Comparison of Formation and Biodegradation of Bromacil Oxidation Products in Aqueous Solutions

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A comparative study of several oxidation methods of aqueous bromacil (**I**) solutions was conducted as part of a series of investigations concerning the chemical and biological remediation of pesticide-laden wastes. Ozonation (A), UV photolysis at 254 nm (B), and sensitized sunlight photodegradation (C) methods were examined. The A products were isolated and their structures elucidated by mass spectroscopy, various ^{13}C and ^{1}H NMR techniques, and other chemical methods. Three main A products were identified: 3-*sec*-butyl-5-acetyl-5-hydroxyhydantoin (**II**, ca. 5%), 3-*sec*-butylparabanic acid (**III**, ca. 20%), and 3-*sec*-butyl-5,5-dibromo-6-methyl-6-hydroxyuracil (**IV**, ca. 5%), which was also synthesized via hydroxybromination of bromacil; a fourth product (**VII**) was obtained in minute amount but was not identified. The aqueous solutions of **IV** are unstable and its decomposition led to re-formation of **I**. The main products found in B [(3-*sec*-butyl-6-methyluracil (**V**) and a dimer compound, **VI**] and C (**II** and **VI**) were similar to those described previously. The biodegradation assays of **I**, A, B, and C solutions were investigated using activated sludge, a pure culture of *Klebsiella terragena* (DRS-I), or soil; they indicated that the B and C solutions were more biodegradable than A solutions, while the parent material (**I**) was nonbiodegradable. A phytotoxicity bioassay, using *Nicotiana tabacum* seedlings, showed complete detoxification of the B and C solutions but only partial detoxification of the A and **IV** solutions. An attempt has been made to evaluate the most suitable method of degradation of solutions of **I**.

Keywords: *Bromacil ozonation; bioremediation; phytotoxicity; detoxification methods*

INTRODUCTION

The material used in this study, bromacil [5-bromo-3-*sec*-butyl-6-methyluracil (**I**), Figure 1], is a nonselective herbicide inhibiting photosynthesis and is used for general weed control on noncrop land and on citrus plantations. Following its agricultural application, the herbicidal effects may persist for more than a season (Worthing and Hance, 1991), demonstrating a good chemical and biochemical stability in soil. Like other pesticides, it may reach and pollute water sources.

The photodecomposition of **I** by ultraviolet light (UV) has been investigated in aqueous solutions (Kearney et al., 1969) and in thin solid films (Jordan et al., 1965) but without identification of the photoreaction products. Information concerning UV photolysis of other related uracil derivatives, namely 5-halogenated uracil, may be obtained from the photobiological studies of deoxyribonucleic acid (DNA) by Ishihara and Wang (1966a–c), Rupp and Prusoff (1965), and Smith (1963).

The sunlight photodecomposition, done by exposure of aqueous **I** solutions to direct solar irradiation for 4 months (summer), yielded only 2.2% of a single dealkylated photoproduct, indicating that **I** is very stable toward sunlight (Moilanen and Crosby, 1974). No traces of mutagenic materials (McGahen and Hoffman, 1966) were detected in the reaction solutions (Moilanen and Crosby, 1974). Facile sunlight photodegradation was observed, however, upon the addition of a sensitiz-

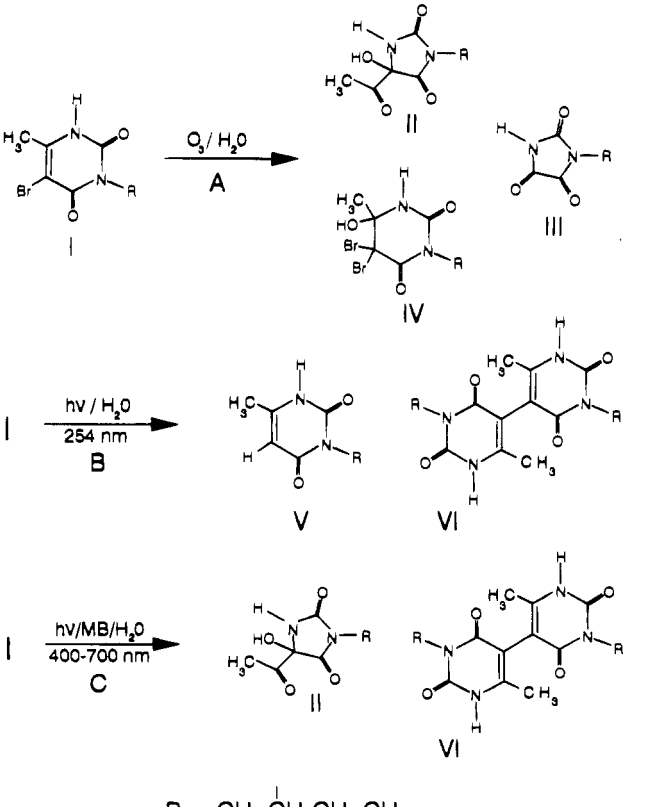

Figure 1. Main oxidation products of **I** in aqueous solutions.

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ing dye (methylene blue, MB) to aerated aqueous solution of **I** (Acher and Saltzman, 1980). Two main products were identified (Acher and Dunkelblum, 1979), and the phytotoxicity was reported (Saltzman et al., 1982).

Previous studies showed a decreased phytotoxicity and an enhanced biodegradation when aqueous pesticide solutions were pretreated with sensitized sunlight (Saltzman et al., 1982; Rejto et al., 1984), UV light, or ozone (Kearney et al., 1988; Somich et al., 1988; Hapeman-Somich, 1992).

The purpose of the present investigation was to perform a comparative study of products formed from three oxidation methods of **I** in aqueous solutions. Ozonation (A), UV irradiation at 254 nm (B), and sensitized (MB) sunlight photodegradation (C) methods were examined and the chemical structures of the main products determined by NMR, mass spectroscopy, and other chemical methods. Solutions treated by A, B, and C methods were subsequently submitted to biodegradation (mineralization) using soil, activated sludge, and a previously isolated microorganism *Klebsiella terragena* (strain DRS-I) (Leeson et al., 1993). Phytotoxicity of the oxidation products was also investigated by examining the effects on the growth of a sensitive plant (*Nicotiana tabacum* seedling).

The energy (kW h/mol) necessary to accomplish degradation of one mole of **I** was used among the other criteria for comparing the A, B, and C methods used in this study.

EXPERIMENTAL PROCEDURES

Materials. *Bromacil* [5-bromo-3-*sec*-butyl-6-methyluracil (**I**)] was a gift from Agan Chemicals Ltd., Israel. Recrystallization from 2-propanol yielded chromatographically pure material (needle crystals, mp 158–159 °C). A 600 ppm stock solution of **I** in ultrapurified water (0.55 Mmho·cm^{-1}, Modulab, Type I HPLC, Continental Water System Corp., San Antonio, TX) was prepared and stored at 4 °C.

[*U-carbonyl-*^{14}*C*]*Bromacil* was a gift from E. I. du Pont de Nemours & Co., Wilmington, DE. Before use, it was purified by preparative TLC, giving a chromatographically (HPLC) pure compound with a specific activity of 2.67 Ci/mol. An aliquot of 210 μL (methanolic solution, 0.339 mM) was added to the [^{12}C]-**I** stock solution (500 mL) to provide a radioactive count of ca. 3100 dpm/mL of solution.

Soil. The soil used for metabolism studies was a sieved (1.0 mm), nonsterile Sassafras silt loam (Salisbury, MD) at a moisture content of 35%. The composition of the soil was 56% sand, 20% silt, and 24% clay. The organic matter content was 14%. The soil had a pH of 4.2, a cation-exchange capacity of 16.6 mequiv/100 g, and a moisture content of 57% at field capacity (0.33 bar). This high organic agricultural soil (not classified taxonomically) was already used in biodegradation assays (Somich et al., 1988).

Activated Sewage Sludge. Sludge was obtained from a waste water treatment plant (Laurel, MD) from the end of the aeration basin and before clarifiers. Fresh sewage sludge slurry (total nitrogen content, 3.1 ppm) was obtained for each series of experiments and was used within 4 h.

K. terragean (Strain DRS-1). DRS-1 is a bacterium that was previously isolated from activated sewage sludge and characterized (Leeson et al., 1993). Its ability to mineralize N-heterocycle compounds (*s*-triazines) as a sole nitrogen source was chosen to metabolize the pyrimidinedione derivatives (**I** degradation derivatives).

N. tabacum cv. Xanthi Seedling. Seeds germinated on Nitsch agar plates (Nitsch, 1969) were used for the phytotoxicity assay.

3-sec-Butyl-5,5-dibromo-6-methyl-6-hydroxyuracil (**IV**). This material was prepared by direct hydroxybromination of **I** by aqueous bromine (Kergomard, 1961). It served for spectroscopic comparison with one of the ozonation products (**IV**) and for phytotoxicity assay.

Instruments and Methods. *HPLC Chromatography.* HPLC chromatographic data were obtained using two Waters Model 6000 pumps equipped with a Waters Model 660 solvent programmer and a Waters Model 990 photodiode array detector and accompanying NEC APC-III controller and software. Analytical separations were achieved on a standard Beckman C_{18} (ODS, 5 μm) endcapped, ultrasphere steel jacketed column (4.6 mm × 25 cm, part no. 235329) using an isocratic regime of 50% acetonitrile in water at a flow rate of 1.0 mL/min unless otherwise indicated. Calibration curves for bromacil concentrations were produced with an isocratic regime of 65% acetonitrile in water, a 100 μL loop, and a flow rate of 0.6 mL/min. Under these conditions the minimum amount of **I** that could be detected was 40 ng (0.4 ppm, 100 μL). Semipreparative separations were carried out on a larger Beckman C_{18} column (10 mm × 25 cm, part no. 235328) using 45% acetonitrile in water at a flow rate of 2.8 mL/min.

Column Chromatography. A rapid method of column chromatography (Still et al., 1978) was used for separation of fractions of ozonation products which were further purified on semipreparative HPLC. The silica gel used was Aldrich product no. 28,862-A (BET surface area, 500 m^2/g; pore volume, 0.75 cm^3; pd, 70–230 mesh; 60 A). The glass column used had 2.5 cm i.d., 30 cm length, and was pressurized by compressed air. The presence of compounds in the eluent fractions was detected either by using TLC plates (precoated silica gel 60 F-254) and UV light or iodine vapors for visualization or by HPLC analyses.

Spectroscopy. Liquid chromatography/mass spectroscopy (LC/MS) electron ionization (EI) spectra (70 eV) were obtained on a Hewlett-Packard Model 5988A mass spectrometer with 3.0 Pascal software equipped with a Hewlett-Packard Model 5980A particle beam LC/MS interface (desolvation chamber temperature = 50 °C; source temperature = 200 °C). LC separations were achieved employing a Zymark Encore HPLC system equipped with the previously described Beckman C_{18} analytical column using a 5 min linear gradient of 20–40% acetonitrile in acetic acid buffer (pH 4) at a rate of 0.4 mL/min. Relative product yields were calculated from peak areas of total ion chromatograms.

MS of pure samples were obtained on a Finnigan Model 4500 with sample introduction using a direct exposure probe which was heated by application of a current from 0 to 1.0 A at a rate of 20 mA/s. EI spectra were collected at 70 eV. Chemical ionization (CI) spectra, using ammonia and perdeuterioammonia, were obtained at 0.6 Torr source pressure and 60 °C source temperature. Postacquisition data processing was by means of an Incos data system.

NMR Spectra. ^{1}H and ^{13}C NMR spectra were obtained on a General Electric QE-300 spectrometer using ca. 4 or 40 mg of sample in $CDCl_3$, respectively, and tetramethylsilane (TMS) as an internal standard. Spectra were acquired at room temperature unless otherwise indicated. APT and COSY experiments were conducted using GE software.

Method A. Ozonation experiments were carried out at room temperature in a previously described 550 mL reactor (Somich et al., 1988). Three different concentrations of **I**, 200, 400, and 600 ppm, were submitted to ozonation at ozone concentrations of 0.16, 0.28, and 0.46% O_3 (w/w). Ozone was generated using a PCI Model GL-1B ozone generator (PCI Ozone Corp., West Caldwell, NJ) with oxygen feed. Ozone (in oxygen) was fed into the reactor at a rate of 1 L/min. The flow stream was maintained at a constant rate by use of mass flow controllers. Ozone concentration in the feed line was monitored continuously using an ozone monitor (Model HC-12, PCI Ozone Corp.). Reaction mixtures were analyzed, during ozonation, by HPLC as described above.

Method B. UV irradiation was carried out in a 325 mL cylindrical quartz reactor (Figure 2). Solution of **I** (375 mL) was continuously supplied at its bottom in the free space (10 mm) between the reactor walls (a) from a 100 mL separatory funnel (b) via a Masterflex pump (c) at a flow rate of 150 mL/min. A 27 cm long Conrad-Hanovia (L5464000) low-pressure mercury lamp (d) (3.15 W emission of UV at 254 nm) was

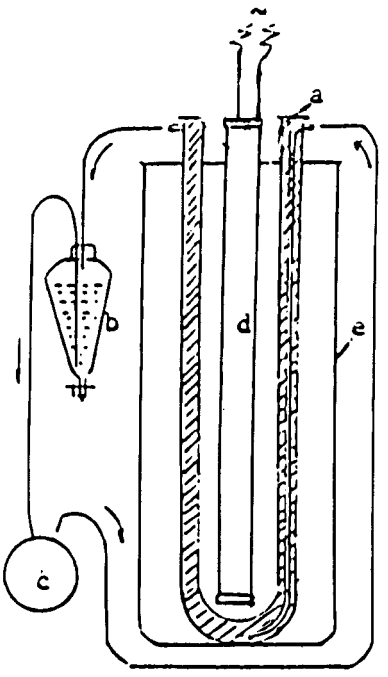


Figure 2. UV irradiation apparatus: (a) reactor; (b) separatory funnel; (c) pump; (d) UV lamp; (e) cooling bath.

vertically and coaxially mounted in the interior free space of the quartz reactor. The quartz reactor was immersed in an ice–water bath (e) and the irradiation performed at 10 ± 2 °C. The actual irradiation time was 86.7% of the operation time, since only 325 mL (86.7%) of the total 375 mL of solution was exposed to irradiation. Samples (0.5 mL) for HPLC analysis were taken from the funnel (b). The **I** solutions submitted to photolysis were of 400 or 600 ppm, with and without addition of hydrogen peroxide (4 mol of H_2O_2/mol of **I**).

Method C. Photosensitized degradation of **I** was carried out using solutions of **I** (100 mL, 400 or 600 ppm) containing 1 ppm of MB under neutral conditions (pH 6.8) or in the presence of 0.1% $NaHCO_3$ (pH 8.12) (Acher and Dunkelblum, 1979). The photoreactions were performed in 250 mL Erlenmeyer flasks exposed to sunlight. Degradation of **I** was followed by HPLC analyses, as described previously. The visible sunlight (λ, 400–700 nm) intensity, registered during the photodegradation experiments, was of 1900 ± 50 μEinstein m^{-2} s^{-1}.

Isolation of A Products. Since the ozonation reactions for preparation of the oxidation products were stopped before their concentrations began to decrease (Figure 3), it was necessary to separate the unreacted **I** by column chromatography. Attempts to obtain a residue of the ozonated solution by lyophilization failed because the product **II** (Figures 1 and 3A) disappeared during the process. The reaction products were eventually obtained by extracting the solution (500 mL) with ethyl acetate (3 × 80 mL); the extract was dried (Na_2SO_4 anhydrous), filtered, and evaporated in a rotavapor (<35 °C) to a constant weight. This residue (170 mg, in a 100 mL ground flask) was dissolved in 10 mL of ethyl acetate, and 5 g of silica gel was added. The slurry was evaporated (rotavapor) to dryness. The free-flowing coated silica gel was poured into the already filled chromatography column (see above) and eluted using a flash method (Still et al., 1978) with a solution of 32 mL of 2-propanol in 968 mL of hexane. Ten eluant fractions of about 100 mL were collected. Fractions 3, 4, and 5 contained a mixture of products **II**, **VII**, and **IV** (HPLC R_t 2.0, 2.9, and 8.5 min, respectively). To the residue obtained from these three fractions (75 mg) was added 15 mL of acetonitrile, and aliquots (250 μL) were injected for further separation (1 mL loop) in the semipreparative HPLC column, using previously described conditions. Product **III** (HPLC R_t 5.5 min) was separated from eluent fractions 1 and 2, using the same semipreparative column method.

Preparation of 3-sec-Butyl-5,5-dibromo-6-hydroxyuracil (**IV**). Into a 100 mL stirred aqueous solution of **I** (400 ppm, 0.153 mmol) at 10 °C was added 14 mL of aqueous bromine (28 mg, 0.35 mmol). After about 2 min, the reaction product was extracted with ethyl acetate (3 × 30 mL). The organic extract was washed with cold water (3 × 20 mL), dried over Na_2SO_4, filtered, evaporated in a rotavapor (<35 °C), and dried under high vacuum, affording 48 mg (95% yield) of a yellowish oil (HPLC R_t 8.45 min). The chemical composition of this compound was unequivocally determined by HPLC, ^1H and ^{13}C NMR, and mass spectroscopy (see below), and it was identical with that of product **IV** obtained from ozonization of **I**. The aqueous solutions of **IV** were unstable, and they totally decomposed with re-formation of **I** and other unidentified products. The addition of $Na_2S_2O_3$ or $NaHSO_3$ to solutions of **IV** instantaneously led to the same results. Attempted preparations of **IV** by reacting Br_2 plus **I** in nonaqueous (acetonitrile, ethyl acetate, chloroform) solutions failed, and **I** was completely recovered.

Biodegradation Experiments. Experiments to assess the ability of indigenous soil microorganisms, activated sludge microorganisms, and strain DRS-1 to mineralize A-, B-, and C-treated or untreated **I** solutions (fortified with [^{14}C]-I) were carried out in biometer flasks (Bartha and Pramer, 1965). The released $^{14}CO_2$ was trapped in 10 mL of 0.1 N NaOH added to the sidearm of a biometer flask. At sampling, the 10 mL of NaOH solution was removed and replaced with 10 mL of fresh solution. Two 1 mL subsamples were added to 10 mL of Beckman Ready-Solv HP (Beckman Instruments Inc., Fullerton, CA), and $^{14}CO_2$ concentration was determined by liquid scintillation counting (Beckman, LS 6800), after the samples had been kept in the counter for 6 h. All of the biometric flasks were incubated at 28 °C.

For soil experiments, to each biometer flask was added 50 g of soil spiked with 5 mL (600 ppm) of above A-, B-, and C-treated solutions of **I**. For sludge and DRS-1 experiments: 25 mL of **I** treated solutions with 25 mL of sludge or with 22 mL of pure water and 3 mL of DRS-1 inoculum was introduced into biometer flasks. These mixtures were amended: (1) for sole nitrogen experiments, with 20 mM phosphate buffer (0.5 mL), trace metals solution (0.5 mL), 2 mM $MgSO_4 \cdot 7H_2O$ (0.5 mL), and 0.2% high-fructose corn syrup (1.0 mL) (Tru-Sweet, Amazo Corp.); (2) for sole carbon experiments, the high-fructose corn syrup and $MgSO_4$ were omitted and 4 mM $(NH_4)_2SO_4$ (0.5 mL) was added (Shelton and Somich, 1988). The pH of the samples was adjusted to 7.0 by addition of a few drops of 0.1 N KOH. The flasks were agitated on a rotatory shaker at 28 °C.

Separate experiments were conducted with unlabeled bromacil solutions to determine the utilization of specific oxidation products as a sole nitrogen source. The incubations were conducted as previously described except that duplicate 250 mL Erlenmeyer flasks were substituted for biometer flasks. The mixture was sampled daily, and to the sample (0.5 mL) in microtube (1.5 mL) were added acetonitrile (0.25 mL) and methanol (0.25 mL); the mixture was vortexed and centrifuged (Microfuge 11, Beckman) for 2 min at a speed of 12 000 rpm. The supernatant was injected (100 μL) in the HPLC analytical column eluted with acetonitrile/water (50:50) at 1 mL/min flow rate, isocratic regime.

Since the biodegradation of C compounds by DRS-1 was inefficient, a series of bioassay experiments were carried out to determine the toxicity of C products and MB (up to 4 ppm) on the growth of DRS-1. These experiments were done under the same conditions as the DRS-1 experiments, both in light and dark, and the growth of the microorganisms was determined visually.

Phytotoxicity Tests. N. tabacum cv. Xanthi seeds were germinated on Nitsch agar plates (Nitsch, 1969) containing solutions of **I** or its A, B, and C degradation products. Solutions of **I** (400 ppm) untreated or A-, B-, and C-treated were diluted 10-, 100-, 1000-, and 10000-fold, and aliquots were used for preparation of Nitsch agar plates. Solutions of pure compound **IV** of 40, 4.0, 0.4, and 0.04 ppm were also used. Control test, without any aliquot addition, were also performed. Three agar Petri dishes (containing 10 seeds each) were used for each concentration. The phytotoxicity (inhibition) effect was determined by comparison (after 3 weeks) of

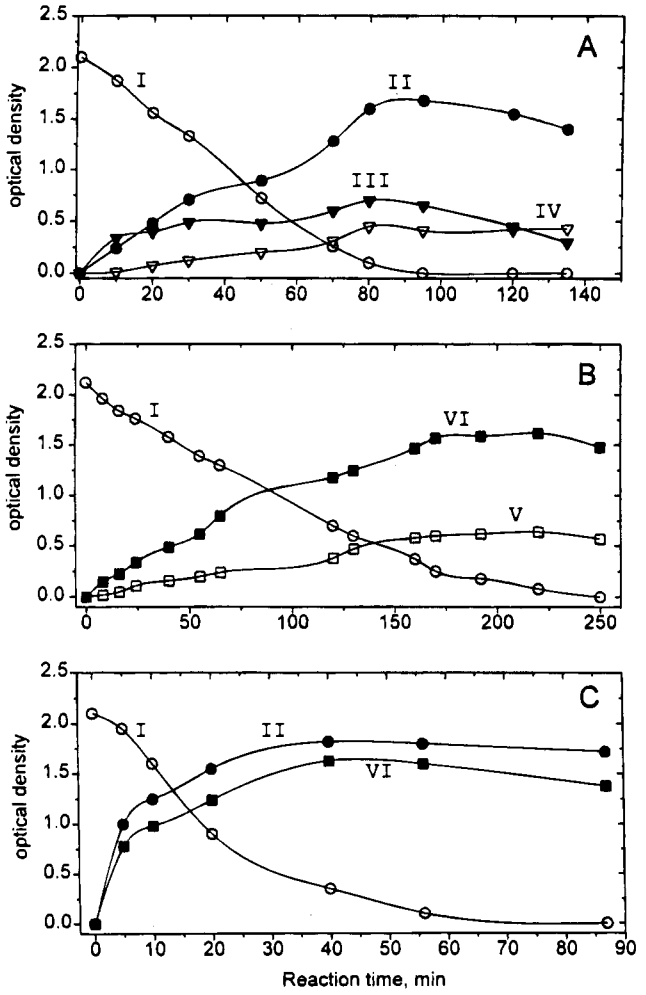


Figure 3. Degradation of **I** and product formation: (A) ozonation; (B) UV photolysis; (C) sunlight.

the length of shoots produced in the different solutions with the length of seedlings grown in the control solution.

RESULTS AND DISCUSSION

The magnitude of the oxidation effects on **I** depended on ozone concentration, on UV irradiation intensity, on oxygen and MB concentrations, and on reaction time (oxidation dose). After degradation of **I**, the concentration of oxidation products also began to decrease (Figure 3). It was therefore necessary to establish an arbitrary time for the reaction termination, which would better fit our research aims. This was chosen, for the reaction mixtures submitted to bioassay, when the concentration of **I** was less than 0.4 ppm (the reaction times required were 90, 250, and 56 min for A, B, and C methods, respectively), while for oxidation products preparations a 10–15 min shorter reaction time was used. The data plotted in Figure 3 represent the heights of the optical density of HPLC peaks (*Y*-axis) with reaction time (*X*-axis). For comparison of A, B, and C methods, it should be noted that only the initial concentration of **I** (400 ppm) was the same, while the other conditions (oxidation dose, reaction conditions, etc.) were different. No quantitative comparison should be made among different product curves since their HPLC optical densities depend on unknown extinction coefficients (ϵ) and on different desorption patterns from HPLC column.

The effect of hydrogen peroxide addition (4 mol of H_2O_2/mol of **I**, from a 3% H_2O_2 solution) on these oxidations was examined. The addition did not enhance the A and C processes (Glaze, 1987; Peyton and Glaze, 1987) but improved the B treatment, reducing the irradiation time.

Table 1. Ozonation Time for Disappearance of Bromacil

	bromacil		ozone		
time, min	ppm/mL	mmol	% wt/wt	mmol	mole ratio[a] O_3/**I**
80	200/500	0.383	0.160	3.57	9.29
90	400/500	0.766	0.281	6.99	9.12
93	600/550	1.240	0.460	12.10	9.49

[a] At the reaction start.

Method A. Four products, **II**, **III**, **IV**, and **VII**, were detected by HPLC when solutions of **I** were treated with ozone (Figures 1 and 3A). The pH of the reaction solution decreased during ozonation from 6.2 to 3.0. In addition, as the reaction proceeded, the colorless solution turned yellow. This was attributed, *a priori*, to bromine formation from the bromide ions or bromine radicals released into the solution. This coloration was also accompanied by an increase in the formation of **IV**.

Three different concentrations of **I**, 200, 400, and 600 ppm, were submitted to ozonation at ozone concentrations of 0.16, 0.28, and 0.46% O_3 (w/w), respectively (Table 1). Eventually, the 400 ppm concentration was found to be the most suitable for the proposes of this study. The disappearance of **I** below detectable limits (<0.4 ppm) was achieved in ca. 90 min at an initial average O_3/bromacil mole ratio of 9.3. The fact that addition of hydrogen peroxide did not affect the degradation rate of **I** implied that ozone and not hydroxyl radical was responsible for **I** oxidation (Glaze et al., 1987; Peyton and Glaze, 1987; Somich et al., 1988; Kearney et al., 1988).

Identification of A Products (Figures 1 and 3A; Tables 2–4). An estimate of the overall significance of the A products was determined by integration of the LC/MS chromatogram of the reaction mixture. For example, a sample from the reaction mixture taken after 40 min of ozonation showed 41% unreacted **I**, 21% **II**, 7% **III**, 31% **IV**, and <1% **VII**. However, this relative concentration of different A products is continuously changing during the reaction. Isolation of **II**, **III**, and **IV** was carried out by flash column chromatography followed by semipreparative HPLC separation affording ca. 5, 5, and 20% of **II**, **III**, and **IV**, respectively. **VII** was neither isolated nor identified. Results from LC/MS of the reaction mixture containing **II**, **III**, and **IV** are shown in Tables 2–4.

Product **II**. The EI-MS of this A product showed a very good match with a compound previously isolated from the C method, namely, 3-*sec*-butyl-5-acetyl-5-hydroxyhydantoin (Acher and Dunkelblum, 1979). The HPLC data of this product were identical with those of the main C product **II** (Figure 1).

Product **III**. This product was crystallized from ether/hexane (10:1) and was identified by ^1H and ^{13}C NMR and by EI- and CI-MS as a new and unknown derivative of parabanic acid (Ishihara and Wang, 1966b), namely 3-*sec*-butylparabanic acid (Tables 2–4). The crystallized material was also submitted to DEP-CI-MS using ammonia and perdeuterioammonia MS (CI, NH_3): *m/z* 200 $(M + NH_4)^+$, 217 $(M + (NH_3)_2H)^+$, 234 $(M + (NH_3)_3H)^+$. MS (CI, ND_3): *m/z* 205 $(M + ND_4 + H_x)^+$, 225 $(M + (ND_3)_2D + H_x)^+$, where H_x represents exchangeable protons present on the molecule. From these data, the molecular ion was determined to be 182 and H_x to be 1. NMR spectra provided further structural confirmation.

Table 2. Mass Spectral Data for Oxidation Products, and Proposed Structures of I–VI

mass	% abundance	proposed structure
I		
260, 262	3	M^+
245, 247	4	$M^+ - CH_3$
231, 233	11	$M^+ - CH_2CH_3$
205, 207	100	$M^+ - C_4H_7$
188, 190	15	$M^+ - HNC_4H_9$
162, 164	16	$M^+ - CONC_4H_8$
161, 163	11	$M^+ - CONC_4H_9$
70	13	$(NC_4H_8)^+$
II		
185	3	$M^+ - CH_2CH_3$
172	24	$M^+ - CH_2CO$
171	43	$M^+ - CH_3CO$
142	9	$M^+ - 72$
116	44	$M^+ - 98$
115	100	$M^+ - 99$
70	28	$(NC_4H_8)^+$
III		
155	2	$M^+ - CH_3$
141	75	$M^+ - CH_2CH_3$
115	29	$M^+ - C_4H_7$
84	11	$(C_2N_2O_2)^+$
70	100	$(NC_4H_8)^+$
56	39	(C_4H_8)
IV		
327, 329, 331	15	$M^+ - CH_2CH_3$
301, 303, 305	28	$M^+ - C_4H_7$
260, 262	9	$M^+ - BrOH$
231, 233	13	$M^+ - BrOH - CH_2CH_3$
205, 207	100	$M^+ - BrOH - C_4H_7$
142	55	$M^+ - BrOH - BrC_3H_3$
127	18	$M^+ - BrOH - BrC_3H_3NH$
70	41	$(C_4H_8)^+$
V		
182	0.2	M^+
167	6	$M^+ - CH_3$
153	16	$M^+ - CH_2CH_3$
127	100	$M^+ - C_4H_7$
110	24	$M^+ - HNC_4H_8$
84	29	$M^+ - CONC_4H_8$ or $(C_2N_2O_2)^+$
70	9	$(C_4H_8)^+$
VI		
362	52	M^+
347	32	$M^+ - CH_3$
307	63	$M^+ - C_4H_7$
306	33	$M^+ - C_4H_8$
291	52	$M^+ - HNC_4H_8$
251	86	$M^+ - C_4H_8 - C_4H_7$
235	100	$M^+ - CONC_4H_9$
207	51	$M^+ - C_4H_8 - CONC_4H_9$
70	37	$N(C_4H_8)^+$
56	26	$(C_4H_8)^+$

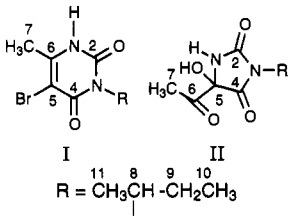
Product **IV**. The LC–EI-MS of **IV** from ozonation reaction mixture revealed the presence of a dibromo compound (3-*sec*-butyl-5,5-dibromo-6-methyl-6-hydroxyuracil). The ^1H NMR spectra of isolated **IV** indicated that nearly 50% of the material had decomposed to starting material (**I**). Additional attempts to isolate pure **IV** from the crude reaction mixture yielded only a sufficient amount for mass spectral analyses. DEP-CI-MS using ammonia and perdeuterioammonia afforded the following MS (CI, NH_3): *m/z* 374, 376, 378 $(M + NH_4)^+$, 391, 393, 395 $(M + (NH_3)_2H)^+$, 408, 410, 412 $(M + (NH_3)_3H)^+$. MS (CI, ND_3): *m/z* 380, 382, 384 $(M + ND_4 + H_x)^+$, 400, 402, 404 $(M + (ND_3)_2D + H_x)^+$, 420, 422, 424 $(M + (ND_3)_3D + H_x)$; the number of H_x was 2. Taking into account the relative instability of **IV** (and the degradation product formed), the molecular ion cluster of *m/z* 356, 358, 360, and the presence of two H_x, **IV** must be formed by the addition of BrOH. Therefore, a compound was synthesized by reacting aqueous solutions of Br_2 and **I**. In the ^1H and ^{13}C NMR data of this compound (Tables 3 and 4), obtained at 10 °C immediately after its isolation, two diastereomers were observed, whereas in the product **IV** formed in the A reaction, only one diastereoisomer was observed. MS and HPLC data of the synthesized compound and the A product **IV** were also identical.

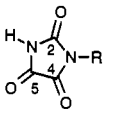
Method B (Figures 1 and 3B). The irradiation process was performed using the reactor described above (Figure 2). The UV absorbance spectrum of **I** has a relatively low extinction coefficient (ϵ) of 2724 cm^{-1} at $\lambda = 254$ nm as compared ot ϵ of 7460 cm^{-1} at $\lambda_{max} = 278$ nm. The high-concentration solutions submitted to irradiation, 1.53 (400 ppm) and 2.30 mM (600 ppm), resulted in high OD values (4.16 and 6.26, respectively) which have a UV-shielding effect (very low transmittance). These poor optical conditions, combined with a low input of the UV lamp (UV light = 3.3 W) and probably a low yield of **I** photodecomposition necessitated long irradiation times of 360 (for 400 ppm) and about 600 min (for 600 ppm) for the photodegradation. The addition of hydrogen peroxide (see above) shortened the irradiation times to 250 and 350 min, respectively. Two main photoproducts (**V** and **VI**) of this reaction were recovered in very low yields (ca. 5%). They were identified by LC–MS. The data (Tables 2–4) corresponded to those described previously for the debromobromacil, **V** (3-*sec*-butyl-6-methyluracil) (Moilanen and Crosby, 1974), and the debromobromacil radical dimer, **VI** (Acher and Dunkelblum, 1979). The HPLC data of the dimer product found in the B reaction mixture were identical with those of the product **VI** from the C reaction mixture.

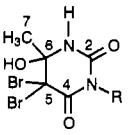
Method C (Figures 1 and 3C). Several experiments were carried out to prepare photodegradation products and treated solutions for bioassay treatments. In the solution of **I** (400 ppm) containing 0.1% $NaHCO_3$ (starting pH 8.12), no bromacil could be detected after about 56 min of sunlight exposure. During the reaction two main products were formed—**II** (ca. 95%) and **VI** (ca. 3%)—and the pH increased slightly to 8.58. The structures of these two C products were verified by LC–MS and compared to previous data (Acher and Dunkelblum, 1979). In the experiments carried out at an initial pH of 6.8, only 5% of **I** was decomposed after 120 min, the same products were formed, and the pH decreased to 4.85. This was an expected result since the efficiency of singlet oxygen ($^1O_2$) formation by MB sensitization is optimal above pH 8 and decreases sharply at a pH lower than 7.0 (Bonnea et al., 1975).

Biodegradation of Oxidation Products Mixtures (Figure 4). Experiments using [^{14}C]bromacil were conducted to determine the extent of mineralization by microorganisms. Ineffective mineralization (<3% $^{14}CO_2$ recovery) of A, B, and C products occurred when **I** treated solutions were incubated with activated sludge as a sole carbon source. When the same treated solutions were incubated with activated sludge as a sole nitrogen source (addition of 0.2% Tru-Sweet), the extent of mineralization of A, B, and C products was significantly greater (ca. 6–17% $^{14}CO_2$ recovered after 14 days), indicating that pyrimidinedione ring cleavage had occurred. Results from mineralization experiments with DRS-1 were generally consistent with sludge experiments (ca. 4–23% $^{14}CO_2$ recovered after 22 days). In HPLC analyses of **I** treated solutions after 4 days of inoculation with either activated sludge or DRS-1, the complete disappearance of all the main A, B, and C oxidation products was observed (no products could be

Table 3. ^{1}H NMR Spectral Data for Ozonation Products II–IV

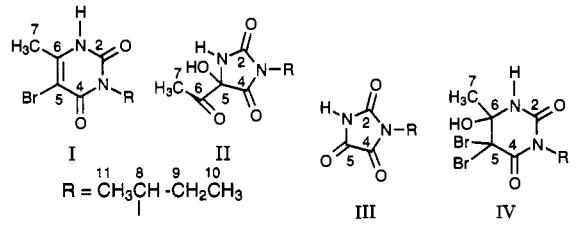



III


IV

N–H	10.7 (s, 1H)	7.2 (bs) (bs, ca. 1H)	8.28	6.75 (s, 1H)
C-7	2.33 (s, 3H)	2.33 (s)		2.02 and 1.96 (2s, 3H)
C-8	5.00–4.88 (m, 1H)	4.03 (m)	4.23–4.11 (m, 1H)	4.75–4.61 (m, 1H)
C-9	2.16–2.02 (m, 1H)	1.82 (m)	2.03–1.88 (m, 1H)	2.15–1.94 (m, 1H)
C-10	0.86 (t, 3H, J = 7.3 Hz)	0.88 (t, 3H, J = 8 Hz)	0.89 (t, 3H, J = 7.3 Hz)	0.90 and 0.88 (2t, 3H, J = 7.3 Hz)
C-11	1.45 (d, 3H, J = 7 Hz)	1.39 (d, 3H, J = 7 Hz)	1.44 (d, 3H, J = 7 Hz)	1.41 and 1.38 (2d, 3H, J = 7 Hz)
O–H				3.32

Table 4. ^{13}C NMR Spectral Data for Ozonation Products II–IV



C-2	159.9	157.2	154.1	161.4
C-4	152.8	169.9	158.0	150.7
C-5	97.6	85.7	128.4	81.9
C-6	148.5	200.0		63.6
C-7	19.8	23.7		22.6
C-8	52.9	50.6	55.5	51.4 and 51.5
C-9	26.0	26.3	28.0	23.7 and 25.1
C-10	11.3	11.0	12.4	9.2 and 9.3
C-11	17.5	17.7	19.3	15.1 and 16.3

detected in a 100 μL sample), concomitant with the appearance of microbial metabolites (no attempt was made to identify them).

Recovery of some $^{14}CO_2$ indicates an ability of microorganisms to cleave the heterocycle pyrimidinedione ring; however, the limited extent of mineralization may be due to the inability of microorganisms to metabolize the *sec*-butyl and possibly other alkyl moieties. Parent material was never observed to be degraded by sludge microorganisms or DRS-1 strain.

Collectively, these data are consistent with previous research which indicates that oxidation or cleavage of alkyl moieties, or removal of halogens, results in inherently more degradable products (Hapeman-Somich, 1992) and that nitrogen-containing products may be more readily metabolized when utilized as nitrogen sources than as carbon sources (Leeson et al., 1993).

Finally, mineralization experiments with organic-rich soil (Figure 4) indicated that a significant percentage of B, C, and A products were utilized as carbon and/or nitrogen sources (35–40%). Nonstoichiometric $^{14}CO_2$ recoveries could be due to either incomplete metabolism or binding to organic matter from soil. The release of $^{14}CO_2$ from sludge and DRS-1 strain experiments showed good mineralization of B products and much less of A and C products. These results may be attributed to a very low biodegradability of C products (experiments carried out by growing DRS-1 in the presence of C products and 4 ppm of MB, in light, were positive, meaning that these solutions were not toxic toward DRS-1 bacterium), or to some toxicity of the A products, especially to product **IV** which is unstable and re-forms **I** (which inhibits the growth of DRS-1).

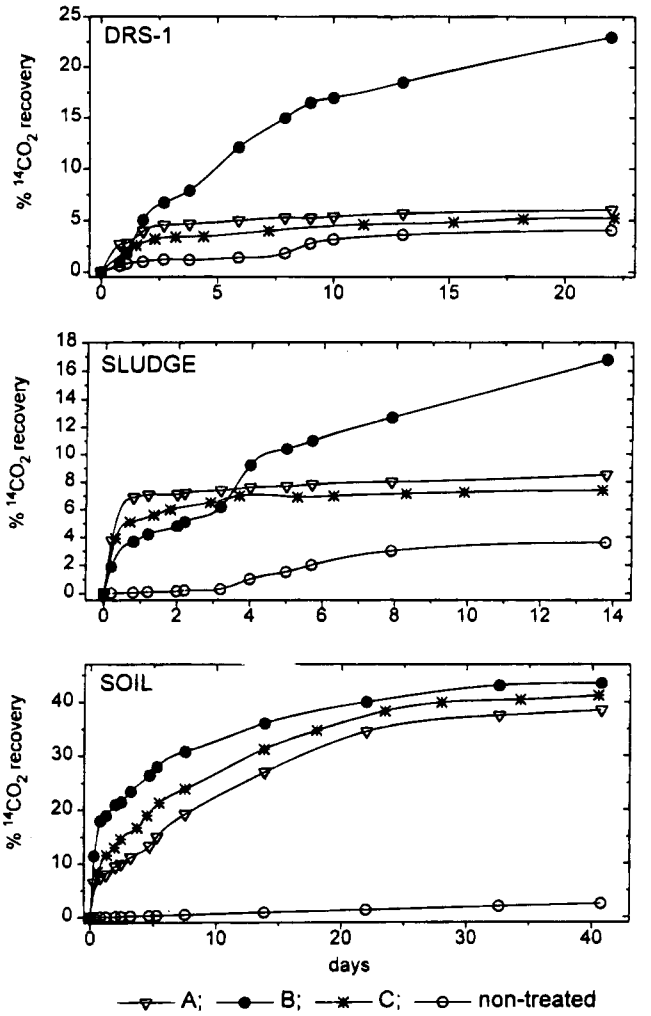


Figure 4. Mineralization of **I** and its degradation products: (▽) ozonation; (●) UV; (*) sunlight; (○) nontreated.

Phytotoxicity Test (Table 5). Solutions of **I** (400 ppm) untreated or treated (A, B, and C) and of the pure compound **IV** solution (400 ppm) at different dilutions (10-, 100-, 1000-, and 10000-fold) were submitted to phytotoxicity assay. Complete growth inhibition effect (100%) of *N. tabacum* was observed using untreated solutions of **I** at concentrations as low as 0.4 ppm, while the no-effect level (0.0% inhibition) was observed at 0.04 ppm. The diluted solutions of **IV** showed the highest inhibitory effect (100, 20, 14, and 0%) as compared with solutions of other degradation products. This toxic effect may be attributed either to compound **IV** itself or to the fact that **IV** decomposes to re-form the parent compound

Table 5. Phytotoxicity of I Treated Solutions[a]

solution tested	inhibitory effect, % at dilution factor of			
	10	100	1000	10000
I	100	100	100	0.0
IV	100	20	14	0.0
A	100	20	10	0.0
B	0.0			
C	0.0			
control	0.0			

[a] Inhibition was determined by comparing the length of shoots produced in the medium containing test solutions with the length of seedlings grown on control medium. The initial concentrations of **I** in untreated or treated (**A**–**C**) solutions was 400 ppm; the same concentration of **IV** was used. The inhibitory effect of 0.0% means no-effect level.

I, which showed a very high toxicity. The dilution solution of A also showed a phytotoxic effects of 100, 20, 10, and 0% at the above dilutions. No inhibitory effect was detected with solutions of B and C at 10-fold dilution; in these cases normal green seedlings similar to those of the control samples (without addition of any solutions) were developed.

Comparison of the Degradation Methods. Any method selected for the deactivation of pollutants should be ecologically friendly. Specifically, the method selected should (i) be chemically efficient (fast and quantitative degradation yield), (ii) produce biodegradable and/or nontoxic products, (iii) be simple and safe to use (minimal fire, explosion, electrical high tension, or health hazards associated with its use), (iv) be economically competitive, and (v) be applied by professionals who should continuously be aware of methods development and current regulations.

One of the simplest approaches for comparing methods A, B, and C for criteron iv is their cost estimation. An important component of this is the energy consumption (En), which is the kW h^{-1} necessary to accomplish the degradation of 1 mole of **I**. For method A, it is the energy needed for the ozone generator to produce O_3 at a concentration of 0.28% (w/w), 1 L/min (165 W s^{-1}); for method B, it is the energy needed by the UV lamp (3.3 W s^{-1} UV emission at λ = 254 nm; 10 W s^{-1} total input); and for method C, it is the sunlight energy (intensity) at the reaction time (1950 μEinstein m^{-2} s^{-1}), assuming that all of the sunlight was absorbed by the sensitizer (at λ_{max} 670 nm, 1 quantum mole (1E) = 178.94 kW s^{-1}; at λ = 254 nm: 1E = 472 kW s^{-1}). Also considered was the time (h) needed for reaction and the amounts of **I** reacted (g). The results of calculations (not shown) of energy consumption (En) are En-A = 1.24 kW h/g, En-B = 0.305 kW h/g, and En-C = 0.187 kW h/g.

Comparison of En for each method showed method C to be the most cost effective. An examination of all the above criteria for the methods used in this study revealed that methods B and C are the most advantageous for this particular case. A combination of C with B would overcome the inconvenience of noncontinuously available sunlight (C) and will give good biodegradable (B) and nonphytotoxic products (B and C). For practical applications, the concentration and the composition of solutions submitted for deactivation treatment will be decisive in selection of the most suitable method.

Summary and Conclusions. The three different methods employed for **I** oxidation (A, ozonation; B, photolysis at 254 nm; and C, sensitized sunlight) brought about its complete degradation and formation of products. The treated **I** solutions common and chemically related products, suggesting similarities in product formation pathways.

The identification of A products of **I** is chemically and ecologically important. Two new unknown products (**III** and **IV**) were isolated and identified. The fact that the product **IV** is not stable and can re-form the initial parent compound (**I**) by elimination of 1 mol of HOBr is to be taken into consideration, since the toxicity of the solution may be restored.

The biodegradation studies confirmed the enhanced biodegradation and the decreased phytotoxicity, previously observed, of the pretreated solutions. The behavior of A-, B-, and C-treated solutions in the mineralization assays, using soil, activated sludge, and DRS-1 showed that the greatest extent of mineralization (% $^{14}CO_2$ recovery) was observed with the indigenous microorganisms in the soil and less with the sludge microorganisms and DRS-1 strain.

The phytotoxicity assay in which *N. tabacum* cv. Xanthi seeds were germinated in the presence of variously treated solutions and pure compound **IV** showed a very good reduction of phytotoxicity in the B and C solutions but a remaining toxicity in A and **IV** solutions.

The comparison of different criteria for selection of the most suitable degradation method of **I** solutions revealed that a combination of the methods B and C might be the most advantageous.

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