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PHOTOLYTIC AND MICROBIAL DEGRADATION OF 3,5,6-TRICHLORO-2-PYRIDINOL

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Abstract—The photolytic and microbial degradation of 3,5,6-trichloro-2-pyridinol (TCP), a metabolite of the insecticide chlorpyrifos and the herbicide triclopyr, has been investigated. TCP (80 mg/L) was decomposed to nondetectable levels within 2 h of ultraviolet irradiation. Release of $^{14}CO_2$ and chloride ion was observed; however, approximately 60% of the initial radioactivity remained in the solution. Dichlorodihydroxypyridine isomers and reductive dechlorination products were found as intermediate products of TCP photolysis. It was proposed that both hydrolytic and reductive dechlorination occurred during photodegradation of TCP. Although TCP can be degraded via photolysis, many degradation products remained in the aqueous medium and only some of them, the reductive dechlorination products, can be mineralized by *Pseudomonas* sp. ATCC 700113. The degradation of the parent compound, TCP, by *Pseudomonas* sp. ATCC 700113 also appears to involve a reductive dechlorination mechanism. A consortium of microorganisms may be needed if a treatment process combining microbial and photolytic activity is to be used to remove TCP in industrial wastewater.

Keywords—3,5,6-trichloro-2-pyridinol Photodegradation Microbial degradation Chlorpyrifos Triclopyr

INTRODUCTION

3,5,6-Trichloro-2-pyridinol (TCP) is a major metabolite of the insecticide chlorpyrifos and the herbicide triclopyr. Like chlorpyrifos and triclopyr, TCP can be decomposed photolytically. The photodecomposition of TCP has been investigated by several researchers. Smith [1] reported that TCP undergoes photodehalogenation and liberates CO_2. Although the author did not identify any intermediate products, the formation of diols, triols, and tetraol was suggested. Oxamic acid, oxalic acid, 2-chlorosuccinic acid, maleamic acid, succinamic acid, and *E*- and *Z*-2-chlorobut-2-enedioic acid were identified as intermediates of TCP photodegradation [2]. These studies, however, have not established the complete pathway of TCP photodegradation; many of the photodegradation products remain uncharacterized.

Biodegradation of halogenated homocyclic aromatic compounds have been studied extensively [3–5], but little information is available about biodegradation of their heterocyclic analogs. Metabolism of TCP by microorganisms has never been thoroughly investigated, but a few reports are available regarding the bacterial transformation of other chlorinated pyridines. Hughes [6] first studied the oxidation of some fluoro- and chloro-substituted nicotinic acids, tentatively identifying 5-chloro-6-hydroxynicotinic acid as an intermediate in the oxidation of 5-chloronicotinic acid by *Pseudomonas fluorescens*. However, none of the halogenated nicotinic acids could serve as a substrate for cell growth. Behrman and Stanier [7] studied the oxidation of 5-chloro- and 5-fluoronicotinic acids, and results obtained for 5-chloronicotinic acid agreed with those of Hughes [6]. 5-Fluoro-6-hydroxynicotinic acid was detected as the product of 5-fluoronicotinic acid. Tibbles et al. [8] isolated a *Mycobacterium* sp. that can use 5-chloro-2-hydroxynicotinic acid as a sole source of carbon and energy. 5-Chloro-2,6-dihydroxynicotinic acid was isolated and identified as a metabolite produced by *Mycobacterium* sp. strain BA from 5-chloro-2-hydroxynicotinic acid. Chloromaleic and chlorofumaric acid were suggested as products after cleavage of the pyridine ring.

Photodegradation products of pesticides have been shown to be identical to some metabolites of living organisms. For example, the major photodegradation products of the herbicide monuron have been isolated as metabolites from plants, animals, and microorganisms [9]. Photolysis of paraquat produced an intermediate, 4-carboxy-1-methylpyridinium, which was also found to be a microbial degradation product [10]. TCP is a major product of chlorpyrifos in plant and animal metabolism as well as in photolysis and hydrolysis [2]. Thus, it is reasonable to assume that some of the photodegradation products of TCP can be similar to microbial degradation products. In addition, photodegradation products may serve as carbon sources for TCP-degrading bacteria.

The purpose of this research was to investigate the photodecomposition of TCP by ultraviolet (UV) light, to identify the photodegradation products, and to study the use of TCP photodegradation products by TCP-degrading bacteria. This information may be useful in designing treatment methods for decontaminating TCP-containing wastewater.

MATERIALS AND METHODS

Chemicals

TCP of 99.9% purity and [2,6-^{14}C]TCP were provided by DowElanco, Indianapolis, Indiana, USA. The ^{14}C-labeled TCP had a specific activity of 27.6 mCi/mmol with a radiochemical purity of >99%. Other chemicals used were of analytical grade; solvents used were of high-performance liquid chromatography (HPLC) grade.

Photodegradation of TCP

To determine TCP degradation and chloride formation, three glass petri dishes, each containing 15 ml of nonlabeled TCP (80 mg/L) in 50 mM phosphate buffer (pH 7), were placed

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47 cm (18.5 in) from a UV light source (GM germicidal lamp, 30 W, 254 nm, 0.5 W/sq ft at 1 ft) at room temperature. The reaction solutions were irradiated for 6 h. Samples were collected at various time intervals, and the concentrations of TCP and chloride ions were determined.

To determine changes of radioactivity during the photodegradation process, the reaction solutions containing 80 mg/L and 0.1 μCi [2,6-^{14}C]TCP were exposed to UV light for 10 h in petri dishes as described above. At various time intervals, samples were taken for determination of radioactivity and TCP concentrations in the reaction.

To determine $^{14}CO_2$ formation, a 250-ml Erlenmeyer® flask with a hanging glass vial inside was used; the flask was sealed with a rubber stopper. The glass vial contained 2 ml 0.1 N NaOH solution to trap $^{14}CO_2$. [2,6-^{14}C]TCP was added to yield a TCP concentration of 80 mg/L and a total radioactivity of 0.09 μCi in 30 ml of phosphate buffer (50 mM). Triplicate Erlenmeyer flasks containing reaction solutions were placed 14 cm below the UV light. Two aliquots of each reaction solution were taken at various time intervals. One aliquot (0.1 ml) was used to measure the TCP concentration by HPLC, and the other aliquot (0.5 ml) was used to determine the radioactivity. An aliquot of 0.5 ml was removed from the NaOH trap and analyzed for radioactivity.

Generation and extraction of photodegradation products

To further analyze products, triplex nonradioactive samples in petri dishes were combined after UV exposure, and the pH was adjusted to 1 with 6 N HCl. The aqueous sample was saturated with NaCl and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate solutions were dried over anhydrous sodium sulfate and concentrated under reduced pressure prior to analyses.

Isolation and characterization of major photodegradation products

Thin-layer chromatography (TLC) of ethyl acetate extracts was performed on glass plates coated with silica gel $60F_{254}$ (EM Science, Darmstadt, Germany) to a thickness of 0.25 mm. Unknown products with high radioactivity were further isolated and characterized. TLC plates were developed in solvent system A (benzene-acetone-acetic acid, 50:50:1), and compounds were visualized by observation under UV light and by spraying with 2% (w/v) ferric chloride ($FeCl_3 \cdot 6H_2O$) solution in ethanol. Iron (III) complexes with 2,3-pyridinediol and forms a blue to purple-red product, depending on the acidity [11]. The products were isolated by extracting TLC scrapings with acetone. Then they were further purified by TLC in solvent system B (hexane-acetone-acetic acid, 30:70:1) and extracted with acetone again. Purified products were subject to mass spectrometry and proton nuclear magnetic resonance (NMR) analyses.

Characterization of other photodegradation products

Residues of ethyl acetate extracts were redissolved in ethyl acetate and analyzed by gas chromatography–mass spectrometry (GC-MS). The same sample was dried under nitrogen flow, derivatized with bis(trimethylsilyl)trifluoroacetamide, and analyzed by GC-MS again.

Metabolism of photodegradation products by TCP-degrading bacteria

Pseudomonas sp. ATCC 700113, a bacterium that mineralizes TCP [12], was used to study metabolism of photodegradation products of TCP. Bacterial cells were grown in a mineral medium containing 100 mg TCP/L, 0.01% (w/v) yeast extract, and 0.018% (w/v) glucose, as described previously [13]. Cell cultures in the late exponential phase of growth were harvested by centrifugation, washed three times with 50 mM phosphate buffer (pH 7), and resuspended in 50-ml reaction solutions that were exposed to UV light for 2 h. At various time intervals, 5-ml samples were taken, and cells were separated from the liquid medium by centrifugation. The supernatants were extracted with equal volumes of ethyl acetate three times. The combined ethyl acetate extracts were then brought to dryness in a rotary evaporator under reduced pressure. Dry residues were redissolved in 50 μl ethyl acetate, and a 1-μl sample was analyzed by GC.

Analysis

Radioactivity was determined using a Beta Trac 6895 liquid scintillation counter (Tracor Analytic, Elk Grove Village, IL, USA). Ecoscint scintillation solution was used (National Diagnostic, Atlanta, GA, USA). Distribution of radioactivity on TLC plates was determined using a System 200 Imaging Scanner (Bioscan, Washington, DC, USA).

Concentrations of chloride were determined by coulometric titration. Samples (3 ml) of the reaction solution were mixed with 1 ml acid reagent (a mixture of 0.4 N nitric acid and 40% glacial acetic acid); chloride concentration was determined using a chloridometer (Buchler Instruments, Fort Lee, NJ, USA).

TCP concentrations in the samples were analyzed by an HPLC system, consisting of a Model 6000A pump (Waters Associates, Milford, MA, USA), a Rheodyne 7125 injector, a Lambda-Max 480 LC UV detector (Waters Associates) set at 254 nm, and a 3392A integrator (Hewlett-Packard, Palo Alto, CA, USA). A Waters 5 mm × 10 cm Nova Pak C_{18} column of 4-μm particle size was used. Compounds were separated using a mobile phase of methanol/water (1:1 or 3:7, v/v) with 0.068% (v/v) triethylamine buffered with 0.13% (w/v) KH_2PO_4 (pH 6.8) at a flow rate of 1.5 ml/min.

GC analyses were conducted on an HP 5890A gas chromatograph (Hewlett-Packard) with a flame ionization detector, using a 30 m × 0.32 mm inner diameter (i.d.) × 0.25 mm d_f Rtx-5 column (Restek, Bellefonte, PA, USA) programmed from 40 to 270°C at 6°C/min. Helium was used as the carrier gas. Temperatures of the injector and detector were 250°C, and samples (1 ml) were injected with a split ratio of 10:1.

GC-MS analyses were carried out on a Kratos MS-25 gas chromatograph-mass spectrometer with electron impact ionization (70 eV) and isobutane chemical ionization using a 60 m × 0.25 mm i.d. × 0.25 mm d_f DB-5 column (J & W Scientific, Folsom, CA, USA) programmed from 40 to 270°C at 6°C/min. Helium was used as a carrier gas. Electron-impact and high-resolution mass analyses were conducted on a Kratos MS-9/50 mass spectrometer. Proton NMR spectra were obtained on a Bruker WM 360-MHz spectrometer with deuterioacetone as the solvent.

RESULTS

Photolysis of TCP

If petri dishes were left open, TCP (80 mg/L) disappeared to nondetectable levels within 2 h upon exposure to UV light (Fig. 1). The color of the reaction solution changed from colorless to reddish brown. Radioactivity in the reaction solution decreased initially and then remained constant at about 60%

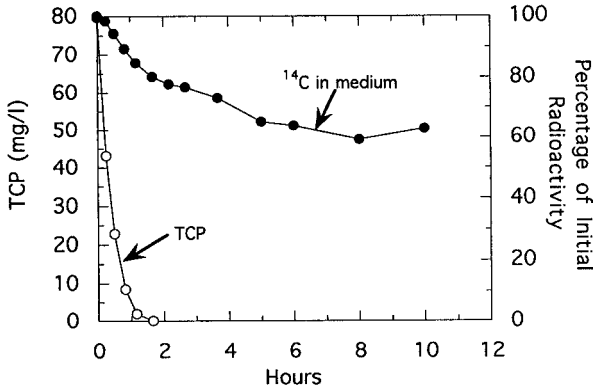

Fig. 1. Degradation of 3,5,6-trichloro-2-pyridinol upon exposure to ultraviolet light (254 nm) at room temperature.

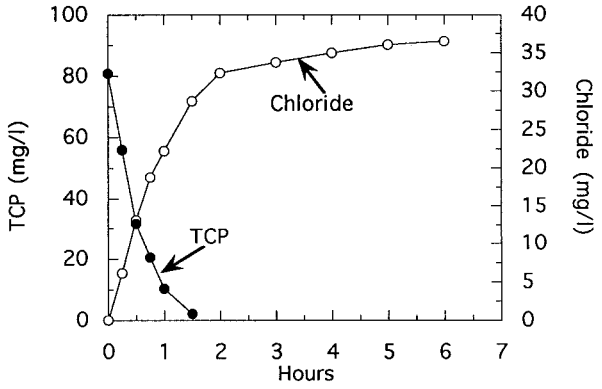

Fig. 3. Chloride release during photodegradation of 3,5,6-trichloro-2-pyridinol. After 6-h ultraviolet exposure, the amount of chloride released was 85.1% of the calculated stoichiometric amount.

of the initial value. Most of the radioactivity loss resulted from the release of $^{14}CO_2$ (Fig. 2). Whereas at the beginning of the experiment 95.6% of radioactivity contained in the aqueous phase could be extracted by ethyl acetate, only 83.2% of radioactivity in the aqueous phase was extractable after 2-h UV exposure. After 10-h UV exposure, only 53.1% of the aqueous radioactivity was found in the ethyl acetate extract. Extraction efficiency of the products could not be determined because standards were not available. The concentration of TCP in the control flask did not change.

Chloride was released during the photodegradation process (Fig. 3). Theoretically, complete dechlorination of 80 mg TCP per liter results in the release of 42.9 mg/L chloride. After 6-h exposure to UV light 36.5 mg/L of chloride was detected in the reaction solutions.

The study of $^{14}CO_2$ formation was carried out in sealed flasks instead of petri dishes. Pyrex® glass filtered out UV light below 290 nm, and the glass barrier reduced the intensity of UV exposure. Since the light source actually emitted broadband lights (≥254 nm) and TCP has a maximum UV absorbance at 320 nm in the region beyond 290 nm, TCP degradation was slower in sealed flasks than in petri dishes but was not inhibited. TCP was decomposed completely after 64 h. Approximately 25% of the initial radioactivity was recovered as $^{14}CO_2$, and 67% remained in solution (Fig. 2). When radioactivity in the NaOH trap was precipitated by the addition of $BaCl_2$, the amount of radioactivity remaining in the supernatant was negligible. The total radioactivity recovered was >92% of the initial radioactivity.

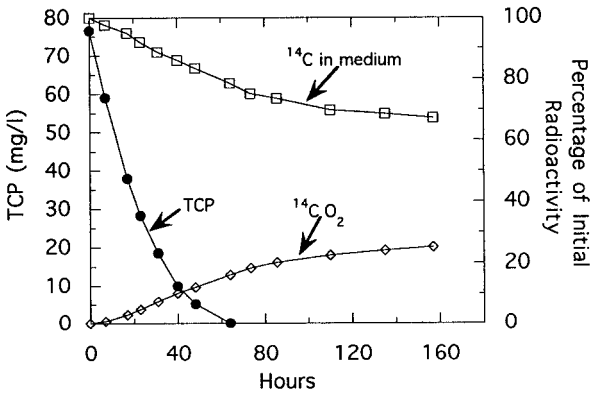

Fig. 2. Release of carbon dioxide during photodegradation of 3,5,6-trichloro-2-pyridinol.

Isolation and identification of major products of TCP photodegradation

Several products resulted from photodegradation of TCP. Product 1 of TCP (Fig. 4) photodegradation was isolated by TLC of the ethyl acetate extract. This product had an R_f value of 0.30 using benzene-acetone-acetic acid (50:50:1). The spot was dark purple under UV light (254 nm) and turned reddish

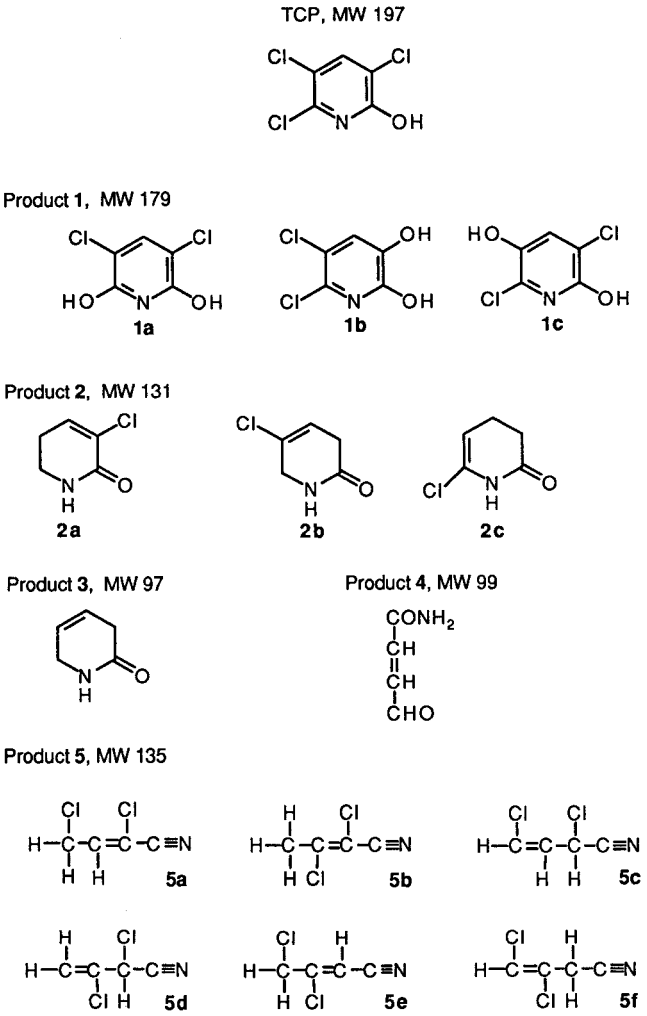

Fig. 4. Proposed structures of some photodegradation products of 3,5,6-trichloro-2-pyridinol.

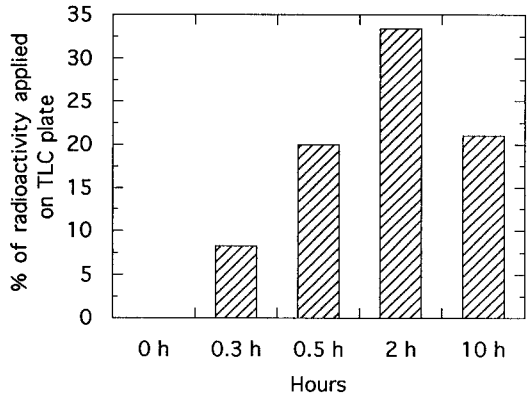

Fig. 5. Change of product 1 during exposure to ultraviolet light. The amount of product 1 was based on percentage of radioactivity associated with product 1 in radioactivity applied on a thin-layer chromatography plate.

brown when sprayed with ethanolic $FeCl_3$ solution. When ethyl acetate extracts of the reaction solution (exposed to UV light for 10 h) were applied to a TLC plate, the spot of product 1 contained 6.4% of the initial radioactivity and 21% of the radioactivity applied on the TLC plate. Figure 5 shows the change in percent radioactivity associated with product 1 over time of UV exposure.

Mass spectral analysis of product 1 revealed a molecular ion $[M^+]$ of 179 and fragment ions at mass-to-charge ratio (m/z) 161 $[M\text{-}18]^+$, m/z 135 $[M\text{-}44]^+$, m/z 133 $[M\text{-}46]^+$, m/z 107 $[M\text{-}72]^+$, and m/z 98 $[M\text{-}81]^+$. The molecular ion is consistent with the molecular formula $C_5H_3NO_2Cl_2$ (calculated mass 178.9541 and measured mass 178.9542), and the fragments are the results of losing H_2O, OH + HCN, CO + H_2O, H_2O + CO + CN, and CO + Cl + H_2O. Chemical ionization provided an MH^+ at m/z 180.

GC-MS analyses of both underivatized and silylated samples indicated the presence of isomers of product 1. GC-MS analysis of ethyl acetate extracts showed a single peak of product 1 with a GC retention time of 26.2 min with a molecular ion $[M^+]$ of m/z 179 and fragment ions identical to what was described above (Table 1). In the silylated sample, three peaks with GC retention times of 23.1, 25.3, and 27.4 min all had molecular weight of m/z 251 as indicated by chemical ionization. This molecular ion is consistent with the molecular formula $C_8H_{11}NO_2Cl_2Si$. These products were determined as the mono-trimethylsilyl (TMS) derivatives of dichlorodihydroxypyridines with one hydroxyl underivatized. Two peaks with GC retention times of 29.1 and 30.5 min gave molecular ions $[M^+]$ of m/z 323, which were confirmed by chemical ionization. This molecular ion is consistent with the molecular formula $C_{11}H_{19}NO_2Si_2Cl_2$. These products were determined to be TMS derivatives of dichlorodihydroxypyridines with two silyl groups attached. Therefore, product 1 was a mixture of the three possible dichlorodihydroxypyridine isomers: 1a, 1b, and 1c.

Proton NMR spectrum of TCP showed that the aromatic proton at the 4 position appeared as a singlet at 8.04 ppm. The ^{1}H-NMR spectrum of product 1 revealed a singlet at 6.82 ppm. The change of the chemical environment, possibly the replacement of a chlorine atom by a hydroxyl group at 3 or 5 position, resulted in the shifting of the peak upfield. Attempts to differentiate the three isomers of dichlorodihydroxypyridine by ^{13}C-NMR analysis were not conclusive due to lack of sufficient material.

TLC of product 3 (Fig. 4) with the solvent system benzene-acetone-acetic acid (50:50:1) revealed a spot containing 5.91% of the initial radioactivity (after 10-h UV exposure) and R_f value of 0.19. Mass spectral analysis revealed a molecular ion $[M^+]$ of m/z 97 and fragments at m/z 80 $[M\text{-}17]^+$, m/z 68 $[M\text{-}29]^+$, and m/z 52 $[M\text{-}45]^+$. Chemical ionization confirmed the molecular weight by providing an MH^+ at m/z 98. The molecular ion is consistent with the molecular formula C_5H_7NO, and the fragments are the result of losing OH, CHO, and CH_2CH_2OH groups. The compound was proposed as tetrahydro-2-pyridone. GC-MS analysis of the silylated sample revealed a peak with a GC retention time of 15.2 min with a molecular weight of 169. The molecular ion is consistent with the molecular formula $C_8H_{15}NOSi$, which corresponds to the silyl derivative of tetrahydro-2-pyridone.

GC-MS characterization of other photodegradation products

Several other photodegradation products of TCP were also detected. Characterization of the intermediates was achieved by GC-MS analysis, and the results are summarized in Table 1. Both underivatized sample and silylated sample were analyzed by GC-MS.

A peak in the underivatized sample with a GC retention time of 16.3 min gave a molecular ion $[M^+]$ of m/z 131 and fragments at m/z 103 $[M\text{-}28]^+$, m/z 88 $[M\text{-}43]^+$, and m/z 60

Table 1. Analysis of photodegradation products of 3,5,6-trichloro-2-pyridinol (TCP) by gas chromatography-mass spectrometry (GC-MS)

Product	Retention time (min)[a]	Molecular weight (amu)	Molecular weight of silyl derivative (amu)	Major fragments
5a–e	9.2, 10.4 11.5, 12.0	135	N/A	100 [M-Cl], 85 [M-CH3-Cl], 74 [M-CN-Cl], 64 [M-HCl-Cl], 49 [M-CNCClCH]
3	13.0	97	169	80 [M-OH], 68 [M-CHO], 52 [M-CH_2CH_2OH]
4	15.0	99	171	81 [M-H_2O], 55 [M-CON_2H], 54 [M-CH_2CH_2OH]
2	16.2	131	203	103 [M-CH_2CH_2], 88 [M-O=C-NH], 60 [M-(CO)-(O=C-NH)]
5f	16.3	135	N/A	108 [M-HCN], 100 [M-Cl], 85 [M-CH_3-Cl], 73 [M-CN-HCl], 64 [M-HCl-Cl]
TCP	25.3	197	269	169 [M-CO], 162 [M-Cl], 134 [M-CO-Cl], 107 [M-Cl-CO-HCN], 98 [M-CO-Cl-HCN-HCl]
1	26.2	179	251, 323	161 [M-H_2O], 135 [M-OH-HCN], 133 [M-H_2O-CO], 107 [M-H_2O-CO-CN], 98 [M-H_2O-CO-Cl]

[a] Retention times were for products in underivatized sample in GC-MS analysis.

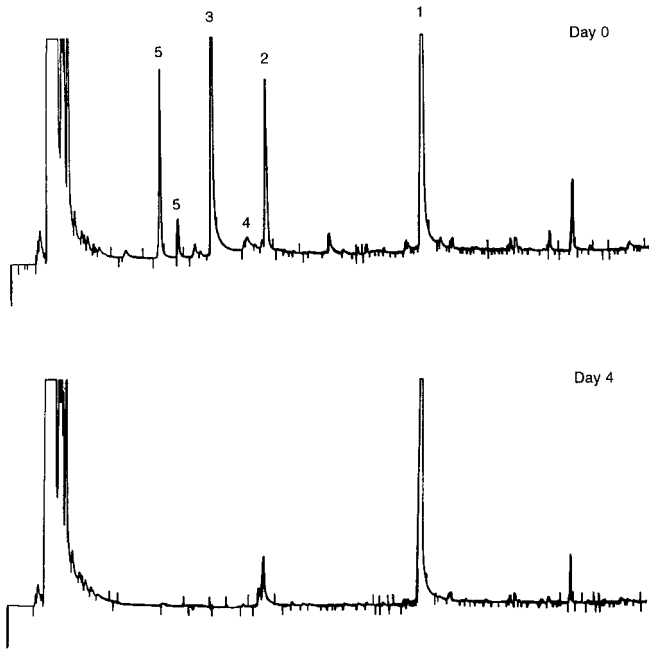

Fig. 6. Gas chromatographic chromatograms of ethyl acetate extracts from resting cell cultures of *Pseudomonas* sp. ATCC 700113 incubated with 2-h ultraviolet exposure solutions for 0 and 4 d.

$[M-71]^+$. Chemical ionization confirmed the molecular weight by providing an MH^+ at m/z 132. The molecular ion is consistent with the molecular formula C_5H_6NOCl, and the fragments are results of losing CH_2CH_2, O=C−NH, and CH_2CH_2 + O=C−NH groups. The compound was proposed as chlorodihydro-2-pyridone, product 2. In the silylated sample, two peaks with GC retention times of 17.0 and 19.3 min had molecular weight of 203. This molecular ion is consistent with the molecular formula $C_8H_{14}NOClSi$. Both products presumably were TMS derivatives of two of the three possible chlorodihydro-2-pyridone isomers: 2a, 2b, and 2c.

The peak in the underivatized sample with a GC retention time of 15.0 min gave fragments at m/z 81 $[M-18]^+$, m/z 55 $[M-44]^+$, and m/z 54 $[M-45]^+$. Chemical ionization indicated the molecular weight by providing an MH^+ at m/z 100. The molecular ion is consistent with the molecular formula $C_4H_5NO_2$, with the fragments resulting from loss of H_2O, O=C-NH_2, and CH_2CH_2OH groups. The compound was proposed as maleamide semialdehyde, product 4. In the silylated sample, a peak with GC retention time of 16.4 min gave an $[M-15]^+$ at m/z 156. Chemical ionization provided an MH^+ at m/z 172. This molecular ion is consistent with the molecular formula $C_7H_{13}NO_2Si$, which was the TMS derivative of product 4.

Five peaks in the underivatized sample gave GC retention times of 9.2, 10.4, 11.5, 12.0, and 16.3 min, respectively. They all produced molecular ions of m/z 135, which was confirmed by chemical ionization. The molecular ions and chlorine isotope patterns are consistent with the molecular formula $C_4H_3NCl_2$. The first four peaks had similar fragmentation patterns, and the base peak was at m/z 100. They all gave fragments at m/z 100 [M-35], m/z 85 [M-50], m/z 74 [M-61], m/z 64 [M-71], and m/z 49 [M-86]. The fragments are the results of losing Cl, CH_3+Cl, CN+Cl, HCl+Cl, and CNCHCCl. The last peak with a GC retention time of 16.3 min gave a fragmentation pattern different from the four others, and the base peak was at m/z 40. The fragments were at m/z 108 [M-27], m/z 100 [M-35], m/z 85 [M-50], 73 [M-62], and m/z 64 [M-71] and are the results of losing HCN, Cl, CH_3+Cl, CN+HCl, and HCl+Cl. There were no corresponding silyl derivatives found in the silylated sample. These products were proposed to be five of the six possible isomeric structures of dichlorocyanopropene, 5a to 5f shown in Figure 4. Structure 5f was tentatively assigned to the compound with a GC retention time of 16.3 min.

Metabolism of photodegradation products by TCP-degrading bacteria

Mixtures of photodegradation products were used as substrates for resting cell cultures of *Pseudomonas* sp. ATCC 700113. Substrate disappearance was monitored by GC. Figure 6 shows the GC chromatograms of ethyl acetate extracts from cultures incubated for 0 and 4 d with TCP solution that had been exposed to UV for 2 h. Dichlorodihydroxypyridines

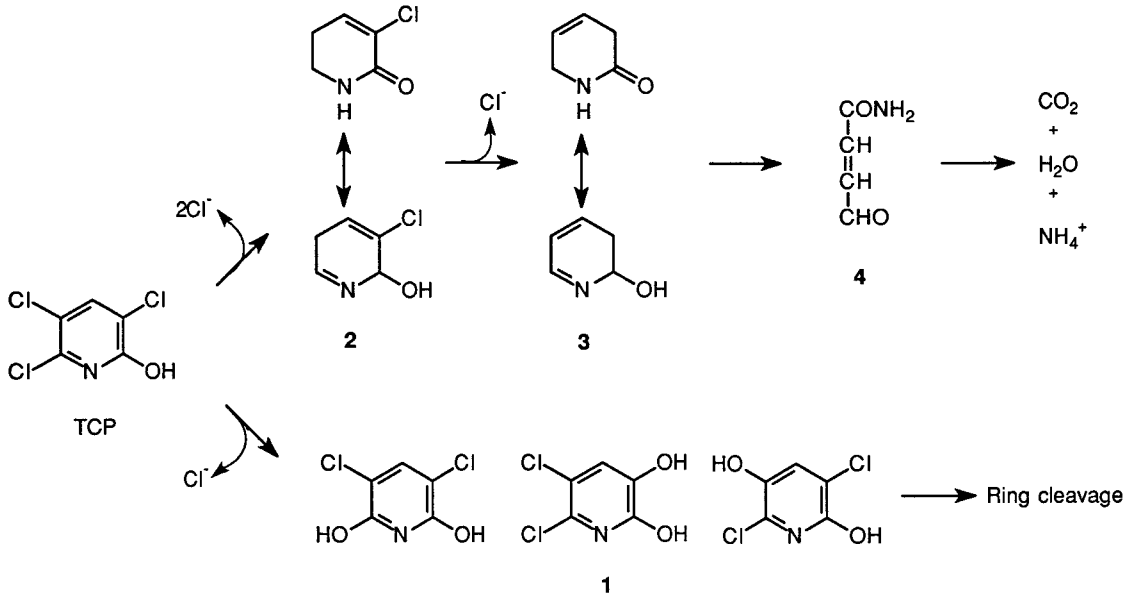

Fig. 7. Proposed photodegradation pathway of 3,5,6-trichloro-2-pyridinol.

(product 1) eluted at a retention time of 23.4 min; early eluting peaks were reductive dechlorination products of TCP. After 4 d of incubation with resting cells of *Pseudomonas* sp. ATCC 700113, most photodegradation products disappeared, with the exception of the dichlorodihydroxypyridines.

Resting cell cultures of *Pseudomonas* sp. ATCC 700113 were also incubated with mixtures of radioactive photodegradation products (2-h UV exposure). After 4 d 22.9% of the initial radioactivity was recovered as $^{14}CO_2$, 54.2% remained in the medium, and 2.3% remained in biomass.

DISCUSSION

[2,6-^{14}C]TCP was decomposed rapidly upon UV irradiation with a half-life of approximately 25 min, and 25.1% of the initial radioactivity was recovered as $^{14}CO_2$. This result was in agreement with the finding of Smith [1], who reported that 25% of ^{14}C-TCP was decomposed to $^{14}CO_2$. The production of $^{14}CO_2$ remained steady after 100 h, whereas 70% of the initial radioactivity still remained in the medium (Fig. 2). This suggests that the degradation of the UV-absorbing compounds in the reaction solution prevented further mineralization. Figure 2 also indicates that CO_2 formation occurred during the initial degradation of TCP, through an intermolecular free radical mechanism during the degradation of TCP, and/or through an intermolecular sensitization reaction between TCP and a degradation product.

After a 6-h exposure to UV light, chloride release in the reaction solution amounted to 85.1% based on stoichiometric calculation. Chlorine atoms that were not released can be associated with small organic molecules, such as dichlorocyanopropene (e.g., 5a–5f in Fig. 4) and/or organic acids. Smith [1], however, reported 100% recovery of chloride ions from TCP photolysis. The generation of chloride is likely due to both homolytic cleavage of the chlorine atom (a radical mechanism) and photonucleophilic substitution (a nonradical mechanism).

As time of UV exposure increased, the extraction efficiency of ethyl acetate decreased. This result suggested that the proportion of the polar products increased during the photodegradation of TCP.

Dichlorodihydroxypyridine (product 1) generated from TCP has three isomers, and they can be separated by GC after silylation. All three isomers with one TMS group attached were found in GC chromatogram; however, only two peaks were detected for isomers with two TMS groups. It is possible that one of the isomers with two TMS groups was present at a very small amount that was below the detection limit. Another possibility is that the TMS derivatives of two of the three isomers were not separated due to similarity in their structures.

The proposed products of TCP photodegradation (Fig. 4) suggested that both reductive dechlorination ($RCH_2X \rightarrow RCH_3$) and hydrolytic dechlorination ($RCH_2X \rightarrow RCH_2OH$) mechanisms were involved in the TCP photolysis. Dechlorination could occur before and after cleavage of the pyridine ring. A proposed pathway of TCP photodegradation is shown in Figure 7. There are two reports regarding photodegradation pathways of chlorinated pyridines; they suggest the occurrence of hydrolytic dechlorination. Smith [1] speculated that TCP undergoes photodehalogenation with the formation of a series of diols, triols, and tetraols; Woodburn et al. [14] reported that aqueous photolysis of the herbicide triclopyr (pH 7) produced 5-chloro-3, 6-dihydroxy-2-pyridinyloxyacetic acid as the major product and other low-molecular-weight acids.

Resting cells of *Pseudomonas* sp. ATCC 700113 metabolized the reductive dechlorination products generated by TCP photolysis. This observation suggests that *Pseudomonas* sp. ATCC 700113 used the reductive dechlorination pathway to degrade TCP. This finding could also explain why *Pseudomonas* sp. ATCC 700113 gave a negative result for the aromatic ring cleavage test [12].

Although TCP was degraded rapidly upon UV irradiation, many degradation products were produced, as indicated by the radioactivity remaining in the medium. *Pseudomonas* sp. ATCC 700113 mineralized TCP to a much greater extent (72–87%) [12]; microbial degradation of TCP was more complete than photodegradation. In addition, microorganisms could further transform some of the photodegradation products.

Several studies indicated that the combined use of photolysis and microbial metabolism provided an alternative method for the treatment of chemical wastes [15–17]. Due to the simultaneous occurrence of multiple degradation mechanisms in photolysis, products of the reaction can be complex. A consortium of microorganisms may be needed to achieve a complete degradation in the treatment process combining microbial and photolytic activity.

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