Photooxidation of Two 4-Dimethylaminoaryl Methylcarbamate Insecticides (Zectran and Matacil) on Bean Foliage and of Alkylaminophenyl Methylcarbamates on Silica Gel Chromatoplates

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Photodecomposition of 4-dimethylamino-3,5-xylyl methylcarbamate (Zectran) and 4-dimethylamino-3-cresyl methylcarbamate (Matacil) deposits on bean foliage involves extensive oxidation of the dimethylamino moiety but, apparently, not of other groups. The methylcarbamate degradation products include the toxic 4-methylamino and 4-amino analogs, the less toxic 4-methylformamido and 4-formamido analogs, and trace amounts of other unidentified, biologically active methylcarbamates. Zectran is less stable than Matacil to photooxidation. In the photodecomposition, the dimethylamino moiety is stepwise demethylated and, concurrently, one of the methyl radicals is oxidized to the formamido group. Many alkylaminophenyl methylcarbamates appear to be susceptible to these reactions.

Methylcarbamates suffer degradation when they are exposed to ultraviolet light or to sunlight, either as solutions in hexane or ethanol, or as deposits on filter paper, silica gel chromatoplates, or plant foliage (*1*, *2*, *5*, *6*, *14*). The methylcarbamate group appears to be more stable to photodecomposition than certain other functional groups contained in the carbamate insecticides; accordingly, the degradation products generally have the methylcarbamate group intact and some of them are potent cholinesterase inhibitors (*1*, *2*, *5*).

Zectran (4-dimethylamino-3,5-xylyl methylcarbamate) deteriorates in insecticidal activity under simulated sunlight, using ultraviolet light (*10*). When Zectran or Matacil (4-dimethylamino-3-cresyl methylcarbamate) is exposed, in hexane or ethanol solutions, to ultraviolet light at 254 mμ or to sunlight, each decomposes to yield five or more products which are cholinesterase inhibitors (*5*). A similar irradiation behavior is noted for Zectran when the photodegradation products on the silica gel chromatoplates are detected with ultraviolet-quenching rhodamine B and iodine vapor (*6*). Within 10 days following the streaking of a Zectran solution onto the stems of broccoli plants immediately below the flower clusters, the compound is degraded to yield 4-dimethylamino-3,5- xylenol, 2,6-dimethylhydroquinone, 2,6-dimethyl-*p*-benzoquinone, and 4-dimethylamino-3,5-dimethyl-*o*-benzoquinone, along with conjugates of some of these compounds and with materials which are incorporated into the lignin fraction (*19*). It is not known to what extent these degradation reactions take place on the surface of the plant or are the result of enzymatic attack following absorption into the plant.

Zectran and Matacil are converted on and in bean plants to more persistent methylcarbamate degradation products and these products, in certain cases, constitute the major persisting residues. In each case, the major degradation product is the 4-methylamino analog, but the 4-amino, 4-methylformamido, and 4-formamido analogs also are present (*1*, *2*). This communication gives more information about the chemical and biological properties of the photodecomposition products of Matacil and Zectran.

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Chemicals

In this report, certain analogs of Zectran and Matacil are designated by trivial names, as follows:

Designation	Chemical Name
Amino Zectran	4-Amino-3,5-xylyl methylcarbamate
Amino Matacil	4-Amino-3-cresyl methylcarbamate
Methylamino Zectran	4-Methylamino-3,5-xylyl methylcarbamate
Methylamino Matacil	4-Methylamino-3-cresyl methylcarbamate
Formamido Zectran	4-Formamido-3,5-xylyl methylcarbamate
Formamido Matacil	4-Formamido-3-cresyl methylcarbamate
Methylformamido Zectran	4-Methylformamido-3,5-xylyl methylcarbamate
Methylformamido Matacil	4-Methylformamido-3-cresyl methylcarbamate

The carbonyl-C^{14}–labeled samples of Zectran and Matacil had a specific activity of 1.0 mc. per mmole and a radiochemical purity greater than 99% based on thin layer chromatography, TLC (*1*, *2*, *12*). Dimethylamino-labeled Zectran and Matacil were prepared by reaction of amino Zectran and amino Matacil, respectively, with either formaldehyde-C^{14} or formaldehyde-H^3, according to a procedure based on that of Williams, Meikle, and Redemann for conversion of 4-amino-3,5-xylenol (from reduction of 4-nitroso-3,5-xylenol) to 4-dimethylamino-3,5-xylenol (*20*). A mixture of either amino Zectran or amino Matacil (0.33 mmole), labeled formaldehyde (1.0 mmole, as a 37% aqueous solution), sodium acetate trihydrate (10 mg.), 10% palladium on charcoal (100 mg.), and anhydrous methanol (5 ml.) was hydrogenated at 25° C. and atmospheric pressure, with stirring, until the theoretical amount of hydrogen was absorbed; the reaction was

achieved within 30 minutes. The reaction mixture was filtered and evaporated, and the residue purified by the use of a Florisil column (described below), giving 64 to 80% yields, based on the starting amino compounds, of 4-dimethyl-C^{14}-amino-3,5-xylyl methylcarbamate (Zectran-*N*-methyl-C^{14}, 0.20 mc. per mmole), 4-dimethyl-H^3-amino-3,5-xylyl methylcarbamate (Zectran-*N*-methyl-H^3, 10 mc. per mmole), and 4-dimethyl-C^{14}-amino-3-cresyl methylcarbamate (Matacil-*N*-methyl-C^{14}, 0.4 mc. per mmole). The identity of all labeled compounds was established by thin layer cochromatography with authentic, nonlabeled compounds, using two different solvent systems for TLC. In preliminary experiments using nonlabeled preparations, the infrared spectrum and melting point for Zectran, prepared by this procedure, were identical to those of an authentic sample. (Methylamino Zectran may be a by-product in the Zectran radiosynthesis because a compound with the chromatographic characteristics of methylamino Zectran was noted, both on Florisil column purification and on two-dimensional TLC separation of the reaction mixture.)

Zectran and Matacil were oxidized with potassium permanganate, in neutral aqueous-acetone mixture, to achieve *N*-demethylation and *N*-formamide formation at the dimethylamino moiety of the molecule. Zectran (5 mmoles in 200 ml. of acetone) was added, with mixing, over a 5-minute period to an aqueous solution of potassium permanganate (10 mmoles in 300 ml. of 0.2*M* sodium phosphate solution, pH 6.9). After 15 minutes at 25° C., the reaction mixture was extracted twice with 500-ml. portions of chloroform and, after evaporation of the chloroform, the products were resolved on a Florisil column. The recovered products and their yields were as follows: Zectran, 0.2%; methylamino Zectran, containing a small amount of amino Zectran (later removed on recrystallization), 67%; methylformamido Zectran, containing a small amount of formamido Zectran (later removed on recrystallization), 14%. Matacil was oxidized by the same procedure, using the same conditions, to yield 29% of unreacted Matacil, along with 2.0 to 2.6% each of methylamino-, amino-, and methylformamido Matacil. Matacil was converted to methylamino Matacil, in 5% yield, by dropwise addition of 20 ml. of acetone containing 100 mg. of chromium trioxide, over a 30-minute period at 5° C., to 600 mg. of Matacil dissolved in 5 ml. of acetone, followed by a further 72-hour reaction period at 25° C., using a procedure based on that of Büchi *et al.* (*4*). Amino Matacil was recovered, in 5% yield, by reaction of 25 grams of activated manganese dioxide, prepared according to Attenburrow *et al.* (*3*), with 0.5 gram of Matacil dissolved in 50 ml. of chloroform for 3 hours at 25° C., using a procedure based on that of Henbest and Thomas (*7*).

Methylformamido and formamido analogs of Zectran and Matacil were prepared also, in yields of 44 to 85%, by refluxing 50 mg. of each of the methylamino and amino analogs with a large excess of 98 to 100% formic acid for 40 minutes, followed by the addition of 2 ml. of saturated aqueous sodium chloride solution (to aid in product crystallization or extraction of the products into ether). A sample of amino Zectran (provided by Dow Chemical Co., Walnut Creek, Calif.) and samples of methylamino and amino Matacil (provided by Dow Chemical Co., Midland, Mich.) were purified on a Florisil column and recrystallized before use. The analytical data (Table I) and infrared spectra (*1*) were appropriate for the structures proposed for each of the Matacil and Zectran derivatives prepared; also, the NMR data supported the identity of methylamino Zectran and methylformamido Zectran preparations. The sources of other methylcarbamates studied were as follows: 4-dimethylamino-3,5-xylyl *N*-hydroxymethylcarbamate, 4-dimethylamino-3-cresyl *N*-hydroxymethylcarbamate, and 4-hydroxy-3,5-xylyl methylcarbamate from H. Balba, Division of Entomology, University of California, Berkeley; 3,5-xylyl methylcarbamate [m.p. 105° C., reported 100.5–102° C. (*11*)] and 4-nitro-3-cresyl methylcarbamate (m.p. 96–8° C.) by reaction of the appropriate phenol and methyl isocyanate for 12 hours at 25° C. in benzene or hexane solution, containing a catalytic amount of triethylamine, followed by product recrystallization from *n*-hexane; and 4-nitro-3,5-xylyl methylcarbamate and 15 other alkylaminophenyl methylcarbamates from the Dow Chemical Co., Walnut Creek, Calif.

Methods and Materials

Chromatography and Detection of Carbamates. The oxidation products of Zectran and Matacil were separated by means of chromatographic columns packed with a 2.5 × 40 cm. bed of 60- to 100-mesh Florisil (Floridin Co., Tallahassee, Fla.), used without activation. The columns were developed with the solvent systems indicated in Table I and Figure 1, and 15-ml. fractions were collected. The methylcarbamates in the eluate fractions were detected with ninhydrin reagent (*1*) and, after hydrolysis, determined quantitatively as methylamine (*16*). To do this, an appropriate aliquot from each fraction was evaporated in a small test tube, residue was dissolved in 0.2 ml. of *p*-dioxane, and 3.0 ml. of aqueous sodium hydroxide (0.66%, w./v.) were added. Each reaction mixture was held in a stoppered tube at 56° C. for 30 minutes and cooled, and 0.5 ml. of an aqueous solution of 3% (w./v.) lactose and 6.6%

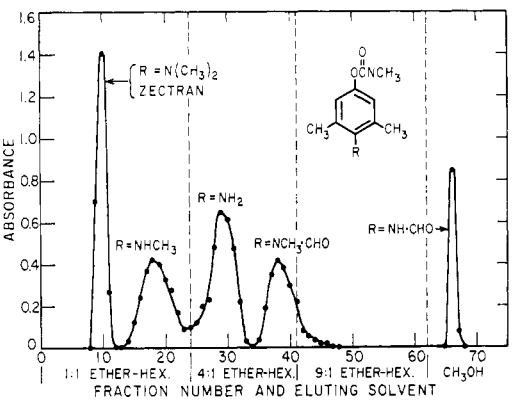


Figure 1. Resolution of Zectran and its oxidation products on a Florisil column based on colorimetric methylamine analysis

Table I. Chemical and Physical Properties and Biological Activities of Zectran, Matacil, and Oxidation Products of Each with Various para-Substituents

Chemical and physical properties	Values for Product with Indicated para-Substituent of									
	4-Substituted-3,5-xylyl Methylcarbamates					4-Substituted-3-cresyl Methylcarbamates				
	$N(CH_3)_2$ (Zectran)	$N(CHO)CH_3$	$NHCH_3$	NHCHO	NH_2	$N(CH_3)_2$ (Matacil)	$N(CHO)CH_3$	$NHCH_3$	NHCHO	NH_2
Melting point, ° C.	91[a]	160–162	95.5	197[b]	114[c]	94–96	99–101	105–106	168	130
Recrystallization solvent	hex.	ether-hex.	hex.	ethanol	ether-hex.	hex.	ether-hex.	ether-hex.	ethanol	ether-hex.
Solvent for elution from Florisil	ether-hex. (1:1)	ether-hex. (4:1)	ether-hex. (1:1)	methanol	ether-hex. (4:1)	ether-hex. (1:2)	ether-hex. (4:1)	ether-hex. (1:1)	methanol	ether-hex. (4:1)
R_f values for TLC on silica gel G, using										
Ether-hexane-ethanol (77:20:3)	0.75	0.25	0.39	0.10	0.32	0.65	0.22	0.52	0.16	0.36
Ethyl acetate–toluene (2:1)	0.77	0.26	0.42	0.14	0.39	0.75	0.30	0.64	0.25	0.45
Toluene-acetonitrile (1:1)	0.80	0.51	0.61	0.38	0.61	0.80	0.50	0.75	0.48	0.65
Elemental analyses, %										
Calculated										
Carbon	64.86	61.01	63.47	59.46	61.85	63.46	59.46	61.85	57.69	60.00
Hydrogen	8.11	6.77	7.69	6.31	7.22	7.69	6.30	7.21	5.77	6.66
Nitrogen	12.61	11.86	13.46	12.61	14.43	13.46	12.61	14.43	13.46	15.55
Found										
Carbon	65.02	60.80	63.27	59.35	61.43	63.40	59.62	61.78	57.57	59.81
Hydrogen	8.11	6.90	7.68	6.30	7.06	7.73	6.70	7.30	5.51	6.61
Nitrogen	12.54	11.82	13.44	12.63	14.52	13.60	12.91	14.59	13.38	15.45
Molecular weight										
Calculated	222	236	208	222	194	208	222	194	208	180
Found	209	218	214	210	199	210	223	206	200	192
Biological activities										
Anticholinesterase activity, pI_{50}										
Human plasma	6.0	5.0	6.7	5.5	5.8	5.0	4.8	5.5	4.7	5.6
Housefly head	8.1	6.9	7.8	6.8	7.5	6.4	5.7	6.4	5.7	6.6
Housefly topical LD_{50}, μg./g.										
Without piperonyl butoxide	113	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	>1000
With 10 μg. piperonyl butoxide	8	14	135	85	>1000	26	105	>1000	400	>1000
Mouse LD_{50}, mg./kg.										
Intraperitoneal	4.2	23	1.4	18	1.6	4.7	21	3.0	13	1.6
Dermal	107	1000	8	1000	7	31	>500	52	500	17

[a] Reported m.p. 85° C. (9).
[b] Reported m.p. 202–205° C. (17).
[c] Reported m.p. 112–14° C. (9).

(w./v.) ammonium sulfate was added. They were heated again at 56° C. for 30 minutes and held for 1 hour at 25° C., and the absorbance was read at 540 mμ. (The resolution of a mixture of 20 mg. of each of the Zectran oxidation products is illustrated in Figure 1 and, in this case, a 3.0-ml. aliquot of each fraction was used for methylamine analysis.) Occasionally, a turbid final solution was produced as a result of substances eluted from the column with hexane, in the first few fractions, or with methanol, in the last few fractions. This interference was avoided by washing the column with hexane, ether, methanol, and hexane, in that order, prior to adding the compounds to the column and, subsequently, eluting the carbamates with the normal sequence of solvents.

For TLC, the chromatoplates were coated with silica gel G (Kensington Scientific Corp., Berkeley, Calif.) at a thickness of 0.25 mm. In studies with Zectran, Matacil, and their oxidation products, chromatograms were developed with ether-hexane-ethanol mixture (77:20:3), unless stated otherwise. The solvent system used in the studies with other alkylaminophenyl methylcarbamates (described below) was ether-hexane-ethanol mixture (64.4:33.3:2.3). Radioactive compounds were detected by radioautography, methylcarbamates with ninhydrin reagent (*1*, *2*), and anticholinesterase agents by an *in situ* procedure on the TLC plates (*15*).

Foliage Treatment and Methods of Analysis. Garden snapbean seedlings (Contender variety) were used when the plants were 5 inches in height and 10 to 12 days old with primary leaves approximately 5 days old. Each of the five radioactive compounds (Zectran-carbonyl-C^{14}, Zectran-*N*-methyl-C^{14}, Zectran-*N*-methyl-H^3, Matacil-carbonyl-C^{14}, and Matacil-*N*-methyl-C^{14}) was individually and uniformly applied, as a solution containing 30 μg. of compound in 80 μl. of ethanol, to the upper surface of each primary leaf as previously described (*1*, *2*). In another study involving nonlabeled materials, Zectran, Matacil, and their methylamino, amino, methylformamido, and formamido analogs were similarly applied, using 1.0 mg. of methylcarbamate and 50 μl. of ethanol for each leaf. Immediately after treatment, the plants were placed outside in an unshaded, unprotected area; they were held under these conditions, prior to analysis, for 20 hours in the case of radioactive materials, and for 48 hours when the larger amounts of nonlabeled materials were used. These studies were made in Berkeley, Calif., and were, in the most part, made during August and September 1965; there was no rainfall. After the appropriate exposure period, the residual deposits were washed from the leaves with chloroform and the washings were subjected to TLC. Labeled degradation products were tentatively identified by cochromatography with authentic, nonlabeled compounds.

Anticholinesterase activity of degradation products was determined after 0, 5, and 25 hours of exposure of Zectran-carbonyl-C^{14} and Matacil-carbonyl-C^{14} deposits on bean leaves. In this assay, the treated leaves were washed with chloroform, and aliquots of the chloroform washings equivalent to 16 and 64 μg. of labeled compound, at zero hour, were analyzed; in the case of the 5- and 25-hour exposure samples, comparable aliquots, representing somewhat lower total radioactivity levels (because of volatilization and hydrolysis), were analyzed. The analyses involved two-dimensional TLC, radioautography at 5° C., *in situ* anticholinesterase assays directly on TLC plates, and comparisons of the chromatographic positions found for the radioactive and anticholinesterase materials.

Photodecomposition of Deposits of Zectran, Matacil, and Their Analogs. The photodecomposition sequence for Zectran and Matacil on plant foliage was determined by examining degradation products from leaves treated with 1.0 mg. of each of the nonlabeled intermediate compounds. An aliquot corresponding to 400 μg. of the carbamate, as originally applied, was used for TLC. With Matacil and its analogs, chromatograms were developed in one dimension and the degradation products responding to the ninhydrin reagent were compared only on the basis of their R_f values. More precise comparisons of the decomposition products were made with Zectran and its analogs by using two-dimensional TLC and cochromatography with the mixture of radioactive photodegradation products obtained from leaves treated with Zectran-carbonyl-C^{14}. A degradation product formed from one of the nonlabeled Zectran analogs was considered to be the same as that derived from another nonlabeled analog if they both yielded ninhydrin positive products which cochromatographed with the same radiolabeled derivative.

Two types of sequence studies were made in which the compounds were spotted on TLC plates and exposed to short-wavelength (2537 A.) ultraviolet light (Chromato-Vue Model C-3, Ultra-Violet Products, Inc., San Gabriel, Calif.). In the first type, 200 μg. of each compound listed in Table I were individually spotted over an area of 0.5 sq. cm. at the origin of the chromatoplate. The plate was exposed for 20 hours to ultraviolet light, the chromatogram was developed, and methylcarbamate degradation products were detected with ninhydrin reagent (*2*). Each of these experiments was repeated two or three times. The second approach was similar to one described by Stahl for examination of photodecomposition products of pyrethroids (*18*). It involved several steps:

Zectran-carbonyl-C^{14} or Matacil-carbonyl-C^{14}, 100 μg., was spotted near one corner of a TLC plate.

The plate was exposed to the ultraviolet light for 20 hours.

Degradation products formed during this first exposure period were resolved by developing the chromatogram in only one direction.

The plate was again exposed to the ultraviolet light for 20 hours, followed by development of the chromatogram in the second direction, using the same solvent, and subsequent radioautography. R_f values obtained for the second direction of development were compared to determine whether any of the products formed during the first irradiation period served as common precursors for the same new product(s) formed during the second irradiation period.

In a related study designed to compare the number of carbamate products formed rather than the sequence for their formation, a series of 15 nonlabeled, alkylaminophenyl methylcarbamates was spotted on TLC plates. The plates were irradiated with ultraviolet light, and the products were resolved and detected with ninhydrin reagent, as described above. Some compounds (Nos. 3 to 6, 10 to 12, 14, and 15 listed in Table II) contained minor impurities; so, these materials were spotted, the chromatogram was developed in one direction to separate the major component from impurities, the plate then exposed to the ultraviolet light for 20 hours, and the chromatogram developed in the second direction to separate the degradation products of the major component. The compounds examined in this study and their R_f values are given in Table II, along with the R_f values of the respective degradation products detected.

Anticholinesterase Assays. In the regular anticholinesterase assays, the product to be tested was deposited as a dry residue on the bottom of a test tube, and 1.0 ml. of enzyme preparation was added. Each milliliter of enzyme preparation contained, in sodium phosphate buffer (0.1*M*, pH 7.3), either 0.1 ml. of human plasma or the homogenized heads from three houseflies (*Musca domestica* L.). The mixture was incubated at 38° C. for 10 minutes, 1.0 ml. of substrate (8.0 μmoles of acetylcholine chloride in sodium phosphate buffer, 0.1*M*, pH 7.3) were added, and the mixture was incubated at 38° C. for 30 minutes. Residual acetylcholine was determined colorimetrically by the Hestrin method (*8*). Anticholinesterase activity was expressed as the negative logarithm of the molar methylcarbamate concentration necessary to effect 50% inhibition (pI_{50}) based on the volume of enzyme and inhibitor (1.0 ml.) prior to substrate addition.

Bioassays and Toxicity Tests. Adult female houseflies of the SCR strain (originally obtained from the Stauffer Chemical Co., Mountain View, Calif.) were treated by applying various amounts of the test compound dissolved in 1.0 μl. of acetone or acetone-dimethylsulfoxide mixture (1 to 1) to the ventrum of the abdomen. (Use of the acetone-dimethylsulfoxide mixture was necessary to obtain solutions of the formamido and methylformamido derivatives.) In certain cases, 10 μg. of piperonyl butoxide in 1.0 μl. of acetone were applied to the pronotum immediately before application of the carbamate to the abdomen. (No mortalities resulted from this amount of acetone, or acetone-dimethylsulfoxide mixture, or this dose of piperonyl butoxide.)

Female white mice weighing 12 to 14 grams (Berkeley Pacific Laboratories, Berkeley, Calif.) were used for both intraperitoneal and dermal toxicity studies. The injections involved 0.10 ml. of dimethylsulfoxide, containing the compound under test, per 10 grams of body weight. For dermal application, the compound was dissolved in 20 μl. of dimethylsulfoxide and applied to a 2 × 2 cm. clipped area on the back of the mouse. For the first 6 hours after dermal treatment, the mice were confined in such a manner that none of the dose was rubbed off, and for the remaining test period they were held, without restriction, in individual cages. All LD_{50} values were calculated on the basis of a 24-hour test period.

Miscellaneous Methods and Procedures. Molecular weight determinations by the osmometer method and elemental analyses were performed by the Microchemical Analytical Laboratory of the Department of Chemistry, University of California, Berkeley. Melting points (uncorrected) were determined by observing

Table II. Decomposition Products of Alkylaminophenyl Methylcarbamates Exposed to Short-Wavelength Ultraviolet Light on Silica Gel G Plates for 20 Hours

	Compound[a]		R_f Values of Spots Detected with Ninhydrin: Original compound	Degradation products
	4-(R_1R_2)N-3,5-$Me_2\phi$OC(O)NHMe			
	R_1	R_2		
1.	Me	Et	0.86	0.00, 0.41, 0.55
2.	Me	*n*-Bu	0.84	0.00, 0.22, 0.30, 0.58, 0.76
3.	Me	*n*-Pent	0.87	0.00, 0.30, 0.63, 0.96
4.	Me	2-Et-Bu	0.85	0.00, 0.28, 0.39, 0.57, 0.73
5.	Et	Et	0.89	0.00, 0.27, 0.45
6.	Et	Iso-Bu	0.83	0.00, 0.48, 0.63
7.	*n*-Pr	*n*-Bu	0.87	0.00, 0.65
8.	Iso-Bu	Iso-Bu	0.87	0.00, 0.67
9.	*n*-Bu	Iso-Bu	0.86	0.00, 0.64, 0.68
10.	Benzyl	Benzyl	0.88	0.00, 0.29, 0.74
	Other dimethylaminophenyl methylcarbamates			
11.	4-Me_2N-2,6-$Me_2\phi$OC(O)NHMe		0.63	0.00, 0.44, 0.91
12.	4-Me_2N-3-Me, 5-EtϕOC(O)NHMe		0.92	0.00, 0.32, 0.48
13.	4-Me_2N-2-Me,5-isoPrϕOC(O)NHMe		0.47	0.00
14.	4-Me_2N-3-*t*-BuϕOC(O)NHMe		0.89	0.00, 0.56, 0.69
15.	3-Me_2NϕOC(O)NHMe		0.82	0.00, 0.38, 0.53

[a]Abbreviations: Me = Methyl; Et = ethyl; Pr = propyl; Bu = butyl; ϕ = phenyl.

single crystals with a microscope as they were heated between microscope cover slips on a hot block. All other methods and procedures were the same as previously described (*1*, *2*).

Results

Photodecomposition Products of Zectran and Matacil. Exposure to short-wavelength ultraviolet light on the chromatoplates yields at least 12 degradation products from Zectran-carbonyl-C^{14} and at least 11 degradation products from Matacil-carbonyl-C^{14}. Figure 2 shows the percentage of the initial radioactivity spotted on the plates which is recovered as individual degradation products after two different exposure periods to ultraviolet light. In each case, four of the major degradation products are tentatively identified, by cochromatography with pure nonlabeled compounds, as the methylamino, amino, methylformamido, and formamido derivatives. These same major products are obtained from Zectran and Matacil when the carbonyl-C^{14} compounds are exposed to sunlight on bean foliage (*1*, *2*). The additional unidentified products are probably carbamates, because they are formed from carbonyl-C^{14} compounds. In the case of Zectran, the minor degradation products are the same in number and chromatographic position when formed either on plant foliage or on TLC plates. The rate of photodecomposition and nature of the products formed on bean foliage are shown in Figure 3 for Zectran-carbonyl-C^{14} and Matacil-carbonyl-C^{14} (*1*, *2*). The amounts of the various carbonyl-C^{14}–labeled degradation products are definitely less from Matacil than from Zectran (Figures 2 and 3).

On exposure of Zectran-*N*-methyl-C^{14} to either sunlight on the plants or ultraviolet light on TLC plates, labeled degradation products with R_f values of 0.00, 0.10, 0.25, 0.39, 0.54, and 0.63 are formed. An additional product at R_f 0.83 is detected after exposure on the plates but not on the plants, and this material chromatographs in the position of 4-dimethylamino-3,5-xylenol. The radiocarbon of the *N*-methyl group is absent from the products with R_f values of 0.32 and 0.69 because these products are detected with the carbonyl- but not with the *N*-methyl–labeled sample. Additional materials which are detected with the carbonyl- but not with the *N*-methyl–labeled sample are those with R_f values of 0.05, 0.14, 0.20, and 0.47. The failure to detect these products with Zectran-*N*-methyl-C^{14} probably results either from the absence of detectable amounts of these products (owing to the lower specific activity of this sample) or because the labeled carbon atoms are removed from the molecule on forming these products.

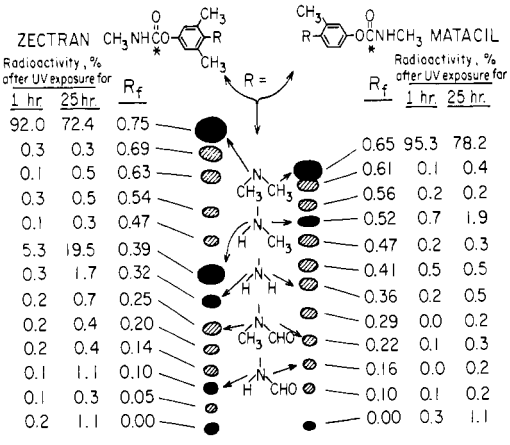


Figure 2. Photodecomposition products of Zectran-carbonyl-C^{14} and Matacil-carbonyl-C^{14} resulting from exposure on TLC plates to short-wavelength ultraviolet light

Products present in amounts exceeding 1% shown as solid spots

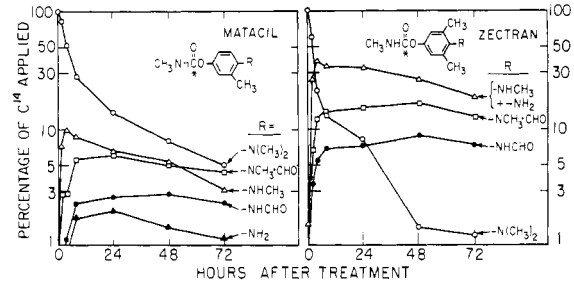


Figure 3. Photodecomposition products of Zectran-carbonyl-C^{14} and Matacil-carbonyl-C^{14} resulting from exposure to sunlight on bean foliage based on results by Abdel-Wahab, Kuhr, and Casida (2)

In addition to the above-mentioned Zectran derivatives, two or three very minor products, between the R_f 0.14 and 0.25 spots, appear under the following circumstances: exposure on either the plants or the TLC chromatoplates, and exposure of either the carbonyl-C^{14} or *N*-methyl-C^{14} samples. There is limited evidence for additional minor carbamate products of R_f 0.28 and 0.59. Nothing is known about the origin or nature of these very minor products.

The following compounds are not formed on photodecomposition of Zectran because the authentic, nonlabeled compounds fail to cochromatograph consistently with any of the labeled decomposition products: 4-dimethylamino-3,5-xylyl *N*-hydroxymethylcarbamate, R_f = 0.45–0.49; 4-hydroxy-3,5-xylyl methylcarbamate, R_f = 0.45–0.49; 4-nitro-3,5-xylyl methylcarbamate, R_f = 0.70; 3,5-xylyl methylcarbamate, R_f = 0.73.

Matacil-*N*-methyl-C^{14} gives a labeled photodegradation product which chromatographs in the position of the methylamino analog, but the data at hand are not adequate to define which of the minor products, if any, lack the radiocarbon from the *N*-methyl grouping. An additional product from the *N*-methyl-C^{14} sample, which is detected following exposure on the TLC plates but not on the plants, chromatographs in the position of 4-dimethylamino-3-cresol. The following nonlabeled methylcarbamates fail to cochromatograph consistently with any of the labeled Matacil decomposition products: 4-dimethylamino-3-cresyl *N*-hydroxymethylcarbamate, R_f = 0.45–0.49 and 4-nitro-3-cresyl methylcarbamate, R_f = 0.58.

Sequence of Photodecomposition Reactions of Zectran and Matacil. Studies of Zectran photodecomposition on plants and on TLC chromatoplates (by two different procedures) consistently show the following changes:

Zectran gives rise to the methylamino and methylformamido analogs, and each of these compounds serves as a precursor for the formamido derivative; the amino analog is formed from the methylamino compound; there is little, if any, conversion of the methylformamido to the methylamino, or of the formamido to the amino analogs. Derivatives having R_f values of 0.54 and 0.63 are formed from the dimethylamino and methylamino analogs, and both of these products contain radiocarbon when formed from Zectran-*N*-methyl-C^{14}; therefore, these two compounds arise directly from the methylamino derivative. The product with an R_f value of 0.69 does not contain radiocarbon when derived from Zectran-*N*-methyl-C^{14}; studies with both exposure on plants and TLC chromatoplates indicate, but do not necessarily establish, that it arises from the amino derivative. Three additional products, with R_f values of 0.05, 0.14, and 0.47, are probably formed from the amino derivative, but the studies with Zectran-*N*-methyl-C^{14} are not adequate to prove that the radiocarbon from both of the *N*-methyl groups is absent in these products.

Sequence studies with Matacil, involving exposure of the known, nonlabeled, Matacil derivatives on TLC chromatoplates to ultraviolet light and on plants to sunlight show the following changes: Matacil gives rise to the methylamino and methylformamido analogs; the methylamino derivative gives rise to the amino and formamido analogs; the amino derivative arises from the formamido derivative and the methylamino analog from the methylformamido analog on chromatoplates, but these conversions occur only in small amount, if at all, on plants. The methylamino analog gives rise to three unidentified materials with R_f values of 0.41, as formed on both the TLC chromatoplates and plant surfaces, of 0.61, as formed only on the chromatoplates, and of 0.20 as formed only on the plant. The amino derivative gives rise, on both the TLC chromatoplates and the plant, to an unidentified material with a R_f value of 0.10, while exposure on the chromatoplates also gives rise to products of R_f 0.51 and 0.56; each of these unidentified materials is detected by ninhydrin reagent.

Products that remain at the origin (R_f = 0.00) are formed on irradiation of each of the Zectran and Matacil samples (nonlabeled, carbonyl-C^{14}– and *N*-methyl-C^{14}–labeled) on TLC chromatoplates or on plants, and they also appear with each of the nonlabeled Zectran and Matacil derivatives when they are exposed to ultraviolet light on the chromatoplates. In addition, each major degradation product of Zectran-carbonyl-C^{14} yields a radioactive product remaining at the origin in the experiments where the TLC plates are successively exposed to ultraviolet light and TLC development. The identities of these products that remain at the origin are not known.

Although the evidence is not conclusive, it appears unlikely that 4-(*N*-methylol)aminophenyl derivatives are present as stable intermediates in the photodegradation of Zectran and Matacil. When a mixture of degradation products recovered from plants treated with Zectran-*N*-methyl-C^{14}, Zectran-*N*-methyl-H^3, or Matacil-*N*-methyl-C^{14} is analyzed for labeled formaldehyde released on the addition of acid none is found, but nonlabeled formaldehyde added to each radioactive hydrolysis mixture yields the formaldemethone derivative (*13*) which is not radioactive. In an attempt to obtain products comparable to those produced on photodecomposition of Zectran and Matacil, formaldehyde (4 mmoles, as a 37% aqueous solution) was reacted for 3 weeks at 25° C. with 0.5 mmole of each of the amino and methylamino derivatives of Zectran and Matacil, as a mixture with 2 mg. of sodium bicarbonate in 4 ml. of anhydrous methanol, to yield two to six methylcarbamate derivatives; however, the yield from each of the methylcarbamate reactants was low and it was not possible to separate the products on Florisil columns.

Degradation of Various Alkylaminophenyl Methylcarbamates Exposed to Short-Wavelength Ultraviolet Light on Chromatoplates. Each of the 15 alkylaminophenyl methylcarbamates listed in Table II is stable when exposed on TLC chromatoplates in the dark for 20 hours. However, a comparable exposure period to ultraviolet light yields decomposition products from each compound, including materials that remain at the origin. The decomposition products are generally more polar (of lower R_f value) than the parent compound, except one product derived from each of 4-(*N*-methyl, *N*-pentylamino)-3,5-xylyl methylcarbamate (compound 3) and 4-dimethylamino-2,6-xylyl methylcarbamate (compound 11). The degradation product at the origin is the only one formed from 4-dimethylamino-2-methyl-5-isopropylphenyl methylcarbamate (compound 13). Only the product at the origin and one additional degradation product are evident with each of 4-(*N*-propyl, *N*-butylamino)-3,5-xylyl methylcarbamate and 4-diisobutylamino-3,5-xylyl methylcarbamate (compounds 7 and 8, respectively). The chemical natures of the products are not known.

Biological Activity of Zectran, Matacil, and Their Degradation Products. On exposure of Zectran-carbonyl-C^{14} and Matacil-carbonyl-C^{14} deposits on bean foliage to sunlight, degradation reactions yield radiolabeled products, many of which are anticholinesterase agents; anticholinesterase agents are not detected in comparable extracts of untreated leaves. Each of the radioactive degradation products of Zectran, except for the minor materials of R_f 0.05 and 0.69, is detected by the anticholinesterase assay (*15*). The individual anticholinesterase products obtained from Zectran increase in amount between the 5- and 25-hour exposure samples, except for the one at R_f 0.63 which decreases slightly on longer exposure. The most active anticholinesterase agents, in the order of their decreasing potency, are as follows: Zectran, methylamino Zectran, the unknown product at R_f 0.63, methylformamido Zectran, formamido Zectran, the residual material at the origin, amino Zectran (which is detected by anticholinesterase assay after 5 but not after 25 hours of exposure), and other very minor anticholinesterase agents found at R_f 0.14, 0.20, 0.47, and 0.54. With Matacil-treated foliage, methylamino Matacil is the only degradation product detected by both anticholinesterase and radioautography assays.

Methylformamido- and amino Matacil, and an unidentified product with an R_f value between that of Matacil and methylamino Matacil, are detected by radioactive but not by anticholinesterase assays.

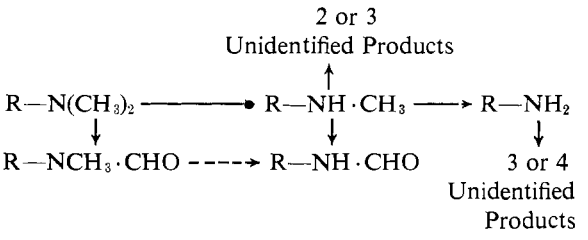
Within the series of Zectran and Matacil oxidation products, considerable specificity is evident from the biological assays (Table I). Compounds in the Zectran series are more toxic to houseflies than compounds in the Matacil series, and the order of decreasing toxicity within each series, which varies in the nature of the 4-substituents, is as follows when piperonyl butoxide is used as a synergist: dimethylamino > methylformamido > formamido > methylamino > amino. Zectran and its amino analog are equal in toxicity to the Mexican bean beetle, *Epilachna verivestis* Muls., and to the Southern armyworm, *Prodenia eridania* (Cram.) (*9*). In assays with acetylcholinesterase and pseudocholinesterase, the methylformamido and formamido compounds are less potent inhibitors than the dimethylamino, methylamino, and amino analogs, and compounds in the Zectran series are more active than compounds in the Matacil series. The analogous Zectran and Matacil derivatives are of almost equal toxicity on intraperitoneal administration to mice; the methylamino and amino analogs are more toxic than the dimethylamino analog, while the methylformamido and formamido compounds are of considerably lower toxicity in each case. The low mammalian toxicity of the methylformamido and formamido analogs, relative to that of the dimethylamino, methylamino, or amino analogs, is also evident in dermal toxicity studies.

Discussion

Zectran and Matacil are partially decomposed when held for several days as dilute solutions in acetone, carbon tetrachloride, chloroform, or methylene chloride, but they are stable when held for comparable periods in acetonitrile, benzene, 95% ethanol, *n*-hexane, toluene, and certain other organic solvents (*1*). Solvents and conditions used in the present photodecomposition study were selected to avoid or minimize any complications arising from storage instability of compounds in solution.

Zectran and Matacil do not readily decompose when they are exposed as spots on TLC chromatoplates in the dark, to fluorescent light, or to long-wavelength ultraviolet light (*1*, *2*). Considerable degradation occurs only on irradiation by short-wavelength ultraviolet light or sunlight. The principal photodegradation products are identified and they result from a series of oxidation reactions at the dimethylamino moiety without modifications of other portions of the molecule. Photooxidation of the dimethylamino group occurs at a greater rate and yields more persistent methylcarbamate products with Zectran than with Matacil. The rate of photooxidation for Zectran and Matacil analogs is influenced by the position of the alkylamino group in the ring, the nature of the *N*-alkyl substitutent, and other ring substituents.

The sequence for photodecomposition of Zectran and Matacil is as follows, where R refers to the Zectran and Matacil structures except for the moiety in the 4-position:



Photooxidation of Zectran and Matacil yields methylamino and amino analogs which are of relatively high toxicity to mammals. The methylformamido and formamido compounds are less toxic. Dimethylsulfoxide is useful as the solvent for intraperitioneal and dermal toxicity studies because of the relatively poor solubility characteristics of the methylformamido and formamido derivatives in other common solvents; this solvent possibly facilitates penetration and distribution of the toxicants to yield lower LD_{50} values—i.e., indicate greater toxicity—than would occur with a more conventional solvent for such studies. Not only are the methylamino and amino analogs potent anticholinesterase agents but certain of the unidentified carbamate degradation products of Zectran are also active as cholinesterase inhibitors.

Only the degradation products on the plant surface are considered in the present study. On penetration of Zectran and Matacil into plants, enzymatic oxidation mechanisms yield the same major methylcarbamate intermediates that are formed by photooxidation (*2*). The major products of potential importance as persisting residues, other than the original compounds applied, are the methylamino analogs. Methods of analysis for residues resulting from the use of Zectran and Matacil need to take into account the methylamino analogs and, possibly, certain other oxidation products. Zectran and Matacil, on the other hand, are unlikely to accumulate on or in plants with repeated applications because of the ease of enzymatic oxidation and photooxidation of the dimethylamino moiety.

Acknowledgment

Acknowledgment is made to the following for suggestions, assistance, and/or materials: H. Balba, R. J. Kuhr, and L. Lykken, Division of Entomology, University of California, Berkeley; T. R. Norton and coworkers, Dow Chemical Co., Midland, Mich., and W. W. Kaeding and C. T. Redemann, Dow Chemical Co., Walnut Creek, Calif.; C. A. Anderson, Chemagro Corp.; A. D. Moore and R. Miskus, Pacific Southwest Forest and Range Experiment Station, U.S. Forest Service, U.S.D.A., Berkeley, Calif.

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Received for review July 7, 1966. Accepted January 26, 1967. Study supported in part by grants from the following sources: Supplement 74 to the Cooperative Agreement between the U.S. Forest Service and the Regents of the University of California; Chemagro Corp., Kansas City, Mo.; the U.S. Public Health Service, National Institutes of Health (Grant No. GM-12248); the U.S. Atomic Energy Commission (Contract No. AT(11-1)-34, Project Agreement No. 113).