Photodecomposition of Salithion® (2-Methoxy-4*H*-1,3,2-Benzodioxaphosphorin-2-Sulfide) and Phenthoate (O,O-Dimethyl S- α -Ethoxycarbonylbenzyl Phosphorodithioate)

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On irradiation with UV light and sunlight, Salithion® and phenthoate were rapidly decomposed in acetone solution and on both silica gel and soil surfaces, whereas both compounds were fairly stable in water solution exposed to sunlight. Under the tested conditions, both Salithion and phenthoate were decomposed predominantly by ways of oxidation of $P=S$ to $P=O$, oxidation of the methylene or methin groups of benzyl moiety, cleavage of $P-O$ -aryl and $P-O$ -aralkyl linkages and decarboxylation. Salithion was isomerized to S-benzyl salithion. On soil surfaces, the carboxy ester linkage of phenthoate was also hydrolyzed. Anticholinesterase activity of the photoproduct S-benzyl salithion against plasma and erythrocyte enzymes was close to that of salioxon and S-methyl salithion, but the product was almost non-toxic to houseflies.

INTRODUCTION

Both Salithion® (2-methoxy-4*H*-1,3,2-benzodioxaphosphorin-2-sulfide) and phenthoate (O,O-dimethyl S- α -ethoxycarbonylbenzyl phosphorodithioate) have a benzyl moiety in their structures and are effective in controlling a broad spectrum of insect pests. It has been demonstrated that Salithion was rapidly metabolized in mammals^{1,2)} and plants³⁾ by ways of oxidation of $P=S$ to $P=O$, cleavage of $P-O$ -aryl and $P-O$ -aralkyl linkages, desmethylation and conjugations of the resulting alcohols. Phenthoate was also metabolized in mice, houseflies and plants^{3,4)} through oxidation of $P=S$ to $P=O$, cleavage of $P-O$ -aralkyl linkage, hydrolysis of ethoxycarbonylbenzyl group and desmethylation. From these data, both insecticide chemicals appear to be biodegradable in animals and plants.

The present investigation was conducted to elucidate photodecomposition of Salithion and phenthoate when irradiated with UV light and

sunlight in solutions and residual deposits on both silica gel and soil surfaces.

MATERIALS AND METHODS

1. Spectroscopy

Nuclear magnetic resonance spectra (nmr) were determined in appropriate deuterated solvents using tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-1-sulfate as an internal standard, on a Hitachi R-20B spectrometer at 60 MHz. Electron impact mass spectra (ms) were obtained with a Shimadzu-LKB 9,000 mass spectrometer at 12 and 70 eV. Infrared spectra (ir) were recorded on a Hitachi EPI-G3 spectrometer. Samples were analyzed as a KBr disk or a thin film on a NaCl crystal.

2. Chromatography

Thin-layer chromatography (tlc) utilized precoated silica gel 60F-254 chromatoplates (with fluorescent indicator, 20 $\times$ 20 cm, 0.25 mm gel thickness, E. Merck) for analytical and preparative purposes, and silica gel 60 chro-

Table 1 *Tlc* solvent systems and *Rf* values for Salithion, phenthoate and their photoproducts.

Compound	Solvent system			
	A	B	C	D
Salithion				
S1 Salithion	0.88	0.82	0.89	
S2 S-Benzyl salithion	0.78	0.75	0.83	
S3 Saligenin	0.76	0.75	0.80	
S4 Salicylic acid	0.74	0.75	0.78	
S5 Salioxon	0.70	0.73	0.72	
S6 2-OHC- ϕ -O-P(S)(OH)OCH ₃	0.35	0.62	0.28	
S7 2-OHC- ϕ -O-P(O)(OH)OCH ₃	0.12	0.50	0.14	
S8 2-HOOC- ϕ -O-P(S)(OH)OCH ₃	0	0.45	0	
S9 2-HOOC- ϕ -O-P(O)(OH)OCH ₃	0	0.30	0	
S10 2-HO- ϕ -CH ₂ O-P(S)(OH)OCH ₃	0.20	0.47	0.18	
S11 2-HO- ϕ -CH ₂ O-P(O)(OH)OCH ₃	0.05	0.41	0.08	
Phenthoate				
P1 Phenthoate	0.76		0.91	0.87
P2 Benzoic acid	0.59		0.72	
P3 Phenthoate oxon	0.55		0.67	0.50
P4 Phenylglyoxylic acid	0.22			
P5 HS-CH(ϕ)-COOC ₂ H ₅	0.72		0.85	
P6 Ethyl mandelate	0.65		0.75	0.54
P7 Carboxyphenthoate	0.47		0.50	0.54
P8 HS-CH(ϕ)-COOH	0.25		0.36	
P9 Mandelic acid	0.18		0.27	0.20
A, toluene/ethyl acetate/acetic acid	(5/7/1)			
B, ethyl acetate/ethanol/water	(4/2/1)			
C, toluene/ethyl formate/formic acid	(5/7/1)			
D, benzene/ethyl ether/acetic acid	(9/2/1)			

matoplates (without fluorescent indicator, 20×20 cm, 0.25 mm gel thickness, E. Merck) for photodegradation study. *Tlc* solvent systems used and *Rf* values for various derivatives of Salithion and phenthoate are listed in Table 1. Unlabeled reference compounds were detected as follows; (1) ultraviolet fluorescence quenching, (2), 1% palladium chloride in 1 N HCl (to detect sulfur compounds), (3) 0.5% 2,6-dibromoquinonchloroimide (DQC) in ethanol (to detect sulfur compounds and phenols), (4) 1% 2,4-dinitrophenyl hydrazine (2,4-DNP) in 2 N HCl (to detect aldehydes and ketones) and (5) 4% nitroprusside (to detect mercaptans). Photoproducts were purified on a silicic acid column (100 mesh, Mallinckrodt Chemical Works, St. Louis, Mo. U.S.A) by eluting with appropriate solvent systems and by preparative *tlc*. Radioactive products were detected by radioautography and radioscanning with a

model TRM-1B *tlc* scanner (Aloka, Tokyo, Japan).

3. Radioanalysis

The radiocarbon content of organosoluble fractions from extraction of various materials and gel regions from *tlc* plates was determined with a Packard model 3385 liquid scintillation spectrometer, using 15 ml of a dioxane scintillation fluid (5 g of 2,5-diphenyloxazole and 100 g of naphthalene in 1 liter of dioxane). Unextractable residues were combusted with a Packard model 306 sample oxidizer prior to radioanalysis. Industrial X-ray 150 films (Fuji Photo Film Co., Ltd., Tokyo, Japan) were used for radioautography.

Percent distribution of radioactive spots was determined by scraping gel regions from *tlc* chromatoplates and counting them with a liquid scintillation spectrometer.

4. Chemicals

^{14}C -Salithion (3.7 mCi/mmole) and ^{14}C -phen-thoate (5.67 mCi/mmole), each labeled at the methylene and methin carbon of the benzyl group, respectively, were prepared in Institute for Biological Science, Sumitomo Chemical Co., Ltd., Takarazuka, Japan.^{2,5)} The radiochemical purity of each labeled compound was greater than 99% based on *tlc* analysis. Unlabeled Salithion (mp. 56°C) was purified by repeated recrystallization from ethanol, and phenthioate was purified on a silicic acid column by eluting with *n*-hexane/acetone (5/2) before use. Salix-on (2-methoxy-4*H*-1,3,2-benzodioxaphosphorine-2-oxide) and *S*-methyl salithion (2-methylthio-4*H*-1,3,2-benzodioxaphosphorine-2-oxide) were prepared according to the methods of M. Eto *et al.*⁶⁾ and K. Kobayashi *et al.*,⁷⁾ respectively. Phenthioate oxon (*O,O*-dimethyl *S*- α -ethoxycarbonylbenzyl phosphorodithioate) and ethylmandelate were synthesized according to the method of Hirose *et al.*⁴⁾ 2-Hydroxybenzyl mercaptan was synthesized in the following. Saligenin (5 g) was quantitatively methylated with diazomethane in ether. A mixture of the obtained 2-methoxybenzyl alcohol (5.1 g) and thionyl chloride (1.2 g) in 20 ml ether was refluxed for 1 hr. After concentration of the reaction mixture, 2-methoxybenzyl chloride was obtained in 90% yield. A solution of the chloride (5.1 g) and thiourea (3 g) in ethanol was refluxed for 30 min. To this reaction mixture, 20 ml of 5 *N* sodium hydroxide solution was added and then the mixture was refluxed for additional 2 hr. The reaction mixture was extracted with ether, and the extracts were concentrated. The residual oil (3.8 g) was treated with boron tribromide in dichloromethane at 10°C for 2 hr, and then the dichloromethane solution was washed with ice water. The solvent was removed *in vacuo* and the residual oil was purified on a silicic acid column by eluting with benzene/ethyl acetate (1/1, v/v). The yield of 2-hydroxybenzyl mercaptan was 2.0 g: nmr (CDCl_3) δ 1.84 (1H, *t*, $J=7$ cps, SH), 3.75 (2H, *d*, $J=7$ cps, CH_2) and 6.7–7.3 ppm (4H, *m*, aromatic); ir (direct) 3400 (OH), 2550 (SH), 1600 and 1500 cm^{-1} (aromatic ring); ms *m/e* 140 (M), 138, 137, 107 (base peak), 79 and 77.

2-Mercaptophenylacetic acid was obtained

in the following. Ethyl α -bromophenyl acetate (2.0 g) in ethanol was added dropwise to 300 mg of sodium ethoxide in ethanol solution saturated with hydrogen sulfide. The reaction mixture was kept at 60°C for 3 hr and then poured into ice water and the product was extracted with ethyl acetate. Distillation of the residue after concentration of the ethyl acetate solution yielded 800 mg of ethyl 2-mercaptophenylacetate: bp 118°C/0.4 mmHg; nmr (CDCl_3) δ 1.15 (3H, *t*, $J=7$ cps, CH_3), 2.55 (1H, *d*, $J=7$ cps, SH), 4.20 (2H, *q*, $J=7$ cps, CH_2), 4.65 (1H, *d*, $J=7$ cps, CH) and 7.3 ppm (5H, *m*, aromatic); ir (direct) 2550 (SH), 1730 (COOC_2H_5), and 1600 and 1490 cm^{-1} (aromatic ring). Ethyl 2-mercaptophenylacetate (400 mg) was then hydrolyzed in 1 *N* sodium hydroxide in ethanol for 10 hr at room temperature. After the solvent was removed, the residue was purified on a silicic acid column chromatography by eluting *n*-hexane/acetone (2/1, v/v). The yield of 2-mercaptophenylacetic acid was 220 mg: nmr (CDCl_3) δ 2.6 (1H, *d*, $J=7$ cps, SH), 4.7 (1H, *d*, $J=7$ cps, CH) and 7.3 ppm (5H, *m*, aromatic); ir (KBr) 2500–3000 and 1710 (COOH), 2550 (SH), and 1600 and 1500 cm^{-1} (aromatic ring).

Carboxyphenthioate (*O,O*-dimethyl *S*- α -carboxybenzyl phosphorodithioate) was obtained by enzyme-catalyzed hydrolysis of phenthioate. A 20% (w/v) rat liver homogenate in 1.15% KCl was centrifuged at 8,000 $\times g$ for 10 min at 2°C and the supernatant fraction was used as follows: To 200 ml of the supernatant fraction, 30 ml of 0.5 *M* phosphate buffer, pH 7.4 and 30 ml distilled water were added, and then 250 μmole phenthioate in ethanol was added. The mixture was incubated at 37°C for 1 hr and then adjusted to pH 1 with 1 *N* HCl, followed by extraction with 300 ml of chloroform. The extracts were concentrated and purified by preparative *tlc* in solvent system A. The structure of carboxyphenthioate was confirmed by ir and nmr, and by converting carboxyphenthioate to phenthioate with diazoethane in methanol/ether mixture (1/1, v/v): nmr (CD_3OD) δ 3.4–3.6 (6H, *dd*, $J=16$ cps, $2\times\text{OCH}_3$), 4.7 (1H, *d*, $J=13$ cps, CH) and 7.2–7.5 ppm (5H, *m*, aromatic); ir (KBr) 1600 and 1400 (COO^-), 1020 (*P*-*O*-alkyl) and 660 cm^{-1} (*P*=*S*).

Saligenin, salicyl aldehyde, salicylic acid, benzoic acid and mandelic acid were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo Japan). Ethyl benzoylformate was obtained from Aldrich Chemical Co., Inc. Milwaukee, Wisconsin.

5. Photodecomposition

^{14}C -Salithion or ^{14}C -phenthoate (150 μCi , 1 g) dissolved in 100 ml acetone was irradiated with a high pressure mercury vapor lamp (45 W, Ushio Electric Co., Ltd., Osaka, Japan) in a quartz photoreactor. During irradiation, air was bubbled as reported previously.⁹⁾ The evolved ^{14}C -carbon dioxide and other volatile ^{14}C -products were trapped in an alkaline solution and dry ice-acetone. Aliquots of the reaction mixture were removed at various intervals and analyzed for photoproducts by *tlc*. After irradiation, the remaining reaction mixture was concentrated and the individual photoproducts were purified by silicic acid column chromatography and by preparative *tlc* in appropriate solvent systems. For preparation of photoproducts, Salithion (0.025 mg/cm²) was deposited on silica gel *tlc* plates and then irradiated with UV light for 60 min below 172.5 watt high pressure UV lamp (Taika, Kogyo, Osaka, Japan). The photoproducts were extracted with methanol from silica gel and then purified as described above.

^{14}C -Salithion (9 ppm) or ^{14}C -phenthoate (11 ppm) in aqueous solutions were exposed to sunlight in 1-liter quartz flasks for up to 20 days, from September to October, 1974, at Takarazuka, Hyogo, Japan. The irradiation time was about 8 hr per day. At intervals, 30 ml aliquots of the solution were removed, adjusted to pH 1 with 1 N HCl and extracted three times with an equivalent volume of ethyl acetate. These extracts were combined and concentrated.

For sunlight irradiation on silica gel *tlc* plates, each radiolabeled chemical (50 μg) was spotted on *tlc* plates and then exposed to sunlight for up to 8 days. Then, these chromatoplates were directly developed in appropriate solvent systems.

Soil thin layer plates (500 μm layer thickness, 10 $\times$ 10 cm) were prepared according to the method of Helling *et al.*¹⁰⁾ Two types of soils

were used in the present study. The characteristics of the soils used were reported in a previous paper.¹¹⁾ The plates were treated with either ^{14}C -Salithion (20 μCi) or ^{14}C -phenthoate (20 μCi) in ether. The chemicals were applied to the soil plates at a rate of 0.01 mg/cm². The treated soil plates were exposed to sunlight for 6 or 12 days. After exposure the soils were extracted with 30 ml of methanol/water (3/1, v/v) and then three times an equivalent volume of methanol/1 N HCl (3/1, v/v) mixture. These extracts were combined and concentrated. The residues were dissolved in 0.5 ml of methanol and aliquots of the solution were subjected to *tlc*.

In all cases, a dark control was used to ensure that given products were derived from photochemical processes.

6. Characterization of Photoproducts

Individual products obtained from Salithion and phenthoate in acetone solutions on irradiation with UV light were purified by silicic acid column chromatography and by preparative *tlc* in solvent systems A and B for Salithion and A and C for phenthoate. Each of these photoproducts were further purified on a Sephadex LH-20 (Pharmacia Fine Chemicals, Sweden) column using methanol as a solvent. Each photoproduct was identified by ir, nmr and ms, and/or *tlc* cochromatography with authentic standards in solvent systems A, B and C for Salithion, and A, C, and D for phenthoate. On the other hand, photoproducts obtained from aqueous solution or surface deposits of both silica gel and soil, were identified by direct *tlc* cochromatography with authentic standards. The structure of certain photoproducts was further confirmed by appropriate derivatization and degradation procedures. Photoproducts with phenolic and/or carboxy groups were methylated with diazomethane in methanol/ether mixture (1/1, v/v) to the corresponding methyl ethers or esters. The obtained products were identified by ir, nmr and ms, and/or *tlc* cochromatography with authentic samples. The products retaining the phosphorus ester linkages were hydrolyzed by heating in 1 N HCl at 100°C for 1 hr and the hydrolyzates were extracted with ethyl acetate and then analyzed by *tlc*.

Oxidation of $P=S$ compounds to the corresponding $P=O$ analogs was accomplished by reacting with excess amounts of *m*-chloroperoxybenzoic acid in methanol at 10°C for 2 hr. The reaction mixtures were concentrated and analyzed by *tlc*. Aldehydes were oxidized to the corresponding carboxylic acids by treating with potassium permanganate in water at 20°C for 3 hr. The reaction mixtures were acidified with 1 N HCl, extracted with ethyl acetate and the extracts were then cochromatographed with the appropriate standards. With potassium permanganate, oxidation of $P=S$ to $P=O$ occurred to a small extent.

7. Acetylcholinesterase Assay

Anticholinesterase activity of Salithion derivatives against both rat plasma and red cell cholinesterases was determined according to the method of Ellman *et al.*,⁸⁾ as reported previously.¹⁹⁾ Separation of red cells from plasma was conducted by centrifuging whole rat blood at $2,500 \times g$ for 10 min. The red cell were diluted with two fold volumes of saline and 10 μ l of aliquots of each were used for enzyme

assay.

8. Insecticidal Activity

Insecticidal activity was determined on a susceptible strain of housefly (*Musca domestica*, WHO strain), as reported previously.¹⁹⁾ Each chemical in acetone solution was topically applied to 15- to 20-day old houseflies. Mortalities were evaluated 24 hr after treatment.

RESULTS

1. Photodecomposition of Salithion

When ^{14}C -Salithion in acetone solution in the presence of air was irradiated with UV light, its degradation proceeded rapidly and extensively. The half-life was approximately 60 min. More than 12 ^{14}C -products were detected on *tlc* in solvent systems A and B. Among them, 9 products were identified as described below. The percentage of these products is listed in Table 2. The main photoproduct, S9, gradually increased with time (reaching) 45.7% of the applied radiocarbon after 240-min irradiation. The amounts of the other photoproducts ranged from 1.4% to

Table 2 Photodecomposition of ^{14}C -Salithion and ^{14}C -phenthoate in acetone solution irradiated with UV light in the presence of air.

Photoproduct	% of recovered radiocarbon as indicated products irradiation time (min)			
	30	60	120	240
Salithion				
S1 Salithion	68.2	47.5	27.2	2.8
S2 S-Benzyl salithion	5.1	5.9	5.5	2.6
S4 Salicylic acid	2.4	3.4	3.3	2.3
S5 Salioxon	1.8	2.0	2.0	1.4
S6 2-OHC- ϕ -O-P(S)(OH)OCH ₃	2.4	3.3	3.1	2.4
S7 2-OHC- ϕ -O-P(O)(OH)OCH ₃	5.0	6.6	11.5	4.4
S8 2-HOOC- ϕ -O-P(S)(OH)OCH ₃	0	2.0	4.2	3.0
S9 2-HOOC- ϕ -O-P(O)(OH)OCH ₃	7.3	13.2	25.6	45.7
$^{14}CO_2$	0	2.0	4.5	12.7
Others	1.1	2.9	4.5	7.9
Total	93.3	88.8	91.4	85.2
Phenthoate				
P1 Phenthoate	29.3	10.5	6.1	2.2
P2 Benzoic acid	37.8	50.8	50.1	40.3
P3 Phenthoate oxon	3.2	1.1	0	0
P4 Phenylglyoxylic acid	5.7	1.5	1.2	0
$^{14}CO_2$	8.6	17.1	25.1	32.6
Others	6.5	9.6	9.6	9.9
Total	90.9	90.6	92.1	84.0

4.4% of the applied during the same period. S7 reached 11.5% of the initial radiocarbon after 120-min irradiation and decreased thereafter.

Photoproduct S4 (salicylic acid) and S5 (salioxon) were each identified by comparing their nmr, ir and ms with those of authentic standards.

S2 showed the following characteristics: ir (direct) 2950 (CH), 1580 and 1480 (aromatic ring), 1270 ($P=O$), 1220 ($P-O$ -aryl), and 1030 cm^{-1} ($P-O$ -alkyl); nmr (CDCl_3) δ 3.9 (3H, *d*, $J=13$ cps, OCH_3), 3.8–4.2 (2H, *m*, CH_2) and 7.2 ppm (4H, *m*, aromatic); ms *m/e* 215 (M), 201, 183, 163, 137, 121, 109, 106, 105, 78 and 77; positive to palladium chloride test. These data indicated that the product S2 appears to be an oxon analog with a sulfur atom, but not identical with *S*-methyl salithion. Hydrolysis of S2 in 1 N HCl yielded a product which was positive to nitroprusside and ferric chloride tests on *tlc* ($R_f=0.7$ in benzene/ethyl acetate, 4/1). This product was identified as 2-hydroxybenzyl mercaptan on the basis of *tlc* cochromatography with the synthesized compound and ms. From these data, S2 was assigned to 2-methoxy-4*H*-1,3,2-benzoxathiophosphorin-2-oxide (*S*-benzyl salithion). Synthesis of this compound was tried, but failed. *S*-Benzyl salithion was obtained in about 30% yield when Salithion in benzene was irradiated with UV light in the presence of air at 310 to 330 nm. However, this product was hardly produced in the absence of air. *S*-Benzyl salithion was converted to Salithion in part, but most was further degraded to more polar products when irradiated with UV light in acetone solution or silica gel *tlc* plates. No effort was conducted to characterize these polar products.

S6 was identified as *O*-2-formylphenyl *O*-methyl hydrogen phosphorothioate [$2\text{-OHC-}\phi\text{-O-P(S)(OH)OCH}_3$] based on the following evidence: ir (KBr) 1650 (CHO), 1580 and 1480 (aromatic ring), 1220 ($P-O$ -aryl) and 1050 cm^{-1} ($P-O$ -alkyl); nmr (CD_3OD) δ 3.7 (3H, *d*, $J=14$ cps, OCH_3) and 6.8–7.8 ppm (4H, *m*, aromatic); positive to palladium chloride, 2,4-DNP and DQC. (indicating the presence of aldehyde and $P=S$ groups); formation of salicylaldehyde by acid hydrolysis of S6.

Oxidation of S6 with *m*-chloroperbenzoic

acid in methanol produced S7, which was positive to 2,4-DNP but negative to palladium chloride tests. Acid hydrolysis of S7 gave salicyl aldehyde. These data suggest that S7 is the oxon analog of S6. Finally, S7 was identified as *O*-2-formylphenyl *O*-methyl hydrogen phosphate [$2\text{-OHC-}\phi\text{-O-P(O)(OH)OCH}_3$] by ir and nmr: ir (KBr) 1640 (CHO), 1600 and 1500 (aromatic ring), 1260 ($P=O$), 1230 ($P-O$ -aryl) and 1040 cm^{-1} ($P-O$ -alkyl); nmr (CD_3OD) δ 3.68 (3H, *d*, $J=11$ cps, OCH_3) and 6.8–7.8 ppm (4H, *m*, aromatic).

S8 was positive to palladium chloride but negative to 2,4-DNP tests. This product was obtained by oxidation of S6 with potassium permanganate in water. Acid hydrolysis of S8 yielded salicylic acid. These findings suggested that S8 is *O*-2-carboxyphenyl *O*-methyl hydrogen phosphorothioate [$2\text{-HOOC-}\phi\text{-O-P(S)(OH)OCH}_3$].

The main photoproduct, S9, was identified as *O*-2-carboxyphenyl *O*-methyl hydrogen phosphate [$2\text{-HOOC-}\phi\text{-O-P(O)(OH)OCH}_3$] based on the following evidence: ir (KBr) 2500–3000, 1700 (COOH), 1600 and 1480 (aromatic ring), 1240 ($P=O$), 1230 ($P-O$ -aryl), 1210 ($P-OH$), and 1060 cm^{-1} ($P-O$ -alkyl); nmr (D_2O) δ 3.7 (3H, *d*, $J=11$ cps, OCH_3) and 7.0–7.7 ppm (4H, *m*, aromatic); negative to palladium chloride and 2,4-DNP; methylation with diazomethane to give the corresponding methyl ester [$R_f=0.53$ in solvent system B; ir (KBr) 1720 (COOCH_3), 1600 and 1490 (aromatic ring), 1240 ($P=O$), 1230 ($P-O$ -aryl) and 1050 cm^{-1} ($P-O$ -alkyl); nmr (CD_3OD) δ 3.7 (3H, *d*, $J=11$ cps, OCH_3), 3.89 (3H, *s*, COOCH_3) and 7.0–7.7 ppm (4H, *m*, aromatic)].

In addition, ^{14}C -carbon dioxide was produced, accounting for 12.7% after 240-min irradiation. On the other hand, desmethyl salithion and *S*-methyl salithion were not detected as photoproducts.

On exposure to sunlight, ^{14}C -Salithion in water was gradually degraded with a half-life of approximately 14 days. More than 9 products were detected by silica gel *tlc* in solvent systems A and B, of which seven products were identified by *tlc* cochromatography with the authentic samples. The relative amounts of these photoproducts are shown in Table 3. Photoproducts S2, S3, S5

Table 3 Photodecomposition of ^{14}C -Salithion and ^{14}C -phenthoate in water solution exposed to sunlight.

Photoproduct	% of recovered radiocarbon as indicated products exposure time (day)			
	3	6	12	20
Salithion				
S1 Salithion	82.7	71.1	57.5	36.4
S2 S-Benzyl salithion	0.8	1.2	1.8	3.6
S3 Saligenine	1.7	5.0	5.4	6.1
S5 Salioxon	1.5	2.8	4.0	4.0
S7 2-OHC- ϕ -O-P(O)(OH)OCH ₃	2.8	4.5	6.8	8.9
S9 2-HOOC- ϕ -O-P(O)(OH)OCH ₃	0	0.4	0.8	1.5
S10 2-HO- ϕ -CH ₂ O-P(S)(OH)OCH ₃	5.5	8.4	11.9	19.9
S11 2-HO- ϕ -CH ₂ O-P(O)(OH)OCH ₃	0.5	1.5	2.3	6.8
Others	4.5	4.8	9.5	12.8
Total	100.0	99.7	100.0	100.0
Phenthoate				
P1 Phenthoate	91.2	86.3	75.6	66.3
P3 Phenthoate oxon	1.9	2.9	4.2	5.3
P5 HS-CH(ϕ)-COOC ₂ H ₅	2.0	2.6	2.6	2.7
P6 Ethyl mandelate	0.9	2.6	7.1	14.6
Others	3.0	4.7	8.9	10.2
Total	99.0	99.1	98.4	99.1

(salioxon), S7 and S9 which were found in acetone solution following irradiation with UV light were also produced. In addition, S10 and S11 were detected. The major photoproducts, S7 and S10, represented for 8.9% and 19.9% of the applied radiocarbon, respectively, after 20-day irradiation. Also, both S10 and S11 were produced by irradiating UV light to Salithion deposits on silica gel *tlc* plates.

S10 was identified as *O*-2-hydroxybenzyl *O*-methyl hydrogen phosphorothioate [2-HO- ϕ -CH₂OP(S)(OH)OCH₃] based on the following evidence: ir (KBr) 3590 (phenolic OH), 1600 and 1490 (aromatic ring, 1190 (*P*-OH) and 1050 cm⁻¹ (*P*-O-alkyl); nmr (CD₃OD) δ 3.5 (3H, *d*, *J*=14 cps, OCH₃), 3.9 (2H, *m*, CH₂) and 7.0 ppm (4H, *m*, aromatic); positive to palladium chloride and ferric chloride; formation of saligenin by acid hydrolysis. S11 was positive to ferric chloride, but negative to palladium chloride tests. Oxidation of S10 with *m*-chloroperbenzoic acid in methanol gave S11. These findings suggested that S11 were as follows: ir (KBr) 3590 (OH), 1600 and 1500 (aromatic ring), 1240 (*P*=O), 1190 (*P*-OH) and 1050 cm⁻¹ (*P*-O-alkyl); nmr (CD₃OD) δ 3.7 (3H, *d*, *J*=11 cps, OCH₃), 3.8 (2H, *m*, CH₂)

and 7.2 ppm (4H, *m*, aromatic). Thus, S11 was assigned to *O*-2-hydroxybenzyl *O*-methyl hydrogen phosphate [2-HO- ϕ -CH₂OP(O)(OH)-OCH₃].

Salithion underwent rapid photodecomposition on silica gel *tlc* plates by irradiation with sunlight. The half-life was approximately 2 days. Among the photoproducts, S2(S-benzyl salithion), S5(salioxon), S7[2-HOC- ϕ -OP(O)(OH)OCH₃], S9[2-HOOC- ϕ -OP(O)(OH)OCH₃], S10[2-HO- ϕ -CH₂OP(S)(OH)OCH₃] and S11 [2-HO- ϕ -CH₂OP(O)(OH)OCH₃] were identified by *tlc* cochromatography with the authentic standards. The percentage of these products is presented in Table 4. One of the major products S5 (salioxon) represented 3.5% of the applied radiocarbon after one-day exposure, and then gradually decreased with time. The radiocarbon lost by volatilization or degradation to volatile products, accounted for 55.6% and 88.6% of the initial radiocarbon after 4- and 8-day exposure, respectively.

When irradiated with sunlight on thin layer plates of Tochigi and Shiga soils, Salithion was rapidly decreased with concomitant increase of degradation products together with loss by volatilization. The half-life was less than 1 day

Table 4 Photodecomposition of ^{14}C -Salithion and ^{14}C -phenthoate on silica gel chromatoplates exposed to sunlight.

Photoproduct	% of recovered radiocarbon as indicated products days after application			
	1	2	4	8
Salithion				
S1 Salithion	75.1	52.0	22.6	1.0
S2 S-Benzyl salithion	1.8	1.7	0.9	0
S5 Salioxon	3.5	2.9	2.5	1.0
S7 2-OHC- ϕ -O-P(O)(OH)OCH ₃	0.3	1.0	1.3	0
S9 2-HOOC- ϕ -O-P(O)(OH)OCH ₃	1.8	3.1	3.8	5.6
S10 2-HO- ϕ -CH ₂ O-P(S)(OH)OCH ₃	1.7	1.8	1.9	0.6
S11 2-HO- ϕ -CH ₂ O-P(O)(OH)OCH ₃	1.1	1.5	2.1	1.2
Others	6.8	7.7	9.3	2.0
Total	92.1	71.7	44.4	11.4
Phenthoate				
P1 Phenthoate	70.7	69.2	48.7	16.0
P2 Benzoic acid	4.6	2.1	2.9	2.7
P3 Phenthoate oxon	6.3	8.8	13.1	21.2
P5 HS-CH(ϕ)-COOC ₂ H ₅	1.2	1.5	1.8	2.4
P6 Ethyl mandelate	0	1.2	1.4	1.6
P7 Carboxyphenthoate	0	1.5	2.4	4.9
Others	5.3	3.4	5.0	7.8
Total	88.1	87.7	75.3	56.6

Table 5 Photodecomposition of ^{14}C -Salithion on soil surface exposed to sunlight.

	% of recovered radiocarbon as indicated products days after application							
	Tochigi soil				Shiga soil			
	1	3	6	dark(6)	1	3	6	dark(6)
Solvent extract								
S1 Salithion	23.9	4.6	2.5	90.7	22.8	3.7	1.6	91.6
S3 Saligenine	9.0	11.0	12.1	3.5	9.1	10.0	11.1	2.6
S5 Salioxon	2.2	1.6	1.1	0	2.5	2.0	1.9	0
S6 2-OHC- ϕ -O-P(S)(OH)OCH ₃	1.0	1.5	2.0	0	2.0	5.0	5.8	0
S7 2-OHC- ϕ -O-P(O)(OH)OCH ₃	1.6	2.7	3.4	0	2.3	3.3	3.1	0
S10 2-HO- ϕ -CH ₂ O-P(S)(OH)OCH ₃	21.5	13.5	5.1	1.4	16.2	8.6	6.0	0.9
S11 2-HO- ϕ -CH ₂ O-P(O)(OH)OCH ₃	2.6	1.8	4.1	0	5.3	5.8	5.6	0
Others	3.3	2.7	4.9	0.3	2.3	2.6	2.5	0.4
Subtotal	65.1	39.4	35.3	95.9	62.5	41.0	37.6	95.5
^{14}C Bound to soil	3.7	6.4	9.3	3.6	3.2	5.2	8.6	2.6
Total	68.8	45.8	44.6	99.5	65.7	46.3	46.2	98.1

on both soil plates and nearly the same products were detected in almost the same proportion, as shown in Table 5. Photoproducts S3, S5, S7, S10 and S11 were produced, and they were all obtained when exposed to sunlight in water solution.

2. Photodecomposition of Phenthoate

On irradiation with UV light in acetone solution in the presence of air, phenthoate was rapidly decomposed with a half-life of approximately 20 min. At least 4 radioactive products were detected by *tlc* in solvent systems

B and C. Among them, three radioactive products were identified. The percentage of these photoproducts is presented in Table 2.

The major product, P2, accounted for 50.8% of the initial radiocarbon after 60-min irradiation. This product was isolated by preparative *tlc* in solvent system A, and then recrystallized from water to give white crystals, whose mp (122°C) and ir were identical with those of benzoic acid.

P3 was identified as phenthoate oxon, because its ir and ms were identical with those of phenthoate oxon. This product accounted for 3.2% of the applied after 30-min irradiation. Thereafter it decreased rapidly, and completely disappeared after 120-min irradiation.

P4 was assigned to phenylglyoxylic acid based on the following evidence: ir (KBr) 3000–2500 and 1710 (COOH), 1670 (C=O), and 1600 and 1500 cm⁻¹ (aromatic ring); positive to 2,4-DNP; conversion of P4 with ethanol in the presence of dry hydrogen chloride to the corresponding ethyl ester, which showed the identical ms with that of ethyl phenylglyoxylate [*m/e*, 179 (M+1), 150, 123, 105 (base peak), 77]. In addition, ¹⁴CO₂ was obtained in a large amount, accounting for 32.6% of the initial radiocarbon after 240-min irradiation.

Phenthoate in aqueous solution was relatively stable when exposed to sunlight. After 20-day exposure, 66.3% of the initial radiocarbon was recovered as intact phenthoate as shown in Table 3. Small amounts of photoproducts were

detected on *tlc*. P3 (phenthoate oxon), P5[HS-CH(ϕ)-COOC₂H₅] and P6(ethyl mandelate) were identified by *tlc* cochromatography with the authentic standards. Both P2 (benzoic acid) and P4 (phenylglyoxylic acid) were not found under these conditions.

Deposits of ¹⁴C-phenthoate on silica gel *tlc* plates were decomposed readily by sunlight irradiation, with a half-life of approximately 4 days. Among the photoproducts, P2(benzoic acid), P3 (phenthoate oxon), P5[HS-CH(ϕ)-COOC₂H₅] and P7 (carboxyphenthoate) were identified by *tlc* cochromatography with the authentic samples. The relative amounts of these products are shown in Table 4. Main photoproducts, P3 (phenthoate oxon), gradually increased with time, and accounted for 21.2% of the applied radiocarbon after 8-day irradiation. The amounts of the other photoproducts were less than 5% during the same period. The loss of the radiocarbon from silica gel *tlc* plates was 43.4% during 8-day period.

¹⁴C-Phenthoate was also readily decomposed on two types of soil thin layer plates under sunlight with a half-life of approximately 3 days, as shown in Table 6. Among the photoproducts, P2(benzoic acid), P3(phenthoate oxon), P7(carboxyphenthoate), P8[HS-CH(ϕ)-COOH] and P9 (mandelic acid) were identified by *tlc* cochromatography. Main product, P8, accounted for approximately 14% of the initial radiocarbon 12 days after exposure, and phenthoate oxon was produced in a small

Table 6 Photodecomposition of ¹⁴C-phenthoate on soil surface exposed to sunlight

	% of recovered radiocarbon as indicated products days after application									
	Tochigi soil					Shiga soil				
	1	3	6	12	dark(6)	1	3	6	12	dark(6)
Solvent extract										
P1 Phenthoate	77.8	44.4	25.4	10.3	96.1	77.9	43.1	27.6	10.9	96.4
P2 Benzoic acid	1.0	2.2	4.7	1.8	0	1.1	1.5	2.6	1.9	0
P3 Phenthoate oxon	2.7	1.7	1.7	1.1	0	2.3	2.3	2.5	1.3	0
P7 Carboxyphenthoate	1.4	1.9	2.9	4.0	0	1.3	1.8	2.3	3.9	0
P8 HS-CH(ϕ)-COOH	3.4	4.1	5.3	14.0	0	2.7	3.5	10.8	14.4	0
P9 Mandelic acid	2.1	3.5	1.0	2.5	0	2.1	3.5	0.9	0.6	0
Others	10.5	12.1	14.6	12.2	3.6	8.5	11.4	10.1	10.9	1.5
Subtotal	98.9	69.9	55.6	45.9	99.7	95.9	67.1	56.8	43.9	97.9
¹⁴ C Bound to soil	2.6	3.3	5.1	3.6	0.3	3.1	2.9	3.0	3.3	1.8
Total	101.5	73.2	60.7	49.5	100	99.0	70.0	59.8	47.2	99.7

Table 7 Anticholinesterase and insecticidal activities of salithion and its derivatives.

Compound	I_{50} (M)		LD_{50} (μ g/insect)
	plasma	erythrocyte	housefly
Salithion	2.3×10^{-8}	1.1×10^{-8}	0.05
Salioxon	5.0×10^{-7}	7.5×10^{-8}	0.19
S-Methyl salithion	4.0×10^{-8}	3.0×10^{-8}	—
S-Benzyl salithion	3.5×10^{-7}	7.5×10^{-8}	>1.0

amount. In two types of soils, the water content was 12% and 5% at the start of irradiation, and decreased to 2.8% and 1.1%, respectively, for Tochigi and Shiga soils during 6-day exposure.

3. Insecticidal and Anticholinesterase Activities of Salithion and Its derivatives

S-Methyl salithion was most active in inhibition of both plasma and erythrocyte cholinesterases, followed in decreasing order of inhibition by S-benzyl salithion and salioxon as shown in Table 7. Although Salithion and salioxon were extremely toxic to housefly, S-benzyl salithion was almost non-toxic.

DISCUSSION

When irradiated with UV light and sunlight in solutions in the presence of air and as residual deposits on silica gel and soil *tlc* plates, both Salithion and phenthoate underwent extensive photochemical degradation. Most of photo-products were identified and the photodegradation pathways for Salithion and phenthoate are proposed as shown in Fig. 1 and Fig. 2. The following photochemical reactions occurred; for Salithion, (1) oxidation of $P=S$ to $P=O$, (2) oxidation of the methylene group of benzyl moiety to the corresponding aldehyde, (3) oxidation of the aldehyde to carboxyl group, (4) cleavage of $P-O$ -aryl linkage, (5) cleavage of $P-O$ -aralkyl linkage and (6) isomerization of Salithion to S-benzyl salithion; for phenthoate, (1) oxidation of $P=S$ to $P=O$, (2) oxidation of the methin group of benzyl moiety, (3) cleavage of $P-S$ bond, (4) cleavage of $S-C$ bond, (5) cleavage of ethyl ester linkage, and (6) decarboxylation. Salithion was mainly decomposed through (1), (2) and (3) in acetone solution with UV light, (1), (2), and (4) in water solutions, (1), (2), and (4) on silica gel

Fig. 1 Proposed photodecomposition pathways for Salithion.

Numbers in parenthesis indicate the types of photochemical reactions described in discussion.

tlc plates, and (1), (4), and (5) on soil surfaces. Phenthoate was chiefly decomposed by way of (2) and (6) in acetone solution with UV light, (1) and (4) in water solutions, (1) and (5) on silica gel *tlc* plates, and (1), (2), (3), (5) and (6) on soil surfaces, respectively. Oxidation of a certain amount of $P=S$ to $P=O$ occurred in various conditions tested with both compounds. Oxidation of the methylene or methin group of benzyl moiety was a predominant reaction found with both Salithion and phenthoate in

Numbers in parenthesis indicate the types of photochemical reactions described in discussion.

imals and plants, occurred in photodecomposition of some organophosphorus esters including Cyanox[®],¹¹⁾ this reaction did not occur in the photodecomposition of Salithion and phenthoate under various conditions tested. In contrast, oxidation of the methylene or methin groups of the benzyl moiety was one of the major reactions found in the photodecomposition of Salithion and phenthoate. It was hardly observed in the metabolism in mammals and plants. Isomerization of Salithion to S-benzyl salithion was not found either in the metabolism in mammals and plants.

Among the photoproducts, S-benzyl salithion and salioxon were inhibitory in plasma and erythrocyte cholinesterases, whereas their biological activities *in vivo* were much less than Salithion.

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サリチオンおよびフエンチエートのアセトン溶液に紫外線を照射した場合およびシリカゲルと土壌表面に吸着させたものに太陽光線を照射した場合、両化合物はすみやかに分解された。しかし、それらの条件に比べて水溶液中ではかなり安定であった。両化合物はおもに $P=S$ から $P=O$ への酸化、ベンジル部分のメチレン基あるいはメチン基の酸化、 $P-O$ -アリル結合および $P-O$ -アラ

ルキル結合の開裂, 脱カルボキシ化などの反応を経て分解された。サリチオンは光異性化により S-ベンジルサリチオンを生成した。また, フェントエートは土壌表面でカルボキシエステル結合の開裂を受けた。光異性化物 S-ベンジルサリチオンのラット血球および血漿コリンエステラーゼの阻害活性はサリオキソン, S-メチルサリチオンとほぼ同程度であったが, 家バエに対してはほとんど殺虫活性を示さなかった。

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