

2d, 121212-35-9; 2e, 121212-36-0; 2f, 121232-49-3.

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Received for review December 5, 1988. Accepted March 21, 1989. This work was supported by a grant awarded by the U.S. Department of Agriculture (87-SBIR-8-0148).

Molecular Orbital Approaches to the Photolysis of Organophosphorus Insecticide Fenitrothion

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Photodegradation pathways of the organophosphorus insecticide fenitrothion (I) [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] in water are discussed from a theoretical point of view, using semiempirical molecular orbital (MO) calculations. Spectroscopic analyses and MO calculations showed that the Norrish type II intramolecular abstraction of the aryl methyl protons by the nitro group in the excited state is involved in the formation of *O,O*-dimethyl *O*-(3-carboxy-4-nitrophenyl) phosphorothioate (IV). The photooxidation of P=S to P=O was considered to proceed via the reaction of I with a hydroxyl radical and not with singlet molecular oxygen.

Photochemical processes play an important role in the degradation of the organophosphorus insecticide fenitrothion (I) in the environment (Mikami et al., 1985a). It undergoes various reactions such as oxidation of the P=S and the aryl methyl moieties and cleavage in the P—O aryl and the PO-CH₃ moieties with subsequent degradation to carbon dioxide. Although the environmental fate of I has been studied in detail, the degradation mechanism, especially photolysis, is still obscure at the molecular level.

In the case of photochemical transformation of a pesticide in pure water, direct photolysis, i.e. where the pesticide absorbs the incident irradiation directly, can occur (Zepp and Cline, 1977). However, active oxygen species generated by direct photolysis can react to give rise to further reactions often referred to as "indirect" photolysis. From this viewpoint, the reactions of pesticides with hydroxyl radicals, singlet molecular oxygen (Draper and Crosby, 1981), hydrogen peroxide (Draper and Crosby, 1984), and superoxide anion radicals (Draper and Crosby, 1983a) have been examined. Although both direct and indirect transformations of pesticides have been studied extensively, the influence of molecular properties such as electronic configurations in both the ground and excited

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states has not been studied in detail.

To clarify the degradation mechanism, therefore, the photolysis and degradation of I in an aqueous medium were examined under various conditions that generate active oxygen species followed by spectroscopic analysis of the resulting products. Furthermore, the semiempirical MO calculations such as AM1 (Austin model 1) and CNDO (complete neglect of differential overlap) were applied to the ground and excited states of I and its model compound to discuss the photodegradation pathways at the molecular level.

MATERIALS AND METHODS

Chemicals. Fenitrothion (I), *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphate (II), *O,S*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothiolate (III), *O,O*-dimethyl *O*-(3-carboxy-4-nitrophenyl) phosphorothioate (IV), and 3-methyl-4-nitrophenol (V) were synthesized in our laboratory according to methods already reported (Mikami et al., 1985b). [¹⁴C]-I uniformly labeled in the phenyl ring (73.6 mCi/g, radiochemical purity >99%) was synthesized in our laboratory (Yoshitake et al., 1976). Eosin, sodium hypochlorite, 30% hydrogen peroxide, and ferrous sulfate heptahydrate were purchased from Wako Chemical Co., Ltd. (Osaka). For the spectrophotometric assay of a trace amount of hydrogen peroxide, leuco crystal violet (Tokyo Kasei, Tokyo) and horseradish peroxidase (EC 1.11.1.7) (Type IV, 280 units/mg protein; Sigma, St. Louis) were used without further purification. Chloroform-*d* (CDCl₃) and water-*d*₂ (D₂O) of spectroscopic grade and H₂¹⁸O (97 atom % ¹⁸O) were purchased from E. Merck (Montreal). Pure water of electrical conductivity 0.055 × 10⁻⁶ mho/cm was supplied from a Puric Model-R equipped with a G-10 filter (Organo Co., Ltd., Osaka).

Spectroscopy. Proton nuclear magnetic resonance (¹H NMR) spectra were measured with a Hitachi R-90H Fourier transform spectrometer at 90 MHz in CDCl₃. Mass spectra were measured in electron-ionization (EI) mode at 70 eV with a Hitachi M-80 mass spectrometer equipped with a Hitachi M-003 data processing unit. Ultraviolet (UV) absorption spectra of I and V in pure water containing 1% acetonitrile were obtained with a Beckman DU-50 spectrophotometer in a quartz cuvette (1-cm path length). The radioactivity of carbon-14 was quantified with a Packard 460CD liquid scintillation spectrometer (LSC), using 10 mL of Packard Emulsifier-scintillator 299.

Photolysis Studies. [¹⁴C]-I (68.0 μg, 5 μCi) and 10 mg of nonlabeled I in 6.0 mL of CD₃OD-D₂O (5:1) were irradiated under air with a 500-W xenon arc lamp (Usio, UXL-5010AA, Tokyo) equipped with a Toshiba UV-D33S filter (experiment A). The emission spectrum with a cutoff of 220 nm and a maximum of 370 nm was obtained through this filter, as evidenced by the measurement with a Licor Model LI-1800 photometer. The higher energy transitions of I at ca. 200 nm, which do not occur in outdoor photolysis, are not anticipated under the conditions tested. An aliquot of 1.5 mL was taken periodically and concentrated under reduced pressure. A similar photolysis study was conducted under a nitrogen (>99.99%, N₂) atmosphere after removal of the dissolved molecular oxygen from the solution of I by freeze-pump-thaw procedures (five cycles) (experiment B). Furthermore, the possible participation of a water molecule in the photolysis of I was examined by using CH₃OH-H₂¹⁸O (5:1) as a reaction medium. The resultant photoproducts were separated by thin-layer chromatography (TLC), using the silica gel TLC plates (60F₂₅₄, 20 × 20 cm, 0.25-mm-layer thickness; E. Merck, Darmstadt) developed with toluene-ethyl formate-formic acid (5:7:1). Chemical identification of each photoproduct was carried out by TLC cochromatography with the nonlabeled authentic compounds. The amount of each product produced was determined by radioassay with LSC after autoradiography of the developed TLC plates. The H-D exchange and the incorporation of the ¹⁸O atom via photolysis were examined by measuring ¹H NMR and/or MS spectra of I or its photoproducts purified from the reaction mixtures.

Photolysis of I in pure water was conducted to examine the generation of hydrogen peroxide. An aqueous solution containing 10 ppm of I was prepared by adding 100 mL of pure water to 1 mg of I as a thin film in an Erlenmeyer flask and shaking the flask

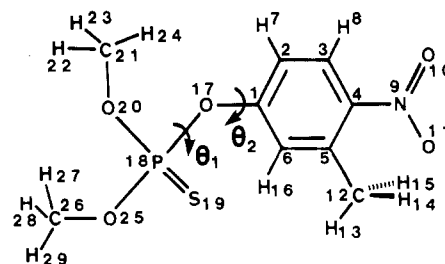


Figure 1. Chemical structure and atomic notations of I ($\theta_1 = \text{C}_1\text{-O}_{17}\text{-P}_{18}\text{-S}_{19}$, $\theta_2 = \text{C}_2\text{-C}_1\text{-O}_{17}\text{-P}_{18}$).

mechanically for 2 h in darkness. After irradiation with UV light, an aliquot of 1 mL was taken periodically in duplicate for spectrophotometric assay of hydrogen peroxide according to the method reported by Mottola et al. (1970). The photolysis study was repeated three times, and a dark control experiment was conducted in parallel. A standard response curve of hydrogen peroxide was prepared for concentrations of between 1 and 40 μM. Some phenol derivatives have been reported to generate hydrogen peroxide under irradiation (Draper and Crosby, 1983b). A similar assay was carried out for the 10 ppm aqueous solution of V after UV irradiation in order to examine the possible participation of V in the generation of hydrogen peroxide.

Reactions with Singlet Molecular Oxygen. The reaction was conducted, with use of the photochemically and chemically generated singlet molecular oxygen. A methanol solution (10 mL) of [¹⁴C]-I (19.9 μg, 1.46 μCi) and eosin (2 mg/mL) was similarly irradiated as in the photolysis studies (experiment C) (Draper and Crosby, 1981). An aliquot of 2.0 mL was taken periodically for TLC and LSC analyses to determine the amounts of I and each of the photolysis products. Meanwhile, the same amount of [¹⁴C]-I dissolved in 300 mL of methanol was treated with sodium hypochlorite (165 μM) and 30% hydrogen peroxide (165 μM) in darkness at 10 °C for 1 h (experiment D) (Ross and Crosby, 1975). As a control, the experiment was repeated without hydrogen peroxide or sodium hypochlorite. The production of singlet oxygen was verified with use of cyclon (2,3,4,5-tetraphenylcyclopentadienone; Aldrich Chemical Co.) as an acceptor, followed by the identification of 1,2-dibenzoylstilbene by GC-MS (Ross and Crosby, 1975). Since the electron donor is absent in this study, the hydrogen peroxide production is negligible based on the report by Usui et al. (1980).

Oxidation by Fenton's Reagent. The aqueous solution (10 ppm, 100 mL) of [¹⁴C]-I (19.9 μg, 1.46 μCi) was prepared as in the photolysis study. Ferrous sulfate heptahydrate granule (60 μM) was added to a rapidly stirred aqueous solution of [¹⁴C]-I at 25 °C after the addition of hydrogen peroxide (30 μM) and allowed to react for 15 min (experiment E) (Draper and Crosby, 1981). The oxidation was twice repeated after fresh addition of oxidant, followed by more ferrous sulfate. As a control, the experiment was repeated without ferrous sulfate (experiment F).

Theoretical Studies. Semiempirical MO calculations were carried out by using AM1 in AMPAC (Dewar et al., 1984), CNDO/2 (Hase and Schweig, 1973), and CNDO/M (Bene and Jaffe, 1968), loaded on a NEC ACOS System 430 or an IBM 4381 (MVS) computer. The modeling and display of a molecule were conducted by ACACS (Yoshida et al., 1985), and each atomic orbital in any MO was drawn by the LOBE program (Kurita and Takayama, 1987).

The lower energy conformation of I was estimated as follows. The initial geometry of V, the molecular fragment of I, was generated by ACACS according to the standard molecular geometries. This structure was then optimized by AM1 calculations and used together with the X-ray crystal structure of the *O,O*-dimethyl phosphorothioic acid moiety of bromophos (Baughman and Jacobson, 1976) to derive the initial geometry of I. The atomic notations are shown in Figure 1, together with two internal rotations defined by the dihedral angles θ_1 ($\text{C}_1\text{-O}_{17}\text{-P}_{18}\text{-S}_{19}$) and θ_2 ($\text{C}_2\text{-C}_1\text{-O}_{17}\text{-P}_{18}$). Conformational analysis by CNDO/2 calculations along each of the above rotations was conducted by stepwisely changing θ_1 or θ_2 in 15–30° with the other rotation fixed. The total energy relative to a minimum (ΔE , kcal/mole) was calculated for each conformation and subject to curve-fitting with a spline method to draw ΔE vs θ_1 or θ_2 plot.

Table I. Degradation of Fenitrothion (I)

compd	% of applied ¹⁴ C ^a					
	A	B	C	D	E	F
I	89.2	97.6	74.7	85.6	62.8	81.1
II	0.8	— ^b	1.6	1.3	9.8	0.8
III	1.0	0.8	—	—	—	—
IV	2.4	—	—	—	—	—
V	1.4	—	8.4	10.7	19.0	14.0
others	5.2	1.6	15.3	2.4	8.4	4.1

^a Key: A, irradiation with UV light for 4 h in CD₃OD-D₂O (5:1) under air; B, (A) but under nitrogen; C, (A) but in CH₃OH under air with eosin; D, incubation at 10 °C for 1 h in CH₃OH with hydrogen peroxide and sodium hypochlorite in darkness; E, aqueous solution of I with Fenton's reagent; F, (E) but without the ferrous sulfate. ^b Not detected.

On the basis of the results of CNDO/2 calculations for a lower energy conformer of I, the reaction indices defined as follows were calculated to estimate the reactive sites of I:

$$f_E = 2\sum_i (C_{i,HOMO})^2 \quad f_N = 2\sum_i (C_{i,LUMO})^2 \quad f_R = (f_E + f_N)/2$$

The f_E , f_N , and f_R are generally utilized to estimate susceptibility to electrophilic, nucleophilic, and radical reactions, respectively. HOMO and LUMO are the highest occupied and the lowest unoccupied MOs, respectively. C_i is the coefficient for atomic orbital i in the HOMO or LUMO.

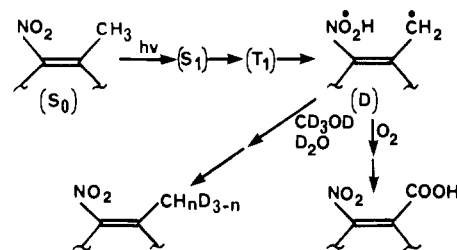
The bond strength of an individual chemical bond is considered to be changed via excitation of a molecule in direct photolysis. Since the photoinduced weakening of a specific bond may be followed by a bond cleavage, the change of bond strength can be a useful photolysis index to estimate which bond is likely to be cleaved in direct photolysis of a chemical. In the natural outdoor environment, chemicals are not subject to irradiation of wavelengths less than 290 nm. Consequently, only those transitions predominant at wavelengths greater than 290 nm should be characterized and used to calculate the photolysis index. On the basis of these considerations, the transitions in absorption of a chemical were analyzed, by CNDO/M calculations where the configuration interactions (CI) of the lowest 60 transitions were considered. Since the CNDO/M calculations of I could not be conducted due to the defect of the atomic parameters for the phosphorus and sulfur atoms, V, whose structure was obtained by AM1, was chosen instead of I for analysis of transitions. For the one-electron transition, considered to be the main participant in direct photolysis, the following photolysis index (ΔM_{ij}) applies (Katagi et al., 1988).

$$\Delta M_{ij} = (M_j - M_i)/2$$

$$M_i = 2\sum_{p,q} C_{ip}(A) C_{iq}(B) S_{pq}$$

ΔM_{ij} means the change of bond strength for an A-B bond via the one-electron transition from molecular orbitals i to j . $C_{ip}(A)$ and $C_{iq}(B)$ are the coefficients for atomic orbitals p (on atom A) and q (on atom B) in molecular orbital i , respectively. S_{pq} is the overlapping integral between atomic orbitals p and q .

The photooxidation of the aryl methyl group of I was studied theoretically with a model compound, *cis*-1-nitro-1-propene (VI), following the proposed reaction pathways shown in Figure 2. The molecular geometry of VI in the ground state (S_0) was obtained by AM1 calculations. The lowest 36 configurations are taken into account for the AM1 calculations of the lowest excited singlet (S_1) and the lowest excited triplet (T_1) states under the condition that the planar structure including the C=C double bond was not

**Figure 2.** Proposed reaction mechanism of the photooxidation of the aryl methyl group.

changed via molecular excitation. The aci-nitro intermediate (D) possessing a biradical electronic configuration was also calculated.

RESULTS

Photolysis Studies. I was photodecomposed with half-lives of 24 and 118 h under air and N₂, respectively. After 4-h irradiation of I under air, II-V, each amounting to less than 2.4% of the applied ¹⁴C, were identified as photoproducts (Table I). In contrast, III was the only product observed in the case of photolysis under N₂. These results are in agreement with results already reported by Mikami et al. (1985a) and show that molecular oxygen plays an important role in the photodegradation of I.

IV was only detected in the case of photolysis under air and considered to be produced via an aci-nitro intermediate (Morrison and Migdalof, 1965) followed by the reaction with molecular oxygen. Since the methyl proton in the phenyl ring is anticipated to be abstracted by the adjacent nitro group in the excited state, the photolysis of I in CD₃OD-D₂O would cause the H-D exchange of this methyl group. The number of the protons (n) in the CH_nD_{3-n} derivative of I and the amount of IV after the irradiation with UV light are summarized in Table II. The n value is estimated by (1) comparing the peak intensity of the methyl protons in the phenyl ring with those in the unchanged methoxy groups in ¹H NMR spectra and (2) analyzing the peak intensities at m/e 277 (parent peak of I), 278, 279, and 280 in EI-MS according to the method reported by Kanamaru et al. (1985). ¹H NMR spectra showed that the n value decreased with duration of irradiation from 2.71 at 8 h to 0.51 at 48 h. Similar results were obtained by EI-MS, and the analysis of peak patterns allows calculation of the amount of each CH_nD_{3-n} derivative of I. After 48 h irradiation, ca. 90% of the methyl groups was found to be CHD₂ or CD₃. As with the H-D exchange, the amount of IV increased to 10.4% relative to the applied I at 48 h.

II was also detected as an oxidation product via photolysis under air. After 60-h irradiation of I under air, MS spectra of II purified from the reaction mixtures by TLC were measured. The analysis of the peak intensities at m/e 261 (parent peak of II) and 263 showed that ¹⁸O was incorporated in ca. 17% of II under either the aerobic and anaerobic conditions, while no incorporation of ¹⁸O into IV was observed.

Amount of Hydrogen Peroxide. Hydrogen peroxide was detected by UV irradiation of an aqueous solution of I and amounted to 4.0 μM at 4 h, as listed in Table III.

Table II. H-D Exchange in the Methyl Group of I Produced by Photolysis

period of exposure, h	yield of IV, ^a %	% of deuterized methyl group ^b				n of CH _n D _{3-n}	
		CH ₃	CH ₂ D	CHD ₂	CD ₃	¹ H NMR	EI-MS
8	3.2	57.9	35.3	6.3	0.5	2.7	2.5
12	4.6	41.9	44.1	12.9	1.1	2.1	2.3
24	7.0	14.7	43.3	34.5	7.5	1.6	1.7
48	10.4	1.2	10.4	43.5	44.9	0.5	0.7

^a Estimated by TLC and LSC analyses. ^b Determined by EI-MS at 70 eV.

Table III. Amount of Hydrogen Peroxide Produced by Photolysis of I and V in Water

period of exposure, h	[H ₂ O ₂], ^a μM	
	A	B
1	1.6	ND ^b
2	2.1	ND
3	4.0	ND

^aPhotolysis: A, I (at 10 ppm); B, V (at 10 ppm). ^bNot detected.

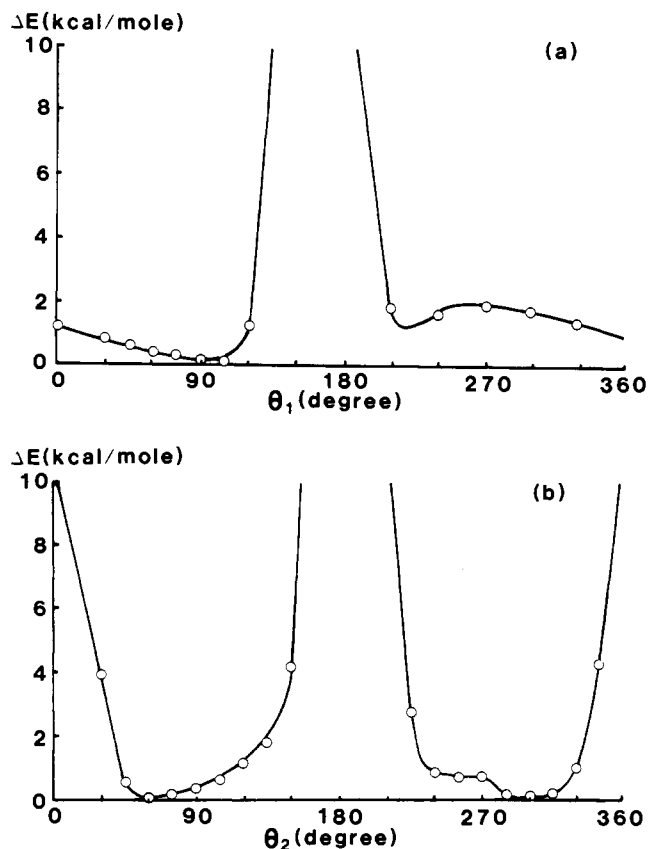


Figure 3. Conformational analysis of I by CNDO/2 calculations: (a) ΔE vs θ_1 in the case of $\theta_2 = 60.0^\circ$; (b) ΔE vs θ_2 in the case of $\theta_1 = 105.0^\circ$.

In contrast, no hydrogen peroxide was generated in darkness or from V under UV irradiation. These results suggest that the formation of hydrogen peroxide requires both I and irradiation.

Reactions with Singlet Molecular Oxygen. In both systems tested, II and V were formed at levels of 1.3–1.6% and 8.4–10.7%, respectively, of the applied ¹⁴C. Although the formation of II is slightly enhanced in the ¹O₂-generating systems as summarized in Table I (experiments A and C and D and F), the increased amount of II is small (0.5–0.8%) and seems to be within experimental error. Therefore, it may be inferred that singlet molecular oxygen does not accelerate the formation of II and V. In the case of experiment C, eosin appeared to act as a photosensitizer. Since V was the main degradation product (ca. 51%) when I was treated with sodium hypochlorite, V detected in experiment D is probably derived from a nucleophilic reaction of the hypochlorite anion with I.

Oxidation by Fenton's Reagent. II and V were produced in higher yields (9.8% and 19.0%, respectively) by Fenton's reagent (experiment E). In the case of experiment F, a similar amount of V (14.0%) was detected. These results strongly suggest that V is mainly formed by the reaction of I with hydrogen peroxide and that II is derived from the action of hydroxyl radicals.

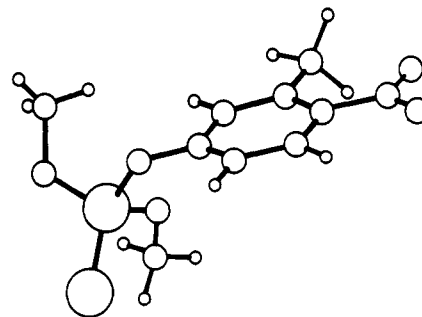


Figure 4. Ball-and-stick model of I.

Table IV. Reaction Indices Calculated for the Lower Energy Conformer of I

atom	f_E	f_N	f_R
C ₁	0.1726	0.0485	0.1106
C ₂	0.1999	0.0105	0.1052
C ₃	0.0116	0.0136	0.0126
C ₄	0.3420	0.0284	0.1852
C ₅	0.0631	0.0098	0.0365
C ₆	0.0735	0.0126	0.0431
H ₇	0.0009	0.0005	0.0007
H ₈	0.0015	0.0003	0.0009
N ₉	0.0019	0.0095	0.0057
O ₁₀	0.3804	0.0083	0.1944
O ₁₁	0.0063	0.0086	0.0075
C ₁₂	0.0138	0.0001	0.0070
H ₁₃	0.0000	0.0000	0.0000
H ₁₄	0.0145	0.0007	0.0076
H ₁₅	0.0125	0.0011	0.0068
H ₁₆	0.0018	0.0011	0.0015
O ₁₇	0.3225	0.1346	0.2286
P ₁₈	0.0564	0.8480	0.4522
S ₁₉	0.2545	0.5498	0.4022
O ₂₀	0.0209	0.1614	0.0912
C ₂₁	0.0013	0.0064	0.0039
H ₂₂	0.0009	0.0019	0.0014
H ₂₃	0.0007	0.0008	0.0008
H ₂₄	0.0000	0.0010	0.0005
O ₂₅	0.0106	0.1351	0.0729
C ₂₆	0.0010	0.0035	0.0022
H ₂₇	0.0000	0.0000	0.0000
H ₂₈	0.0000	0.0036	0.0018
H ₂₉	0.0000	0.0004	0.0002

Lower Energy Conformer of I. The results of conformational analysis of I for the rotations of θ_1 and θ_2 are shown in Figure 3. I possesses the lower energy conformation at $\theta_1 = 105.0^\circ$ and $\theta_2 = 60.0^\circ$, which are in good agreement with those observed in the X-ray crystal structure of bromophos (Baughman and Jacobson, 1976). On the basis of these data, the ball-and-stick model is shown in Figure 4.

Reaction Indices. The reaction indices of I are summarized in Table IV. The larger f_E values are observed for one of the nitro oxygen atoms (O₁₀), the P–O aryl oxygen atom (O₁₇), and the sulfur atom (S₁₉), whereas significantly larger f_N values are observed for the phosphorus atom (P₁₈) and the sulfur atom (S₁₉). The f_R index also exhibits the largest values for the P₁₈ and S₁₉ atoms.

The CNDO/M calculations showed that V possesses a predominant absorption at 256.857 nm with an oscillator strength of 0.3293, while the absorption spectrum of V in water containing 1% acetonitrile exhibited an absorption at 312.9 nm with a molar extinction coefficient (ϵ) of 6468.5 M⁻¹ cm⁻¹. Since the calculations are based on a molecule in the gas phase, this difference can be attributed to the effect of electrostatic polarization by water molecules. The estimated absorption mainly consists of HOMO → LUMO and HOMO-1 → LUMO transitions whose CI coefficients are 55.2 and 29.6%, respectively. This result shows that

Table V. Photolysis Index ΔM_{ij} of I Calculated for the One-Electron Transition from HOMO to LUMO

bond	$\Delta M_{ij} \times 10^3$	bond	$\Delta M_{ij} \times 10^3$
P ₁₈ -O ₁₇	-70.53	C ₁ -O ₁₇	+17.85
P ₁₈ -O ₂₀	-62.39	C ₄ -N ₉	+3.377
P ₁₈ -O ₂₅	-54.40	N ₉ -O ₁₀	-3.178
P ₁₈ -S ₁₉	-117.3	N ₉ -O ₁₁	+0.1540
O ₂₀ -C ₂₁	-2.361		
O ₂₅ -C ₂₆	-1.651		

the lower energy transitions such as HOMO \rightarrow LUMO predominate in the excitation. By analogy of the chemical structures between I and V, the photolysis index ΔM_{ij} of I was calculated for the one-electron transition from HOMO to LUMO based on the CNDO/2 calculations of I. The results are summarized in Table V. The P-O and P=S bonds of I are estimated to be significantly more weakened by direct photolysis than any of the other bonds. This would be attributed to the partial distribution of LUMO at the P=S moiety, which adds a charge-transfer character to the HOMO \rightarrow LUMO transition.

Excited States of Model Compound VI. VI possesses a planar structure in the ground state (S_0). The internal rotation of the methyl group is almost free (rotational energy barrier 0.45 kcal/mol), as evidenced by AM1 calculations. The total energy of S_1 relative to that of S_0 is 95.07 kcal/mol, which corresponds to absorption at 300.6 nm. Since the $S_0 \rightarrow S_1$ excitation of nitroethylene occurs at 305 nm (Morrison, 1969), AM1 calculations are likely to give a precise estimate of the total energy of the S_1 state of VI. For the T_1 state, the relative total energy to S_0 is 47.59 kcal/mol. In both states, 5.0–8.0% of the unpaired electrons is located on each nitro oxygen atom and ca. 3% on the methyl protons. In the case of the aci-nitro intermediate (D), the total energy relative to S_0 is 25.70 kcal/mol and 21.7–22.8% of the unpaired electrons is located on the methylene carbon.

DISCUSSION

Molecular Excitation of I. CNDO/M calculations simulate the electronic transitions of a chemical in the gas phase, while an absorption spectrum derives from the solvated chemical. Therefore, the estimated absorption maximum must be shifted to take account of the effect of the electronic polarization by water molecules. Bayliss (1950) has described a quantum mechanical method of evaluating the shift of an absorption maximum by using the wavenumber and oscillator strength of a transition, the apparent radius of a spherical solute molecule, and the refractive index of the solvent. On the basis of a knowledge of the molecular geometry of the AM1 structure, the spherical radius of a molecule of V can be calculated to lie between 2.5 and 3 Å. Using this value and taking the refractive index of water at ca. 1.33, it can further be estimated that the absorption maximum of the molecule in aqueous solution will be between 298 and 337 nm. The observed peak occurs at 312.9 nm and lies well within this range, in contrast with the absorption peak in the gas phase occurring at 256.857 nm. There is, therefore, a strong suggestion that the observed absorption of V at 312.9 nm is mainly due to an HOMO \rightarrow LUMO transition. Assuming that the lowest energy excitations of I and V would occur at roughly the same wavelength in the gas phase and that the spherical radius of I is 4.0–4.5 Å, the absorption maximum of I in water can be calculated to be between 267.8 and 272.7 nm, which is in good agreement with the observed value (270 nm, $\epsilon = 6500 \text{ M}^{-1} \text{ cm}^{-1}$). Therefore, the ΔM_{ij} for the one-electron HOMO \rightarrow LUMO transition, evaluated from the results of CNDO/2 calculations of I, would predict that direct photolysis of I will predominate.

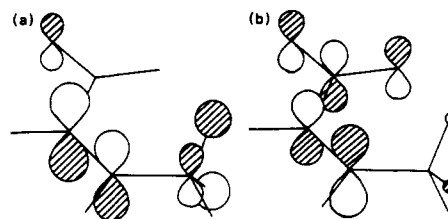


Figure 5. Molecular orbitals of the model compound VI prepared by LOBE program based on AM1 calculations: (a) HOMO; (b) LUMO.

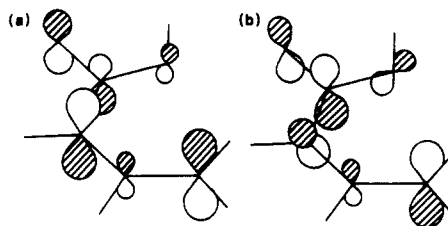


Figure 6. Singly occupied molecular orbitals (SOMO) of the model compound VI in the D state prepared by the LOBE program based on AM1 calculations: (a) SOMO at a lower energy level; (b) SOMO at a higher energy level.

The reaction index f_N shows that nucleophilic attack by a water molecule would occur mostly at the phosphorus atom of I. Since the P-O aryl bond is considered to be weakened by molecular excitation, it is anticipated that V is more easily formed under irradiation than by hydrolysis in darkness. A similar effect will be observed for the phosphate derivatives. The photochemical acceleration of the hydrolysis of nitrophenyl phosphates has been reported previously (Havinga et al., 1956). This can now be predicted at the molecular level by the present methods.

Photooxidation of the Aryl Methyl Group. Photolysis studies indicate that the formation of IV from I requires molecular oxygen and light energy but that active oxygen species such as singlet molecular oxygen or the hydroxyl radical do not participate in this photooxidation process. Consequently, it is the reaction of I in the excited states with molecular oxygen that initiates the photooxidation of the aryl methyl group. Meanwhile, the H-D exchange in the aryl methyl group proceeds concurrently with the increase of IV, which can be elucidated by the reaction mechanism via an aci-nitro intermediate (Morrison and Migdalof, 1965). The reaction of the 1,4-biradical, which is formed via the Norrish type II reaction from the excited triplet state, with the deuterized solvent (CD_3OD or D_2O), is known to cause efficient H-D exchange (Small and Scaiano, 1977). In the case of the reaction of the 1,4-biradical with molecular oxygen, it is likely that the hydroxymethyl derivative of I is formed and then successive oxidation in a similar mechanism leads to the formation of IV.

AM1 calculations of the model compound VI in the S_1 state show that the two unpaired electrons distribute in the singly occupied MO (SOMO) whose electronic configuration is similar to that of HOMO or LUMO in the S_0 state. Therefore, the predominant excitation is likely to $n \rightarrow \pi^*$ (Figure 5) where the n orbital has π character, similar to that reported previously by Kasha (1960). The hydrogen abstraction by the oxygen atom of the nitro group would proceed via molecular excitation to form the biradical intermediate (D), as evidenced by Figure 5. The atomic orbitals of SOMO of the D state are shown in Figure 6, which implies the high reactivity of the methylene carbon with a solvent molecule or molecular oxygen. These pathways are thermodynamically possible on the basis of

the order of energy level of each state: $S_1 > T_1 > D$. On the basis of these results, it is likely that the similar excitation proceeds in the case of I, which results in the photooxidation of the aryl methyl group.

Photooxidation of the P=S Moiety. II was photochemically formed in the presence of molecular oxygen in the same way as IV, but singlet molecular oxygen does not participate in the formation of II. The low degree of incorporation of the ^{18}O atom in II shows that the P=O oxygen of II mainly originates from molecular oxygen. However, hydrogen peroxide (ca. 4 μM) is produced during photolysis. It is considered that I acts as a photosensitizer like eosin and produces hydrogen peroxide from molecular oxygen (Usui et al., 1980). As shown in Table I, V is the main degradation product and a trace amount of II is detected when I is treated with hydrogen peroxide. Therefore, it is unlikely that the nucleophilic reaction of hydrogen peroxide with I leads to the formation of II (Stec et al., 1976). In the case of the treatment of I with Fenton's reagent, II is formed in a higher yield (experiment E). These results strongly suggest that a hydroxyl radical may be involved in the photoinduced formation of II. The hydroxyl radical is probably produced from hydrogen peroxide via homolysis of the weak peroxygen bond by photon absorption (Volman, 1963). The reaction index f_R also indicates that the possible sites attacked by a hydroxyl radical are the phosphorus and sulfur atoms. Since the configuration at the phosphorus atom of the organophosphorus compound S-2571 (*O*-ethyl *O*-2-nitro-5-methylphenyl *N*-isopropyl phosphoramidothioate) has been reported to be preserved in the photooxidation of P=S to P=O (Mikami et al., 1977), the nucleophilic attack by a hydroxyl radical on the phosphorus atom of I would proceed along the P-O axis from the opposite side to the most apicophilic oxo ligand (Stec et al., 1976). Meanwhile, the low incorporation of ^{18}O indicates that a water molecule participates in the formation of hydrogen peroxide. Deep-UV light is known to generate a hydroxyl radical and superoxide (Holroyd and Bielski, 1978). Since the photolysis study was conducted by using UV light with wavelengths of >220 nm, the direct photolysis of H_2^{18}O might proceed as a side reaction.

Other Photoreactions. The migration of the methoxy methyl group to the sulfur atom to form III is observed only under irradiation. Although this process was not studied in detail, the greater localization of LUMO at the sulfur atom implies a possible migration of the methoxy methyl group to the sulfur atom.

Mikami et al. (1985a) have reported that the hydroxylation at the 4-position of the phenyl ring and the reduction of the nitro group proceed via photolysis of I. The former reaction may be elucidated by the reaction of a hydroxyl radical at the C_4 atom of I, which possesses the larger f_R value. Meanwhile, the AM1 calculations of the radical produced via hydrogen abstraction by the methylene carbon of VI in the D state show that the unpaired electron is mainly located at the nitro nitrogen (32.8%) and the two oxygen atoms (39.0%). This result suggests that the further abstraction of a hydrogen atom from a solvent molecule, leading to the formation of the amino derivative of I, is feasible.

Registry No. I, 122-14-5; II, 2255-17-6; III, 3344-14-7; IV, 54812-31-6; V, 2581-34-2; O_2 , 7782-44-7; ferrous sulfate heptahydrate, 7782-63-0.

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Received for review September 23, 1988. Revised manuscript received January 17, 1989. Accepted February 23, 1989.

Determination of GX-071 and Its Major Metabolite in Rat Blood by Cold On-Column Injection Capillary GC/ECD

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GX-071 (*N*-ethylperfluorooctanesulfonamide) is currently undergoing general toxicologic studies to support its registration for use as a pesticide. To aid in these investigations, methodology was developed for the determination of GX-071 and its major metabolite as their pentafluorobenzyl derivatives from 20–40 μ L of rat blood at ppm to ppb levels using cold on-column injection capillary GC/ECD. The compounds were extracted from an alkaline aqueous phase to a methylene chloride phase as ion pairs and derivatized with pentafluorobenzyl bromide. The major metabolite of GX-071, perfluorooctanesulfonamide (GX-071M), was identified by cold on-column injection capillary GC/MS from the blood of both rats and dogs that had been given oral doses of GX-071. The percent RSD of the procedure was 4.7 and 2.8 at 100 ppm and 17 and 21 at 400 ppb for GX-071 and GX-071M, respectively.

The establishment of critical toxicokinetic parameters of a chemical or a drug such as absorption, distribution, biotransformation, and elimination requires many repeated analyses of blood and other biological fluids from laboratory animals dosed with the compound of interest. The laboratory animal of choice for these investigations is often the rat. However, the size of laboratory rats places constraints on the volume of blood that can be taken at any one time without seriously compromising the results of later analyses. Therefore, methods for the determination of a compound in rat blood must be able to do so from small sample sizes if they are to be routinely useful in toxicokinetic/pharmacokinetic investigations.

Identification of major metabolites and the determination of the parent compound and major metabolites in biological fluids are essential parts of pharmacological studies of a chemical in regard to its toxicity from in vivo exposure. This work was done as part of a study of the pharmacokinetics and toxicokinetics of GX-071 (*N*-ethylperfluorooctanesulfonamide) to support its registration as a pesticide. GX-071 is being evaluated as a pesticide for the red imported fire ant. Such investigations are crucial to the evaluation of a chemical in regard to its intended uses and anticipated human exposures (Yakatan and Iglesia, 1984; Garrett, 1974). GX-071 and its major metabolite, perfluorooctanesulfonamide (GX-071M), were identified in the blood of rats and dogs that had been given

oral doses of GX-071. This methodology included the separation of an ethyl acetate extract of whole blood by cold on-column injection capillary gas chromatography (GC) (Arrendale and Chortyk, 1985). Capillary gas chromatography/mass spectrometry (GC/MS) was used to confirm the identification of GX-071 and GX-071M.

Fluorocarbons, such as GX-071, are known for their chemical and thermal stabilities (Metcalf, 1970; Smith, 1970). Among its physical properties, GX-071 possesses thermal stability and resistance of hydrolyses. The hydrogen attached to the sulfonamide nitrogen is acidic, and the compound forms salts with strong bases. However, the compound is lipophilic and has very low solubility in water, and solutions exhibit a neutral pH. Although it is a sulfonamide, GX-071 does not fit the general description often applied to derivatives of sulfanilamide (Anand, 1979). Thus, its pharmacokinetic/toxicokinetic properties including metabolism were not expected to parallel those normally associated with the classical sulfonamides (Hekster and Vree, 1982).

Both GX-071 and GX-071M are lipophilic in nature and can be extracted from blood with ethyl acetate. Furthermore, their sulfonamide hydrogens are acidic, and their anionic species form ion pairs with tetrabutylammonium as the counterion. Methodology was developed for the extraction of GX-071 and GX-071M as ion pairs with the tetrabutylammonium counter ion from 20–40 μ L of rat blood in an alkaline aqueous phase to a methylene chloride phase. This was followed by the addition of pentafluorobenzyl bromide to the methylene chloride with subsequent formation of their PFB derivatives, which were analyzed by cold on-column injection capillary GC with electron capture detector (ECD) using an internal standard spiking technique at ppm to ppb levels.

A recent study (Schnellmann, 1989) indicated that GX-071 and GX-071 M were potent uncouplers of oxidative phosphorylation. Therefore, both compounds were han-

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