	1.0	1.2	0.5			
Water phase	...	0.2	43.9	53.1	51.4	45.8	45.4	44.7
Unextracted	...	0.4	7.3	10.2	11.9	12.3	15.1	13.6
Loss	...	...	23.1	24.0	28.1	35.8	35.4	37.3
	VI.	4-Methylthio-3,5-xylyl Methylcarbamate (Mesurol)						
VI	0.74	98.0	49.0	19.8	2.2	<0.1	<0.1	<0.1
VI-Sulfoxide	0.08	1.3	19.8	23.3	5.5	4.7		
VI-Sulfone	0.34	<0.1	2.3	8.2	5.5	1.8	3.2[d]	1.3[d]
Water phase	...	0.3	4.2	7.2	8.2	8.7	8.1	7.7
Unextracted	...	0.4	11.5	18.0	25.3	24.0	26.1	27.5
Loss	...	...	13.2	23.5	53.3	60.8	62.6	63.5
	VII.	4-Dimethylamino-3,5-xylyl Methylcarbamate (Zectran)						
VII	0.78	66.7	22.1	3.1	2.2	<0.1	<0.1	<0.1
VII-A'[c]	0.00	2.5	2.5	2.9	2.3	0.9		
VII-D	0.05	2.5	3.2	3.4	3.6	1.5		
VII-E	0.27	4.5	4.5	4.5	5.4	3.5		
VII-F[e]	0.44	10.9	7.6	6.4	4.1	2.7	6.7[d]	2.7[d]
VII-G' or -H'[c]	0.59	4.8	3.5	5.6	3.1	4.3		
Water phase	...	1.3	2.5	3.2	4.5	5.3	4.4	4.8
Unextracted	...	6.8	41.3	56.0	64.4	62.1	76.0	78.5
Loss	...	...	12.8	14.9	10.4	19.7	12.9	14.0
	VIII.	4-Dimethylamino-3-cresyl Methylcarbamate (Matacil)						
VIII	0.62	90.2	5.0	2.2				
VIII-D	0.11	1.2	0.7	0.5	2.0[d]	1.8[d]	1.8[d]	1.2[d]
VIII-H	0.43	5.4	2.9	1.5				
Water phase	...	0.7	8.9	11.4	8.1	8.2	6.8	8.2
Unextracted	...	1.7	55.8	58.8	62.6	63.7	66.0	66.5
Loss	...	...	26.2	25.2	27.1	26.1	25.3	24.0

[a] Chromatographic solvent: 4 to 1 ether-hexane mixture; two-dimensional development.

[b] Corrected to 100% recovery, based on experiments with fortified samples; average of three replicate results for all data except ratio of products within organoextractable fraction where only two replicate results were averaged; all samples held in frozen state.

[c] Products believed to be identical to those bearing same letter designation in Table IV. All other products without prime designation were established, by co-chromatography with unlabeled known compounds, to be same as those bearing same letter designation in Table IV.

[d] Total found in chloroform extract after cleanup column. Thin-layer chromatography omitted.

[e] Mixture of two components.

Table VI. Products Recovered from Growing Bean Plants Injected in Stem with C^{14}-Carbonyl-Labeled Zectran and Matacil, and with Sodium Carbonate-C^{14}, and Sampled at Intervals after Treatment in March 1965

Designation	R_f Value[c]	Amounts,[a] % of Radioactivity Injected for Indicated Time after Treatment, Hours: 0	0.33	1	3	9	27	0-frozen[a]
VII. 4-Dimethylamino-3,5-xylyl Methylcarbamate (Zectran)								
VII	0.78	97.3	85.2	83.2	72.0	48.3	24.8	30.2
VII-A′[a]	0.00	<0.1	0.1	0.4	0.4	0.8	0.6	1.5
VII-D	0.05	<0.1	<0.1	<0.1	0.2	0.6	0.5	1.1
VII-E	0.27	<0.1	0.2	0.7	0.6	1.2	1.4	3.2
VII-F[b]	0.44	2.6	2.6	5.1	5.8	10.8	15.4	14.1
VII-G′ or -H′[a]	0.59	<0.1	<0.1	0.6	1.0	1.4	1.1	4.5
Water phase	...	<0.1	<0.1	0.3	0.8	1.5	3.7	2.9
Unextracted	...	0.1	0.7	2.8	6.8	14.7	28.6	15.0
Loss	...	...	11.2	6.9	12.4	20.7	23.9	27.5
VIII. 4-Dimethylamino-3-cresyl Methylcarbamate (Matacil)								
VIII	0.62	97.8	88.8	88.3	78.7	66.0	35.7	82.2
VIII-A′[a]	0.00	0.3	0.2	<0.1	<0.1	0.1	0.2	0.2
VIII-D	0.11	<0.1	<0.1	<0.1	<0.1	0.1	0.7	0.1
VIII-H	0.43	0.6	1.9	1.5	2.2	5.1	16.3	1.7
Water phase	...	1.2	1.0	1.2	0.8	2.0	7.8	0.8
Unextracted	...	0.1	0.5	1.1	1.9	4.7	15.2	4.2
Loss	...	...	7.6	7.9	16.4	22.0	24.1	10.8
Sodium Carbonate								
Organic phase	...	0.5	0.5	0.4	0.5	0.6	0.5	0.4
Water phase	...	10.1	5.5	4.1	1.9	2.4	1.7	1.0
Unextracted	...	29.5	90.7	72.8	75.9	77.4	61.4	55.8
Loss	...	59.9	3.3	22.7	21.7	19.6	36.4	42.8

[a] All conditions and designations same as those in Table V but time intervals in hour rather than days and samples were immediately analyzed, without freezing, except those for which results are given in last column. The latter were handled in a manner closely analogous to the procedure on which Table V is dependent.
[b] Includes product found at R_f = 0.33, amounting to 4 to 11% of sum.

Table VII. Rate of Degradation of C^{14}-Carbonyl-Labeled Methylcarbamates Injected into Stem of Growing Bean Plants or Incubated in Basic Solution

Designation	Compound	50% Recovery Times, Hours: From Bean Plants: Organic soluble compounds[a]	From Bean Plants: Original compound[b]	From pH 9.3 buffered solution[b]
I	Carbaryl	34	34	0.5
II	Baygon	24	24	3.1
III	UC 10854	12	10	2.8
IV	HRS-1422	84	84	6.8
V	Banol	6	6	0.1
VI	Mesurol	22	10	0.4
VII	Zectran	11	7	2.3
VIII	Matacil	3.6	2.4	4.0

[a] Average of three replicate results.
[b] Average of two replicate results.

In fact, from Table VI, it is clear that considerable decomposition occurs with Zectran and Matacil during freezing and storage. While it is known that the compounds used quantitatively survive the extraction and chromatography operations, the recovery efficiency for the degradation products is not known in this regard. However, it is not likely that these considerations greatly affected the results. It is known that these compounds do not break down during prolonged storage in hexane in the dark; this is also true of degradation products which have been identified. The loss values (Tables V and VI), are possibly the result of expired $C^{14}O_2$.

With sodium carbonate-C^{14}, the large loss of radioactivity at 0-time (Table VI) was probably an artifact of the extraction procedure. The same large loss was also evident when a plant homogenate was fortified with radioactive sodium carbonate and extracted by the same procedure; however, the radiocarbon was quantitatively recovered by adding the radioactive sodium carbonate to water, followed by an identical partitioning procedure.

In Tables V and VI, some products are designated with a letter followed by a prime. This indicates that the products so marked may be the same as those designated by the letter without the prime in Table IV, but that suitable compounds were not available to prove identity by co-chromatography or other means. All compounds designated by the same number and letter (without the prime) in these three tables have been shown to be identical by co-chromatography.

From this work, it appears that the carbamate ester group is, to some extent, stable when substituted-phenyl methylcarbamates are placed on glass or bean plant surfaces and when they are injected into the bean plant. This appears to be true because the radioactivity from carbonyl-C^{14}-labeled preparations of these compounds is recovered in organic solvent extracts of the treated plants, and, in certain cases, methylcarbamate metabolites have been identified. Undoubtedly, concomitant with their persistence, a certain portion of the applied or injected methylcarbamate hydrolyzes to the substituted phenol and methylcarbamic acid, the former being conjugated and the latter probably being decomposed and partially expired as $C^{14}O_2$. The botanical origin of certain methylcarbamates—e.g., eserine—might also indicate that the methylcarbamoyl group is resistant to degradation in plants, at least under certain conditions.

The relative resistance of the methylcarbamate group to photooxidation on surfaces and to metabolism within plants allows for possible reactions involving less stable groupings within the molecule prior to hydrolysis. Thus, Mesurol converts to its corresponding sulfoxide and sulfone. In Zectran and Matacil the *p*-dimethylamino group undergoes *N*-dealkylation without other alteration in the molecule, and *N*-formamido compounds appear as intermediates in the dealkylation.

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SYNTHESIS AND ACTIVITY

Synthesis and Insecticidal Activity of O-Alkyl O-2,4,5-Trichlorophenyl Phosphoramidothioates

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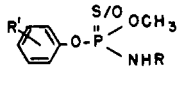
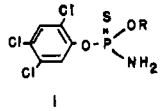
The synthesis, physical properties, and insect toxicities of a series of O-alkyl O-2,4,5-trichlorophenyl phosphoramidothioates are described. The amidothiophosphate esters were prepared from O-2,4,5-trichlorophenyl phosphoramidochloridothioates and from O-alkyl O-2,4,5-trichlorophenyl phosphorochloridothioates. The latter intermediates were prepared by partial esterification of O-2,4,5-trichlorophenyl phosphorodichloridothioate with an alcohol and by treating an O-alkyl phosphorodichloridothioate with sodium 2,4,5-trichlorophenate. While the amidothiophosphates have a wide spectrum of insecticidal activity, their use on plant insects is limited by phytoxicity. They are active against a variety of household insects and grain pests and may be useful in such applications.

RECENT reports from our laboratories have been concerned with the synthesis of aryl methyl phosphoramidates (*4*, *9*) and *O*-aryl *O*-methyl phosphoramidothioates (*2*) and the relationship among chemical structure, anticholinesterase properties, and insecticidal activities.

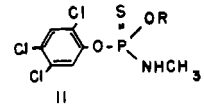


In an early study (*2*), it was demonstrated that insecticidally active compounds could be obtained by replacing a methoxy group of Ronnel (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate) (*7*) with an amide (*5*) or a substituted amide group (*8*). In many instances, these compounds, especially *O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate, were more active toward certain insects and mites than the parent triester.

In the early studies the methyl ester was kept constant and the aromatic and amide portions of the molecules were changed. The purpose of the present investigation was to change only the aliphatic ester groups of *O*-alkyl *O*-2,4,5-trichlorophenyl phosphoramidothioates I and II and relate the change in structure to insecticidal activities.



I

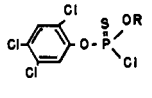


II

R = CH_3, C_2H_5, *n*-C_3H_7, i-C_3H_7, *n*-C_4H_9, i-C_4H_9, *sec*-C_4H_9

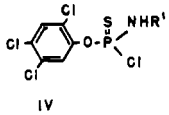
Chemical Studies

O-Alkyl *O*-2,4,5-trichlorophenyl phosphoramidothioates (I) and *O*-alkyl *O*-2,4,5-trichlorophenyl methylphosphoramidothioates (II) were prepared by the amidation of *O*-alkyl *O*-2,4,5-trichlorophenyl phosphorochloridothioates (III) with ammonia or methylamine and by the reaction of *O*-2,4,5-trichlorophenyl phosphoramidochloridothioates (IV) with sodium alkoxides.



III

R = R in I and II



IV

R′ = H, CH_3

O-Alkyl *O*-aryl phosphorochloridothioates (type III intermediates) have been prepared by treating *O*-aryl phosphorodichloridothioates with excess alcohol in the absence of an acid acceptor (*2*) and by the esterification of *O*-alkyl phosphorodichloridothioates with phenols in the presence of hydrogen chloride acceptors (*6*).

O-2,4,5-Trichlorophenyl phosphorodichloridothioate and an alcohol (2- to 3-mole excess) dissolved in methylene dichloride were heated at 30° to 45° C. (*14*). When the evolution of hydrogen chloride was complete, the acid chloride (III) was washed with water and/or aqueous sodium hydroxide to remove dissolved HCl, unreacted alcohol, and hydrolysis products. Phosphoramidothioates I and II were obtained on treatment with aqueous (*1*) or anhydrous ammonia or methylamine.