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Identification of Sensitized Photooxidation Products of Bromacil in Water

Aureliu J. Acher* and Ezra Dunkelblum

The dye sensitized photolysis of aerated aqueous solutions of bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) exposed to solar irradiation has been investigated. The major product, formed in 83% yield, has been isolated and identified as a mixture of diastereoisomers of 3-*sec*-butyl-5-acetyl-5-hydroxyhydantoin. The techniques used were a combination of gas chromatography–mass spectrometry, proton and carbon-13 nuclear magnetic resonance, and infrared spectroscopy. In addition, silylation, reduction, and oxidation of the photooxidation product were carried out for further confirmation of the proposed structure.

The fate of agricultural chemicals in the environment is of great interest and importance. The extensive use of pesticides is a serious ecological problem. Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil; I) is one of the most important herbicides used to control a wide range of grasses and broadleaf weeds (Martin, 1972). The photodecomposition of I has been studied in order to evaluate its fate in the environment. The UV-light photodecomposition of I was investigated in aqueous solutions (Kearney et al., 1969) and in solid films (Jordan et al., 1965), however, without the identification of the photoreaction products. Experiments done in simulated natural conditions with solar radiation (Moilanen and Crosby, 1974) yielded only 2.2% of a single dealkylated photoproduct (5-bromo-6-methyluracil). Addition of dye photosensitizers to aerated aqueous solutions of I leads to a quantitative and fast sunlight photochemical reaction (Acher and Saltzman, 1979).

Identification of the photoreaction products of I is important not only from the ecological point of view, but is also of photobiological interest since uracil derivatives are included in DNA and RNA. This accounts for some aspects of the photobiology of viruses and bacteria which contain these genetic materials (Wang, 1976).

This paper describes the formation and the identification of the sensitized photooxidation products of I in water.

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EXPERIMENTAL SECTION

Materials. Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil; I), provided by Agan Ltd., Israel, was chromatographically pure, mp 158–159 °C. Methylene Blue (MB) (BDH, No. 26132 Q) was used as a 0.5% water solution.

Instrumentation. Combined gas chromatography–mass spectrometry (GC–MS) was performed on a Varian 2740 GC, equipped with a flame ionization detector, 2 m × 3 mm glass column with 3% OV-17 on Gas-Chrom Q 80–100 mesh coupled to a DuPont 490 B low-resolution (EI) mass spectrometer. The elution started at 100 °C; the program rate was 6 °C/min up to 220 °C; the carrier gas was helium.

The chemical ionization mass spectrum (CI) was recorded on a similar spectrometer with isobutane as reagent gas. The sample was introduced through the direct probe. High-resolution mass spectra were recorded on a Varian MAT-731. The IR spectra were run on a Perkin Elmer 257 instrument in KBr pellets or dichloromethane 1% solutions. The ^{1}H-NMR spectra were carried out on a Varian A60 or on a Bruker WH-270 MHz spectrometer, and the ^{13}C-NMR spectra were run on a Varian FT-80A (20 MHz) spectrometer. All spectra are reported in δ from Me_4Si (internal standard). A Varian vis-UV spectrophotometer, Techtron, Model 635, was used for I deter-

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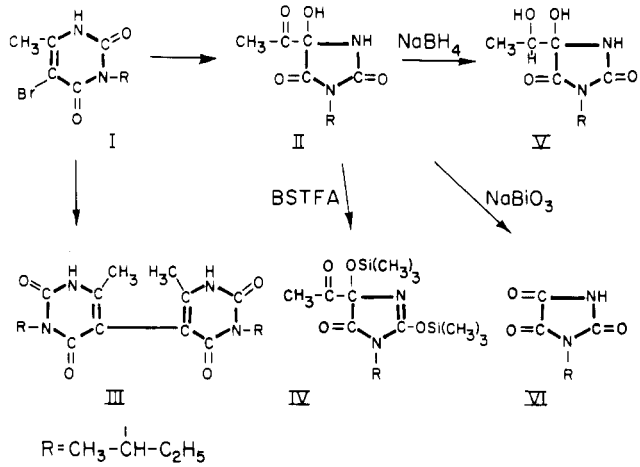
Scheme I



minations (at 278 nm). The average solar radiation intensity in the 400–700-nm range was measured by a quantum sensor (Lambda Instr. Corp., Nebraska) connected to a digital-integrator (Type TS 100A).

Irradiation. The photoreactions were carried out in Pyrex vessels with direct-outdoor-solar radiation.

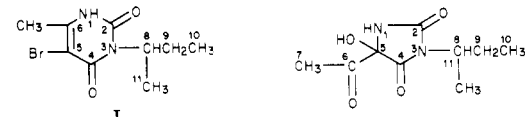
Procedure. Aerated aqueous solutions (2 L) of I (250 ppm) containing MB (2 ppm) at an initial pH of 9.4 (NaOH) were exposed to direct solar radiation (1800 μE m^{-2} s^{-1}) for 60 min at ambient temperature. The reaction was monitored by TLC (dichloromethane–ethyl acetate, 7:3) on commercial aluminum sheets (Merck, silica gel 60 G 254). The detection of I on TLC plates was done by UV light and of the main product II by iodine. The concentration of I was also determined by UV spectroscopy. The photoreaction was terminated before total disappearance of I in order to avoid extensive decomposition of II. The reaction mixture was lyophilized and the crude oily residue was dissolved in dichloromethane and submitted to column chromatography on silica gel (20 g, 70–200 mesh, Merck art 7734). The elution with solvent mixture of dichloromethane–ethyl acetate (8:2) yielded 0.34 g (83%) of an oily colorless product which was homogeneous on TLC with an R_f of 0.7 relative to R_I = 1.0.

RESULTS

Identification of the Photooxidation Products. GC analysis of the oily product obtained as described above showed one major peak of 97–98% of II, eluted at 167–170 °C (t_R 12 min) and a second peak (III), approximately 2% of the mixture, which was eluted at 220 °C (t_R 32 min) (see Scheme I). Traces of other products were observed but not analyzed.

The NMR spectra of the column purified product II showed great resemblance to that of the starting material I (see Table I). The ^{1}H-NMR spectrum proves the presence of the *sec*-butyl side chain and of the methyl group (C-7). The ^{13}C-NMR spectrum shows the presence of two amidic carbonyl carbons, C-2 (157.2) and C-4 (169.9), which have almost the same chemical shifts as the corresponding carbons (C-2, 156.0 and C-4, 171.0) in 1,3-dimethyl-5-hydroxyhydantoin used for comparison. The carbonyl carbon (C-6) at δ 200 is attributed to the acetyl group. The low-resolution GC–MS showed clearly that both the main product II and the minor one III lost the bromine. The product II showed the following significant peaks (at 17 eV), m/e (relative abundancy): 214 (8, M^+), 197 (1), 198 (1), 185 (8.5), 172 (39), 171 (71), 159 (8), 156 (6), 141 (13), 166 (35), 115 (100), 100 (7), 87 (6), 70 (11), and 57 (36). The CI spectrum had only two strong

Table I. ^{1}H and ^{13}C NMR Spectra of Bromacil (I) and Major Photooxidation Product (II)[a]



	I ^{1}H	I ^{13}C	II ^{1}H	II ^{13}C
NH	11.27 (brs)		7.2 (brs)	
C-2		159.9		157.2
C-4		152.8		169.9
C-5		97.6		85.7
C-6		148.5		200.0
C-7	2.36 (s)	19.8	2.33 (s)	23.7
C-8	4.95 (m)	52.8	4.03 (m)	50.6
C-9	1.95 (brm)	26.0	1.82 (brm)	26.3
C-10	0.87 (t), J = 7.0 Hz)	11.3	0.88 (t) J = 8 Hz	11.0
C-11	1.46 (d, J = 7.0 Hz)	17.5	1.39 (d, J = Hz)	17.7

[a] The chemical shifts of ^{1}H and ^{13}C are δ from Me_4Si. The ^{13}C spectra reported are proton decoupled. The NH signals disappeared on shaking with D_2O; the OH proton in II was not identified. The 270-MHz ^{1}H-NMR spectrum showed two multiplets at δ 1.810, 2.101 and δ 1.710, 1.940 for the two diostereotopic protons at C-9 in I and II, respectively.

Table II. High-Resolution MS, Significant Peaks, and Major Fragmentation of II

mass found	% abundancy	composition	structure	calcd mass
214.091	2.1	$C_9H_{14}N_2O_4$	M^+	214.095
197.091	2	$C_9H_{13}N_2O_3$	$M^+ - OH$	197.093
185.065	7.6	$C_7H_9N_2O_4$	$M^+ - C_2H_5$	185.056
172.085	89	$C_7H_{12}N_2O_3$	$M^+ - CH_2CO$	172.085
171.078	100	$C_7H_{11}N_2O_3$	$M^+ - CH_3CO$	171.077
159.040	4.1	$C_5H_7N_2O_4$	$M^+ - C_4H$	159.040
156.093	10.7	$C_7H_{12}N_2O_2$	$M^+ - C_2H_2O_2$	156.090
141.029	6.8	$C_5H_5N_2O_3$	$M^+ - C_4H_9O$	141.030
116.016	100	$C_3H_4N_2O_3$	$M^+ - C_6H_{10}O$	116.022
115.009	100	$C_3H_3N_2O_3$	$M^+ - C_6H_{11}O$	115.014

peaks, with the same abundancy at m/e 215 (M^+H) and 197 ($M^+H - H_2O$). The metastable ion at 180 indicates the 215 → 197 transformation, confirming the molecular weight of II and the presence of an OH group sensitive to protonation. The metastable ions at 137 and 77 in the EI spectrum correspond to the 214 → 171 and 117 → 115 transformations, indicating the loss of a CH_3CO group (43) and of isobutylene (56) by a McLafferty rearrangement (Safe and Hutzinger, 1973). The major fragments in the high-resolution MS (at 70 eV) confirmed the loss of CH_2CO and CH_3CO and subsequently the loss of C_4H_8 (see Table II).

The IR spectrum of a 1% dichloromethane solution of II confirmed the presence of an OH group at 3430 cm^{-1} (in KBr, a broad band at 3360 cm^{-1} due to hydrogen bonding) and two carbonyl bands at 1730 and 1800 cm^{-1}. Further confirmation for the proposed structure of II has been obtained by the following reactions, which proved (a) the presence of hydroxyl group at C-5 (by silylation), (b) the presence of an acetyl group at C-5 (by $NaBH_4$ reduction), and (c) the presence of an α-hydroxy ketone at C-5 (by $NaBiO_3$ oxidation) (see Scheme I).

(*a*) *Bis(2,5-trimethylsilyloxy)-3-sec-butyl-5-acetylhydantoin (IV).* Silylation with BSTFA [bis(trimethylsilyl)trifluoroacetamide] in acetone gave the bis(trimethylsilyl) derivative which was analyzed by GC–MS. The main fragmentation was similar to that of II, namely, loss of the acetyl group with subsequent loss of the side chain by a McLafferty rearrangement. The highest mass obtained was $M^+ - CH_3$. The most significant peaks (at

70 eV), m/e (relative abundancy) were 343 (4, $M^+ - CH_3$), 315 (56, $M^+ - CH_3CO$), 259 (16, $M^+ - CH_3CO$, C_4H_8), 244 (11), 186 (7), 147 (6), 116 (6), 100 (19), and 73 (100, $(CH_3)_3Si$).

(*b*) *3-sec-Butyl-5-(1-ethanol)-5-hydroxyhydantoin* (*V*). Mild reduction of II (50 mg) with $NaBH_4$ (0.5 g), in methanol (20 mL) at 0 °C for 1 h, gave the ethanol derivative. The ^1H NMR of the crude product (in D_2O) proved the transformation $CH_3CO \rightarrow CH_3CH(OH)$. The singlet at δ 2.33 disappeared and was replaced by a doublet at δ 1.27 (J = 7.0 Hz). The MS (17 eV) showed the following major peaks, m/e (relative abundancy): M^+ - absent, 198 (19, $M^+ - H_2O$), 156 ($M^+ - H_2O - CH_2CO$), 142 (21, $M^+ - H_2O - C_4H_8$), 100 (100, $M^+ - H_2O - Ch_2CO - C_4H_8$).

(*c*) *3-sec-Butyl-5-ketohydantoin* (*VI*). Selective mild oxidation of II (50 mg) in 50% acetic acid (4 mL) with $NaBiO_3$ (0.60 g) for 60 min was carried out at room temperature (Heller et al., 1962). After neutralization ($NaHCO_3$) and lyophilization, the residue was extracted with dichloromethane. The formation of 3-*sec*-butylparabanic acid (VI) (R_{VI} = 1.25 relative to R_{II} = 1.0) was confirmed by ^1H NMR (absence of the CH_3CO group at C-5) and the MS which had the following significant peaks (at 17 eV): 170 (1.3, M^+), 141 (51, $M^+ - C_2H_5$), 115 (24, $M^+ - C_4H_7$), 56 (100). A metastable ion at m/e 78 confirms the 170 → 115 transformation.

The combined data from NMR, MS, and IR spectra and the chemical transformations proved unequivocally the structure of II as 3-*sec*-butyl-5-acetyl-5-hydroxyhydantoin. The minor product III is probably a 5,5 dimer of 3-*sec*-butyl-6-methyluracil. Both in the EI and CI mass spectra a molecular peak at m/e 362 was observed, indicating the presence of III.

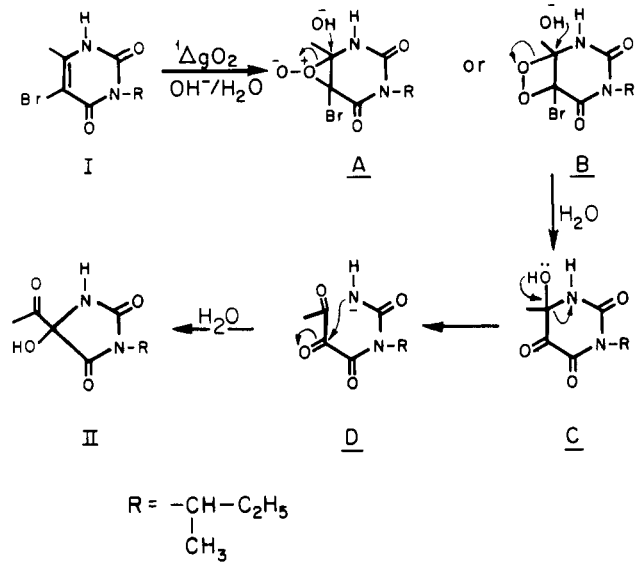
DISCUSSION

Moilanen and Crosby (1974) investigated the sunlight photodecomposition of I in water and found, after prolonged irradiation (4 months), only a dealkylated product, 5-bromo-6-methyluracil. The very low yield of the photoreaction product (2.2%) indicated that I is practically stable in sunlight.

The addition of MB (or other dye sensitizers), which is known to form singlet oxygen, to the photoreaction mixture (Foote, 1968), caused a fast chemical transformation of I. The necessary presence of MB and O_2 in the photoreaction mixtures was proved by carrying out blank experiments (without MB or O_2) which gave no photoreaction products. The rate of the reaction is pH dependent, being faster at higher pH (9.4) and almost negligible at lower pH (5.0). In the optimal outdoor experimental conditions, the photooxidation is complete after about 1 h; irradiation for longer periods resulted in the decomposition of II and the formation of more polar products (on TLC) which have not been identified so far. The presence of bromine in the substrate is essential for this process. No photoreaction products could be found when 3-*sec*-butyl-6-methyluracil was irradiated under identical conditions as I (Acher and Saltzman, 1979).

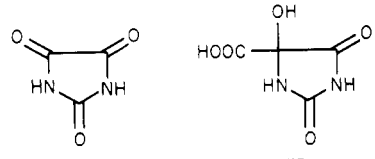
A plausible mechanism, proposed for the formation of II from I, is based on a reaction of singlet oxygen ($^1\Delta_gO_2$) with the double bond of I, followed by a fast OH^- catalyzed rearrangement with concomitant loss of bromine (Scheme II). The first step is the formation of the transient intermediate A or B. The formation of peroxiranes (type A) or dioxetanes (type B) is usually considered the first step in the reaction of $^1\Delta_gO_2$ with double bonds (Kearns, 1971), while radical mechanisms are normally ruled out (Kopecky and Reich, 1965). In the reaction of hetero-

Scheme II



substituted olefins (vinyl ethers, vinyl thioethers, and enamines), both peroxiranes and dioxetanes have been proposed as intermediates (Ando et al., 1973, 1974; Wasserman and Terao, 1975). In one case a dioxetane has even been isolated, in low yield, from a steroidal enamino ketone (Abello et al., 1975). The second step in Scheme II is an OH^- catalyzed rearrangement from A or B with the loss of bromine to produce the key intermediate C (3-*sec*-butyl-5-keto-6-hydroxy-6-methyluracil). Under the reaction conditions, C undergoes ring opening to D with subsequent ring closure to give the product II. In the last step a new chiral center is formed at C-5, in addition to the one already present at C-8, creating the possibility of the existence of two diastereoisomers. The ring closure to II can proceed either from the top or bottom of the plane leading to a 1:1 mixture of diastereoisomers, as was indeed observed. The 270-MHz ^1H-NMR spectrum (C-7, 2 s, δ, 2.335 and 2.340; C-11, 2 d, δ 1.381 and 1.395) and ^{13}C-NMR spectrum (C-9, δ, 26.257 and 26.352; C-11, δ, 17.615 and 17.759) revealed two sets of signals with the same intensity, confirming the formation of the two isomers in equal amounts.

The formation of minute amounts of the dimer III (Scheme I) may be explained by a different mechanism which involves a homolytic C–Br cleavage in I with subsequent dimerization. Radical mechanisms with C–Br cleavage have been postulated in the UV photochemistry of 5-halogenouracils (Ishihara and Wang, 1966a,b), in which the main products were dimers. The UV photochemistry of 5-iodouracil (Rupp and Prusoff, 1965) also involved free radicals which could be trapped with sulfides and thiols. Some hydantoin type compounds were obtained by both research groups. Ishihara and Wang (1966b) reported in one case on the formation of parabanic acid (VII) in about 2% yield; Rupp and Prusoff (1965) reported on the isolation of alloxanic acid (VIII) from



VII VIII

5-iodouracil (no yield was given). It is evident that these products are the result of oxidation processes; however no mechanism for their formation has been proposed.

Further investigation on the photooxidation of II might elucidate the possible correlation among the formation of VII, VIII, and II.

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Appreciation is expressed to E. Berman for the measurements of the ^{1}H-NMR 270-MHz and ^{13}C-NMR spectra and S. Zitrin for the CI spectra measurement.

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An Improved Analytical Procedure for Captafol Residues in Apple Wood, Leaves, and Fruit

Nanou Ngoran, Charles D. Ercegovich,[1] Kenneth D. Hickey,[2] and Ralph O. Mumma*

The fungicide captafol is currently used for the control of various crop fungal diseases and has been introduced recently in apple pest management programs for control of scab on both leaf and fruit in a single application technique. We report an improved analytical technique for captafol residues in apple wood, leaves, and fruits. The procedure involves extraction of plant tissue with acetonitrile, partitioning with a mixture of methylene chloride–petroleum ether, cleanup on a Florisil column, and quantitation by gas–liquid chromatography, employing an electron-capture detector. Captafol residues as low as 4 ppb were determined. Average recoveries of captafol from fortified samples were 93.0, 97.1, and 87.1% for apple leaves, wood, and fruit, respectively. Samples from apple trees treated with captafol at two concentrations and at two locations were analyzed periodically to demonstrate the practical application of this method and to determine captafol distribution in the orchards.

Captafol [*N*-(1,1,2,2-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide] is the active ingredient of the commerical formulation of Difolatan (Chevron Chemical Co., Ortho Division, Richmond, CA). It is currently used as a nonsystemic fungicide for the control of many of the major fungal diseases on fruits, ornamentals, vegetables, and turfgrass of economic importance (Chevron Chemical Co., 1965).

Techniques for residue analysis of various crops have been reported in the literature. Both gas and thin-layer chromatography (GC, TLC) methods have been used for the quantitative analysis of captafol residues in plant and animal tissues. Kilgore and White (1967) developed a procedure for the extraction and determination of captafol residues in apricots, cherries, nectarines, peaches, and prunes. The fruits were extracted with benzene (600 mL/300 g sample), analyzed directly, or cleaned up on attaclay–charcoal columns before analysis by GC employing electron-capture detection. Chevron Chemical Co. (1975) described a method using ethyl acetate as the extracting solvent (350 mL/25–50 g sample). Sample cleanup was composed of four steps: (1) ethyl acetate–water partition, (2) acetonitrile–hexane partition, (3) acetonitrile–water–hexane partition, and (4) Florisil column chromatography. Quantitation of the residues were performed by GC using either an electron-capture or flame-photometric detector.

Other techniques using GC for captafol residue determinations have been reported by Crossley (1972), Cooke (1973), Baker and Flaherty (1972), Pomerantz et al. (1970), and Lemperle and Strecker (1971). Chiba and Northover (1977) published a combined TLC and GC analysis of captafol residue in apple leaves and wood. They used tumbling and sonification extraction methods. Samples were purified by TLC prior to GC analysis. Although these methods are satisfactory with regard to recoveries, they generally involve time-consuming extraction and cleanup procedures and large volumes of purified solvents.

We now report the development of an improved analytical procedure for captafol in apple wood, leaves, and fruit and its practical application in the analysis of plant

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