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Received for review October 18, 1988. Revised manuscript received March 24, 1989. Accepted April 14, 1989.

Photodecomposition of Metalaxyl in an Aqueous Solution

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UV irradiation of metalaxyl in aqueous solution resulted in 70% substrate transformation in 5 days, with rates of transformation affected by irradiation time, pH, and substrate concentration. Addition of 1% acetone accelerated photodecomposition, while riboflavin and methylene blue had no effect. After 5 days of irradiation of metalaxyl at pH 6.8, two products (A and B) were formed: A contained 3% and B 6% of the initial radioactivity. The two compounds were isolated by TLC and their structures identified by mass and NMR spectroscopy. Irradiation of A resulted in the formation of B. In each case photolysis caused a rearrangement of the *N*-acyl group to the 4-position on the aromatic ring.

Metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester] is the active ingredient of the fungicide Ridomil. Since its introduction in 1977, it has been widely used for the control of plant diseases caused by oomycetous fungi of the order Peronosporales. The primary mechanism of action of metalaxyl is an inhibition of RNA-polymerase activity of various oomycetous species. Research on mobility and metabolism of metalaxyl in soil and microbial degradation of the fungicide have been reviewed by Cohen and Coffey (1986).

In our previous studies we investigated the microbial transformation of metalaxyl by the fungus *Syncephalastrum racemosum* and found that the major transformation mechanism involves benzylic or aromatic ring hydroxylation of the fungicide (Zheng et al., 1989). There is little information available regarding UV degradation of metalaxyl. A project report of CIBA-GEIGY Corp. has documented that, after irradiation of an aqueous solution of metalaxyl for 7 days using artificial sunlight, 5% of the added chemical was converted to metalaxyl acid [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine] and 17% to unidentified polar compounds (Burkhard, 1979).

The purpose of our research was to examine the effect of UV irradiation on the transformation of metalaxyl, to isolate and identify the transformation products, and to elucidate the mechanisms of photolytic transformation reactions.

MATERIALS AND METHODS

Chemicals. Metalaxyl (technical grade) of 96.4% purity and [*ring*-U-^{14}C]metalaxyl with a specific activity of 39 μCi/mg was supplied by Agricultural Division, CIBA-GEIGY Corp., Greensboro, NC. Riboflavin (RF), methylene blue (MB), and benzophenone (BP) were purchased from Sigma Chemical Co. (St. Louis, MO). These chemicals were added as freshly prepared solutions in deionized water or dissolved directly into the metalaxyl solutions (200 mg/L for RF, 100 mg/L for MB and BP or 1% acetone) prior to irradiation.

UV Irradiation of Metalaxyl and Recovery of Samples. Ultraviolet irradiation was performed with a 30-W germicidal lamp (Angstrom 2537, General Electric). Open dishes (250 mL), each containing 200 mL of the reaction solution, were placed 30 cm beneath the UV light. The reaction solutions were irradiated for 1–5 days at 30 °C. The samples containing photosensitizer were irradiated for 4–8 h under the same conditions. Controls were placed in a dark chamber and incubated for the same period of time; no chemical changes were observed.

Because of volume changes due to evaporation, it was necessary to adjust each reaction solution to the original volume before extraction. Ethyl acetate was used as extractant. The reaction solution was shaken for 1 min with an equal volume of ethyl acetate in a separatory funnel. The efficiency of extraction exceeded 94% as indicated by radioactivity measurements of the aqueous and solvent phase. The distribution of radioactivity in the reaction mixture, in the ethyl acetate extract, and in the aqueous phase was determined by liquid scintillation spectrometry.

Experiments with Various Metalaxyl Concentrations and pH Values of the Reaction Solutions. Photodecomposition studies with various concentrations of metalaxyl (5, 10, 20, 30, 40, 50 mg/L) were carried out in a citrate–phosphate buffer (0.2 M Na_2HPO_4/0.1 M citric acid) at pH 6.8. To investigate the effect of pH on the photodecomposition of metalaxyl, 50 mg/L of the substrate was prepared in citrate–phosphate buffers of pH 2.8, 4.8, and 6.8 and in a Tris buffer of pH 8.8 (0.1 M Tris/0.1 M HCl). Following the first ethyl acetate extraction of the reaction solution (pH 4.8, 6.8, 8.8), the remaining aqueous phase was adjusted to pH 2.8 with 2 M HCl and reextracted with an equal volume of ethyl acetate. The two ethyl acetate extracts were pooled and evaporated to dryness on a

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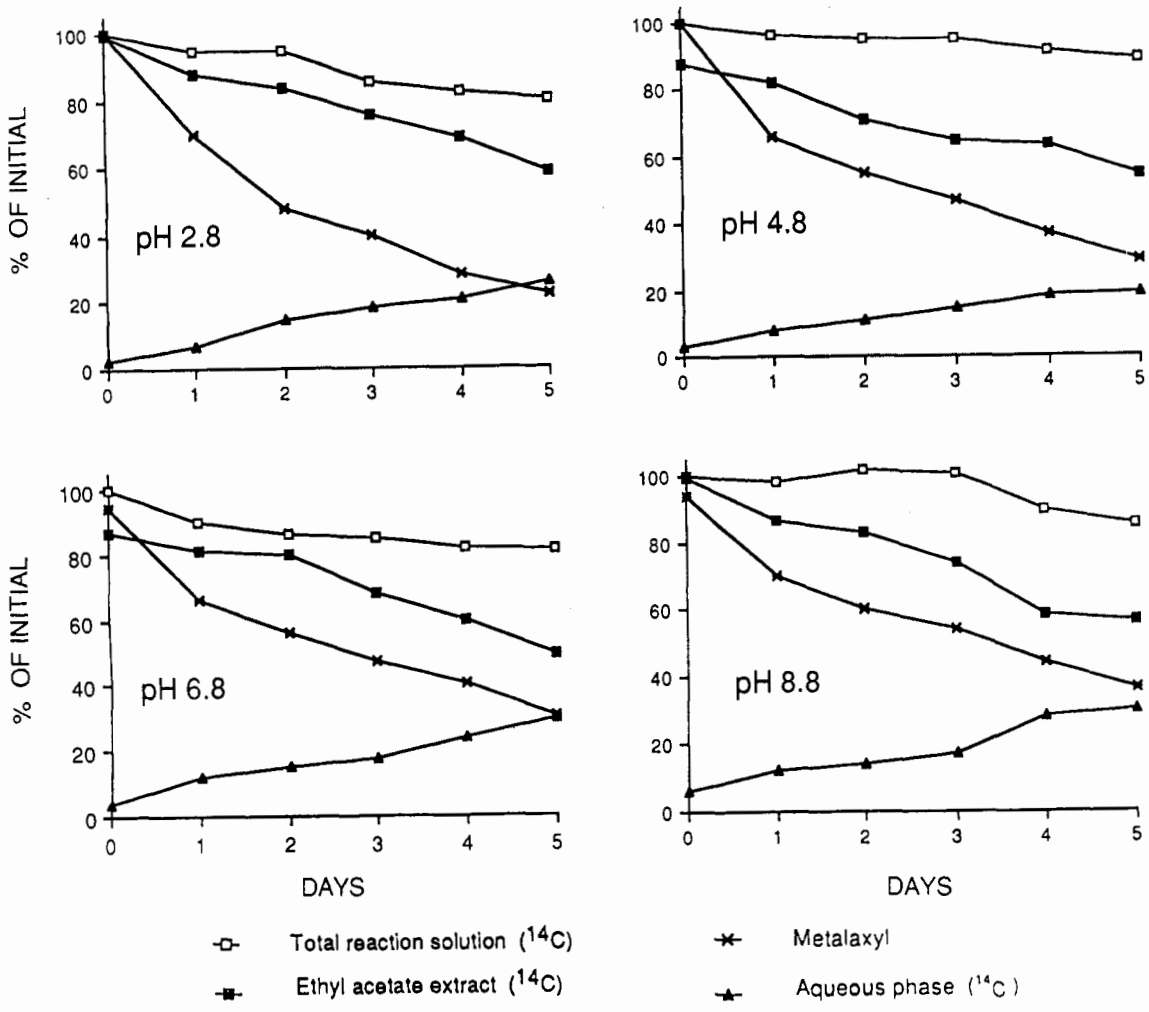

Figure 1. Photodegradation of metalaxyl (50 mg/L) at various pH values (percent of initial radioactivity or initial metolachlor concentration).

rotary evaporator. The residues were dissolved in ethyl acetate and adjusted to 10 mg/L for quantitative analysis.

Isolation and Quantitation of Products. In order to produce greater amounts of the photodecomposition products, the reaction solution (containing 50 mg/L metalaxyl and 1% acetone) was irradiated for 5 days. Products were isolated by thin-layer chromatography (TLC) on glass plates precoated with 0.25-mm silica gel 60F-254 (E. Merck, Darmstadt, West Germany). The plates were developed in solvent system A (benzene–acetone, 9:1, v/v) and the spots visualized under ultraviolet light. These spots were scraped from the thin-layer plates and extracted with ethyl acetate. Products isolated from solvent system A were further purified by TLC in solvent system B (hexane–ethyl acetate, 8:2, v/v) and solvent system C (benzene–acetone, 19:1, v/v). Products A and B after isolation by TLC were quantitatively measured by liquid scintillation spectrometry of radioactivity and gas chromatography (GC). Quantitative analyses by GC were performed by the external standard method. Triplicate samples were evaluated for each treatment.

Analytical Methods. Radioactivity was measured with a Beta Trac 6895 liquid scintillation counter (Tracor Analytic, Elk Grove Village, IL). Samples were prepared in a ScintiVerse II Universal LSC cocktail (Fisher Scientific Co., Fair Lawn, NJ).

Gas chromatography (GC) was performed on a Hewlett-Packard Model 5890A instrument equipped with a flame ionization detector (FID) and a Hewlett-Packard 3390A integrator. A capillary column (RTx-5, 30 m × 0.32 mm (i.d.), fused silica tubing cross-bonded with 95% dimethylpolysiloxane–5% diphenylpolysiloxane) was purchased from Restek Corp., Bellefonte, PA. Samples were injected in the split mode with a ratio of 80:7. Helium at a constant pressure (48 psi) was used as carrier gas. The injector and detector (FID) temperatures were maintained at 250 and 275 °C, respectively. Oven temperature was set at 220 °C for determination of metalaxyl concentration and programmed from 100 to 220 °C at a rate of 8 °C/min for determination of photodecomposition products of metalaxyl.

Electron-impact mass spectra were obtained on a Kratos MS 9/50 double-focusing mass spectrometer at an ionization potential of 70 eV using a direct-insertion probe or a gas chromatograph–mass spectrometer (Finnigan 3200). Proton NMR spectra were recorded on a Bruker WM 360-MHz spectrometer with deuteriochloroform as the solvent.

RESULTS

Photolysis of Metalaxyl due to UV Irradiation. The effect of pH on the disappearance of metalaxyl (50 mg/L) and distribution of radioactivity in the aqueous and organic phase (ethyl acetate) and reaction solution is presented in Figure 1. The results demonstrate that metalaxyl concentration and radioactivity in the ethyl acetate extract gradually decreased while radioactivity in the aqueous phase increased. The rate of metalaxyl transformation was affected by the initial pH of the reaction solution, with photolysis of metalaxyl occurring more rapidly at pH 2.8. There was no obvious difference in substrate disappearance between pH 4.8 and 6.8, but at pH 8.8 metalaxyl was transformed somewhat more slowly. After 5 days of irradiation, recoveries of metalaxyl were 22, 29, 30, and 36% of that added initially, with half-lives of 2.62, 2.93, 2.98, and 3.40 days, at pH 2.8, 4.8, 6.8, and 8.8, respectively. About 88% of the total radioactivity was recovered in the reaction solution after 5 days

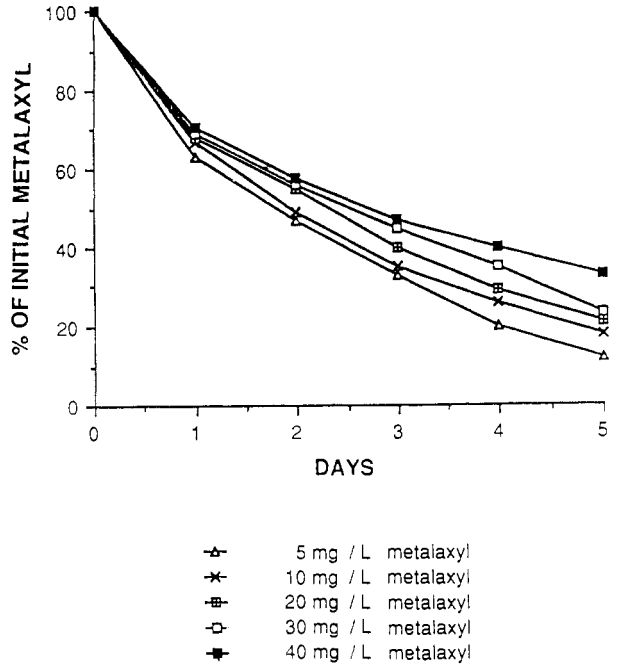

Figure 2. Photodegradation of various concentrations of metalaxyl at pH 6.8.

of irradiation at pH 6.8; 56% of this amount was extractable into ethyl acetate and 30% remained in the aqueous phase. About 30% of ^{14}C in the ethyl acetate fraction was identified as metalaxyl. The loss of radioactivity from the reaction solution as a result of volatilization accounted for 12% of the added chemical.

The effect of various substrate concentrations on the photodegradation of metalaxyl was studied at pH 6.8. The results in Figure 2 show that relatively more metalaxyl is photolyzed at lower concentrations. After 5 days of irradiation, recoveries of metalaxyl were 12, 18, 21, 23, and 33% of that added initially with half-lives of 2.3, 2.5, 2.7, 2.9, and 3.3 days, at initial metalaxyl concentrations of 5, 10, 20, 30, and 40 mg/L, respectively. This result is consistent with a constant photon flux photochemical process.

The photolysis of metalaxyl was investigated in the presence of photosensitizers. Results indicate that irradiation of the reaction solution of metalaxyl (10 mg/L) for 48 h in the presence of 1% acetone at pH 6.8 resulted in 67% decomposition of metalaxyl (half-life 30 h). Irradiation of the reaction solution lacking acetone for the same time period resulted in only 47% decomposition of metalaxyl (half-life 61 h). Addition of riboflavin, methylene blue, and benzophenone had no significant sensitizing effect (data not shown).

GC analysis of the ethyl acetate extract showed the presence of three major peaks: The peaks appearing at a retention time of 18 and 15 min were designated products A and B, respectively. A peak appearing at 9 min did not contain radioactivity and therefore was not further investigated.

The yields of product B were found to be similar at pH 2.8, 4.8, and 6.8 while the quantity of product A formed was greater at pH 4.8 and 6.8 than at pH 2.8. Products A and B were formed in the least amount at pH 8.8 (Figure 3). At initial metalaxyl concentrations of 20 and 40 mg/L after 5 days of irradiation, the formation of product B was found to be greater than that of product A.

Isolation and Identification of Products. The concentrated ethyl acetate extracts from samples exposed to UV light for 5 days were analyzed by TLC using sol-

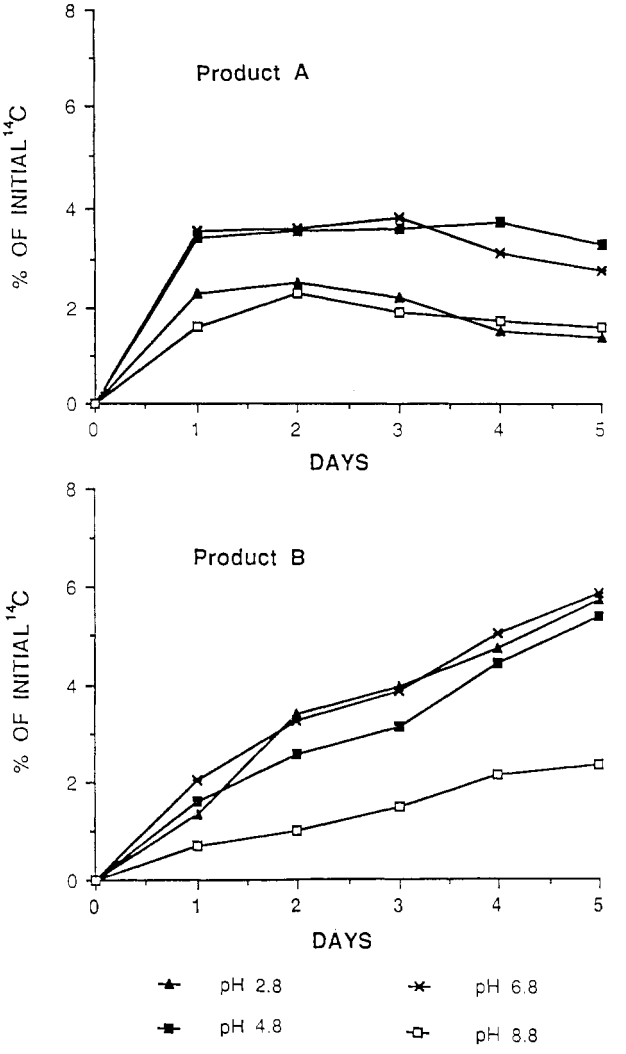

Figure 3. Formation of products A and B after exposure of metalaxyl to UV irradiation at various pH values.

vent system A (benzene–acetone, 9:1, v/v). As shown in Table I, 11 spots were detected under ultraviolet light. They were scraped and extracted with ethyl acetate for determination of radioactivity. About 86% of the radioactivity applied to a thin-layer plate was recovered in the ethyl acetate extract, of which 44% was determined to be metalaxyl, 5.2% to be product A, and 8.1% to be product B. Samples recovered from solvent system A with R_f 0, 0.13, 0.39, and 0.85 were rechromatographed in solvent systems B and C. GC analysis of each sample isolated from the most intensive thin-layer spots showed several peaks. Therefore, no further attempt was made to characterize these products.

Product A was purified first in solvent system B (R_f 0.1) and then redeveloped in solvent system C (R_f 0.47). The purified product gave a single peak upon GC analysis at a retention time of 18 min. Product B was purified by TLC in solvent system B (R_f 0.19). The purified product gave a single peak upon GC analysis with a retention time of 15 min.

Mass spectral analysis revealed molecular weights of 279 and 249 for products A and B. High-resolution accurate mass measurements gave a molecular composition of $C_{15}H_{21}NO_4$ for A, which is identical with that of metalaxyl. The molecular composition of B was measured as $C_{14}H_{19}NO_3$, differing from metalaxyl and A by the equivalent of CH_2O.

The NMR chemical shift and coupling information for metalaxyl and photoproducts A and B are given in Fig-

Table I. Distribution of [^{14}C]Metalaxyl (50 mg/L) and Its Products after TLC Analysis[a]

			metalaxyl		product A		product B				
R_f	0	0.13	0.33	0.39	0.46	0.54	0.67	0.74	0.81	0.85	0.95
^{14}C, dpm	3323	10601	28740	1565	3394	995	5347	390	501	1718	137
% ^{14}C appl on TLC	5.1	16.1	43.7	2.4	5.2	1.5	8.1	0.6	0.8	2.6	0.2

[a] Radioactivity applied to the TLC plate, 65 723 dpm. Total ^{14}C recovered from the TLC plate, 86%.

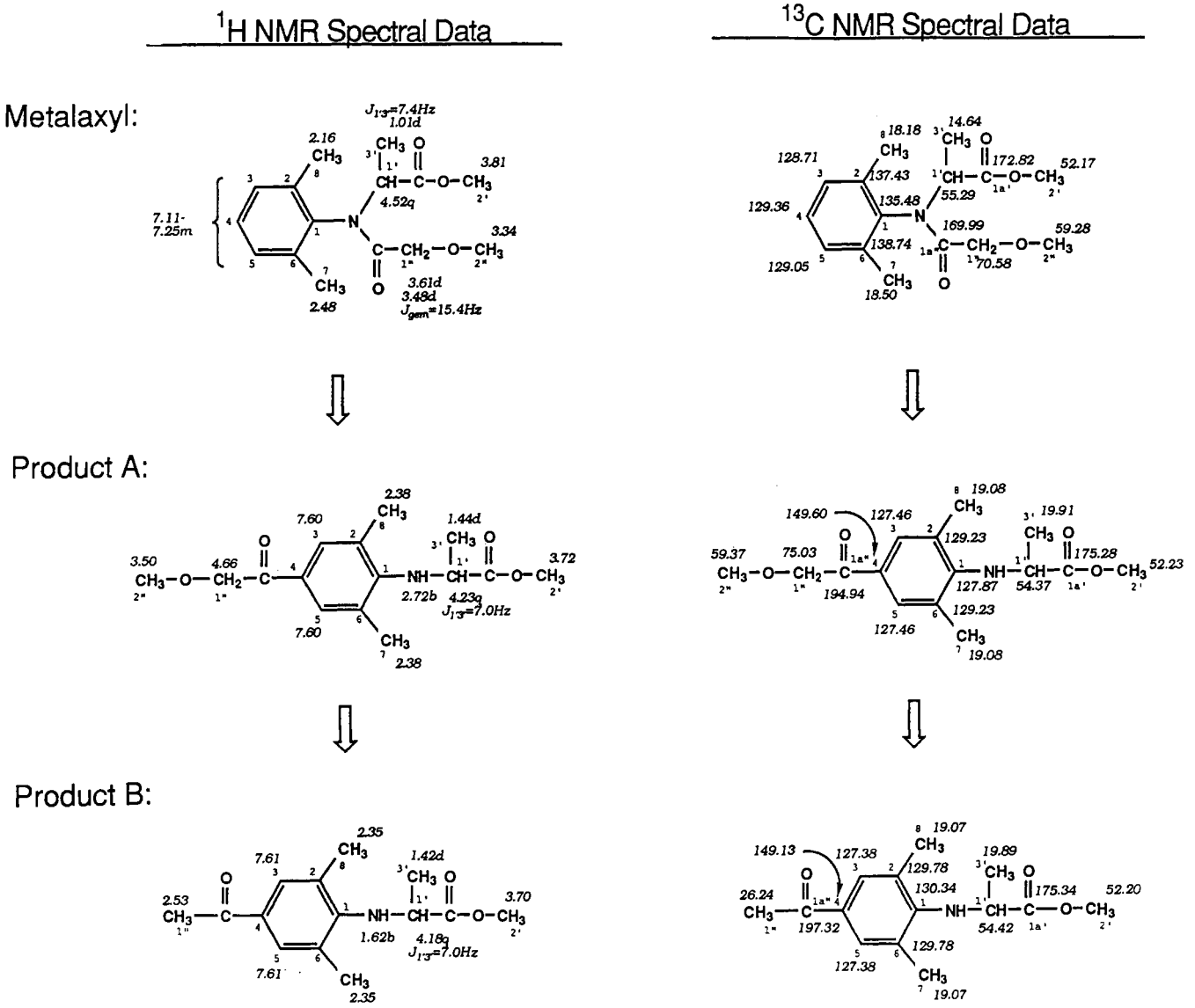

Figure 4. Structures and proton NMR spectral data for metalaxyl, product A, and product B.

ure 4, and the values derived from NMR nuclear Overhauser effect studies on products A and B are given in Table II. The presence of the alanine moiety in all three molecules is evidenced by a methyl doublet sharing a 7-Hz coupling with a methine quartet (C1′–C3′) and a methyl ester methoxyl appearing between δ 3.81 and 3.70. However, products A and B show a broad N–H resonance not found in metalaxyl, implying that nitrogen in A and B is attached to two, not three, carbon substituents. Aryl methyl groups in both products are equivalent and resonate at δ 2.35–2.38, whereas in metalaxyl they are inequivalent and appear at δ 2.48 (7-methyl) and 2.16 (8-methyl) because of hindered rotation. The aromatic hydrogens of metalaxyl appear as a multiplet integrating for three protons, but both products A and B show a singlet at δ 7.60 or 7.61, respectively, which integrates for two protons. This implies a symmetrical substitution pattern about the ring. Thus, it appears that the α-methoxyacetyl has been cleaved from the nitrogen and that the aromatic ring has undergone substitution in the 4-position in both A and B.

The remaining peaks in the NMR of product A consisted of a three-proton singlet (δ 3.50) in the methoxy chemical shift region and a two-proton singlet at δ 4.66. These facts, combined with the mass spectral composition data discussed previously, led us to postulate the structure shown in Figure 4 for product A. To substantiate this, nuclear Overhauser effect experiments were performed (see Table II) (Noggle and Schirmer, 1971). Reciprocal enhancements between 3,5-H and 7,8-CH_3 confirmed the suspected ring substitution to be 1,4. Mutual nuclear Overhauser effects between the 1′-H and 3′-CH_3 on the one hand and the benzylic methyl groups 7-CH_3 and 8-CH_3 on the other hand establish their relationship to be the same as in the case of metalaxyl. Finally, 2″-methoxyl exhibits strong interactions with 1″-CH_2,

Table II. Nuclear Overhauser Effect (NOE) for Products A and B

product A	product B
$H_{3,5}$–$CH_{2(1'')}$ = 25.0%	$CH_{3(1'')}$–$H_{3,5}$ = 63.8%
$H_{3,5}$–$CH_{3(7,8)}$ = 41.7%	$CH_{3(7,8)}$–$H_{3,5}$ = 87.2%
$CH_{2(1'')}$–$H_{3,5}$ = 49.2%	$CH_{3(3')}$–$CH_{3(7,8)}$ = 61.7%
$H_{1'}$–$CH_{3(7,8)}$ = 17.5%	
$H_{1'}$–$CH_{3(3')}$ = 6.7%	
$CH_{3(2'')}$–$CH_{2(1'')}$ = 12.5%	
$CH_{3(7,8)}$–$H_{3,5}$ = 53.5%	
$CH_{3(7,8)}$–$H_{1'}$ = 28.3%	
$CH_{3(7,8)}$–$CH_{3(3')}$ = 13.3%	
$CH_{3(3')}$–$CH_{3(7,8)}$ = 16.6%	
$CH_{3(3')}$–$H_{1'}$ = 15.8%	

which in turn is affected by irradiation of aryl hydrogens H3 and H5.

Further analysis of the mass spectrum of A is consistent with this structure. It has many similarities to the mass spectrum of metalaxyl. Although both indicate facile losses of the methoxy methyl grouping (m/z 234), the fact that this ion is the most intense in the spectrum of A implies greater ion stability. This is exactly what one would predict for the substituted benzoyl ion that is formed from A. Conversely, this stability leads to minor cleavage between the aromatic ring and the carbonyl group (m/z 206), but for metalaxyl, methoxyacetyl cleavage from nitrogen occurs readily.

The NMR spectrum of product B differs from the NMR spectrum of A in two significant ways: the disappearance of the two-proton singlet corresponding to 1'' and the dramatic upfield shift of the three-proton singlet from δ 3.50 to 2.53. Taken together, these data show an acetyl side chain at aromatic C4, yielding the structure shown in Figure 4. The mass spectral data are consistent with this and show a significant loss of a methyl group (m/z 234), due to the formation of the stable substituted benzoyl group as in A. The absence of the easily cleaved methoxymethyl group in B allows the slightly less facile α-elimination of the carbomethoxy group to predominate (m/z 190). Nuclear Overhauser effect studies (Table II) confirm this structure by showing the proximity of 1''-methyl and aromatic hydrogens 3 and 5.

To provide further support to the proposed structures, the ^{13}C chemical shifts for metalaxyl as well as product A and B were obtained. Complete assignment of metalaxyl was secured through $^{13}C/^{1}H$ direct correlation and COLOC experiments (Kessler et al., 1984; Bax, 1983). Of particular interest is the noticeable downfield shift of the amidic carbonyl C1a'' in metalaxyl from δ 169.99 to approximately δ 196 in product A and B, which is characteristic of a benzylic ketone. Correspondingly, there was a downfield shift of C4 in product A and B to approximately δ 149. Additionally the spectrum of product B lacks the 2''-methoxy resonance at δ 59 and the 1''-methylene group at δ 75, which is replaced by the 1''-methyl resonance at δ 26. Thus, the proposed structures for product A and B are clearly supported by these observations.

DISCUSSION

Photodecomposition plays an important role in the fate of pesticides in the environment. In recent years considerable interest has been generated concerning the effects of photolysis on pesticide degradation. In particular, combinations of treatments have been found to be very useful in accelerating the degradation rate of certain pesticides. Kearney et al. (1982) found that, under laboratory conditions, UV–ozonation causes dechlorination and ring cleavage of a number of pesticides [2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,5,2',5'-tetrachlorobiphenyl (PCB), pentachlorophenol (PCP), 2,3,7,8-tetrachlorobenzo-p-dioxin (TCDD)]. It was shown that in field soils 2,4,5-T and PCP were degraded faster than TCDD and PCB when they were exposed to UV light for 1 h in the presence of oxygen. Kearney et al. (1982) proposed that this pretreatment process could make these compounds more biodegradable and thus enhance their disappearance from the soil.

An earlier study by Sharom and Edgington (1982) showed that metalaxyl was very stable in water at pH 3, 5, and 7, with more than 84% of the initial amount remaining in solution after 12 weeks. The fungicide was rapidly degraded in water of pH 10, with less than 5% of the initial amount remaining after the same time period. In the present study, however, exposure of metalaxyl to UV light in aqueous solutions of pH 2.8–8.8 resulted in more efficient photodecomposition of metalaxyl at lower pH values. The fungicide was most slowly degraded at pH 8.8. The amount of product A reached its maximum value after 1–2 days of irradiation at pH 6.8, and thereafter no further accumulation was observed (Figure 3). Based on GC analysis, it was determined that product A was further decomposed to product B upon UV irradiation at pH 6.8. UV irradiation of product B for 5 days elicited no change at pH 6.8 but caused decomposition at pH 2.8, as indicated by the appearance of several new peaks with GC analysis. However, since metalaxyl is degraded more rapidly at the lower pH (Figure 1), the amount of product B accumulated at pH 2.8 was comparable to that formed at pH 4.8 or 6.8, despite this subsequent decomposition.

Addition of 1% acetone as a photosensitizer to a reaction solution containing 10 mg/L of metalaxyl led to a more rapid photodecomposition reaction. GC analysis showed that the presence of acetone did not change the identity or relative amounts of products formed in the ethyl acetate extract.

After extraction of the reaction solution with ethyl acetate, approximately 30% of the radioactivity remained in the aqueous phase. The identity of these products, however, was not determined.

Photochemical rearrangements of anilides similar to those discovered here for metalaxyl were first reported by Elad et al. (1965). Acetanilide, for example, yields 20% 2-aminoacetophenone and 25% 4-aminoacetophenone after mercury lamp irradiation for 3 days. Since the ortho positions of metalaxyl are substituted with methyl groups, acyl migration to these positions on the aromatic ring is blocked and the only product thus observed is the one in which the acyl group from nitrogen migrates to the 4-position. Although this migration likely involves the formation of a free-radical pair, the details of the photoanilide rearrangement are unclear. Thus, further research is needed to elucidate the mechanism of the observed UV-induced photodecomposition of metalaxyl.

Registry No. A, 122623-84-1; B, 122623-85-2; RF, 83-88-5; MB, 61-73-4; BP, 119-61-9; metalaxyl, 57837-19-1; acetone, 67-64-1.

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Received for review December 8, 1988. Accepted June 2, 1989.

Simultaneous Determination of Tri-*n*-butyltin, Di-*n*-butyltin, and Triphenyltin Compounds in Marine Products

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A method for simultaneous determination of tri-*n*-butyltin (Bu_3Sn^+), di-*n*-butyltin (Bu_2Sn^{2+}), and triphenyltin species (Ph_3Sn^+) in marine products is described. The sample was homogenized with methanol, mixed with sodium chloride and hydrochloric acid, and then extracted with a mixture of ethyl ether and *n*-hexane (60:40). After fish fat was removed on a Florisil column, organotin compounds were alkylated with ethylmagnesium bromide. Extracted tetrasubstituted tin compounds were further cleaned by passage through a Sep-Pak Florisil cartridge prior to determination by gas–liquid chromatography with a flame photometric detector (detection limit: 0.2 ng for each organotin compound). Analysis of fish samples, purchased in retail stores, showed accumulations of organotin compounds, suggesting that marine pollution with these tin compounds, especially Ph_3Sn^+, is prevalent over Japan. It was also found that Bu_3Sn^+ is metabolized to Bu_2Sn^{2+} and its hydroxylated product at an alkyl side chain in fish liver.

Organotin compounds are used as stabilizers of polyvinyl chloride, catalysts, pesticides, and marine antifoulants (WHO, 1980). The biocidal properties of tri-*n*-butyltin species (Bu_3Sn^+) and triphenyltin species (Ph_3Sn^+) make them useful for the control of marine organisms such as barnacle and seaweeds, and these compounds are included in ship paints and antifoulants used in Japanese marine farms. Recently, however, pollution of the aquatic environment and marine products by these chemicals has become a major public concern.

We previously reported an analytical method for determination of Bu_3Sn^+ and di-*n*-butyltin species (Bu_2Sn^{2+}) in fish (Sasaki et al., 1988), which employs a combination of tetraalkylation with a Grignard reagent (Meinema et al., 1978) and gas–liquid chromatography with flame photometric detection (FPD–GC). In this report a concise, simultaneous analytical method for Bu_3Sn^+, Bu_2Sn^{2+}, and Ph_3Sn^+ determination, using the same principle but a different kind of cleanup method, is described. This was then applied to a survey of pollution levels in marine products.

For brevity, each of the organotin species is referred to in the paper as if it existed only in cationic form, but this formalism is not meant to imply exact identities for these species in marine products.

MATERIALS AND METHODS

Reagents. Tri-*n*-butyltin chloride, di-*n*-butyltin dichloride (>97%), triphenyltin chloride (98%), and diphenyltin dichloride (96%) were purchased from Sankyo Organic Chemicals Co., Ltd. (Tokyo), Wako Pure Chemical Industries, Ltd. (Tokyo), Tokyo Kasei Kogyo Co., Ltd. (Tokyo), and Aldrich Chemical Co. (Milwaukee, WI), respectively. Ethylmagnesium bromide [3 M in ethyl ether (Et_2O)] was purchased from Tokyo Kasei Kogyo Co., Ltd. Butyl(3-hydroxybutyl)tin dichloride and butyl(4-hydroxybutyl)tin dichloride were synthesized by the method described in the previous paper (Fish et al., 1976; Ishizaka et al., 1989). Morin reagent was purchased from Nakarai Chemicals Ltd. (Kyoto). All chemicals were analytical reagent grade, and organic solvents were HPLC grade or distilled in glass before use. Double-distilled water was used throughout. Silica gel (Wakogel C-100, Wako) was activated for ca. 4 h at 120 °C after addition of half its volume (v/w) of HCl (36%) and overnight equilibrium (Hattori et al., 1984). Florisil PR (Floridin Co., Handcock, WV) was used without further modification. Sep-Pak Florisil cartridges (Waters Associates Inc., Milford, MA) were used in conjunction with a 5-mL glass syringe.

Organotin standard solutions were prepared in ethanol solution. Working standard mixture solution was made by combining each stock solution and diluting it with *n*-hexane to 5 μg/mL for each organotin compound.

Gas–Liquid Chromatography. A GC-9A gas chromatograph (Shimadzu Co. Ltd., Kyoto), equipped with a flame photometric detector (FPD), was operated in the tin mode (filter for 610 nm) with a fused silica capillary column CBP 10 (Shimadzu; equivalent to OV-1701; 0.53 mm (i.d.) × 12 m). Operating temperatures: column oven, programmed from 130 °C (hold 4 min) at the rate of 20 °C/min to 240 °C (hold 5 min); injection port, 240 °C; detector, 300 °C. Gas flow rates: He carrier gas, 20 mL/min; H_2, 150 mL/min; air, 100 mL/min. A Shimadzu C-R2AX was used for data collection, and the concen-

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