Biological Activity of Optically Active Salithion and Salioxon

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Salioxon enantiomers were obtained from salithion enantiomers by MCPBA oxidation. The larvicidal and adulticidal activities of salithion enantiomers, and the anti-cholinesterase activity of salioxon enantiomers were determined on house flies. On larvae, the potency of (*S*)(-)-salithion ($LC_{50} = 1.1$ ppm) was about two-fold higher than the (*R*)(+)-enantiomer ($LC_{50} = 2.1$ ppm). A similar ratio was obtained when these compounds were applied topically to female adults. The relative potency of these enantiomers correlated well with the anti-cholinesterase activity of their respective oxidative enantiomers, (*R*)(+)- and (*S*)(-)-salioxon, which are assumed to have been formed after their application to the insects.

Salithion (2-methoxy-4*H*-1,3,2-benzodioxaphosphorin 2-sulfide)¹⁾ has been developed as an insecticide by modifying the structure of the six-membered cyclic metabolite (M-1) formed from tri-*o*-tolyl phosphate (TOCP). In order to study the relationship between the absolute configuration and the insecticidal activity of salithion, syntheses of optically active salithions were carried out by a two-step phosphorylating method.²⁾ However, their optical purities were only about 80%.³⁾ Therefore, optically pure (>98% *e.e.*) salithions were synthesized by the proline method and their absolute configurations were determined.⁴⁾

Salithion itself is not a potent acetylcholinesterase inhibitor, but its biologically oxidized form salioxon (2-methoxy-4*H*-1,3,2-benzodioxaphosphorin 2-oxide)⁵⁾ has shown strong inhibitory activity.

The biological activity of salithion and salioxon enantiomers has not been studied yet in detail. This paper describes the insecticidal

activity of salithion enantiomers and the anti-acetylcholinesterase activity of their respective oxidative salioxon enantiomers, which are assumed to be formed *in vivo*.

Materials and Methods

Thin-layer silica gel plates (60F-254, Merck), 5 × 2 cm and 5 mm thick, were used for analysis, and 20 × 20 cm and 0.5, 1.0 or 2.0 mm thick for preparative thin-layer chromatography (TLC). ¹H-NMR was measured with a JEOL JNM-FX 100 spectrometer at 100 MHz. Tetramethylsilane (TMS) was used as an internal standard, and chemical shift values are expressed in δ (CDCl₃) ppm. Optical rotations were measured with a Hitachi-Union Giken PM-101 polarimeter and are expressed as specific rotations.

Synthesis of 2-methoxy-4H-1,3,2-benzodioxaphosphorin 2-oxide (salioxon).

a) *From salithion.* An ice-cooled solution of salithion (140 mg), prepared by the proline method,⁴⁾ in 8 ml of benzene was mixed with an ice-cooled solution of 210 mg of *m*-chloroperbenzoic acid (MCPBA) in 5 ml of benzene. The mixture was stirred at 5~10°C for 3 hr, and then at room temperature for 1 hr. After filtration and concentration, the salioxon was purified by TLC with a

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mixture of hexane–ethyl acetate (1:1.2) as the developing solvent, yielding about 60 mg (30%). From the (+)- and (–)-salithion enantiomers, (–)- and (+)-salioxon enantiomers were obtained, respectively, the $[\alpha]_D$ being -5.0° (1.73, CHCl_3) and $+4.0^\circ$ (1.93, CHCl_3).

b) From a diastereomeric mixture of (S)-2-[1-(2-methoxycarbonylpyrrolidyl)]-4H-1,3,2-benzodioxaphosphorin 2-sulfide (PS). To a solution of PS⁴⁾ (200 mg) in 10 ml of methylene chloride, MCPBA (210 mg) in 5 ml of methylene chloride was added at 0°C . The mixture was stirred at room temperature for 3 hr, then refluxed for 6 hr and washed with an aqueous solution of sodium bicarbonate. The organic layer was dried, concentrated, and then purified by TLC with a mixture of hexane–ethyl acetate (1:1.4) as a developing solvent. This procedure gave 50 mg (26%) of (S)-2-[1-(2-methoxycarbonylpyrrolidyl)]-4H-1,3,2-benzodioxaphosphorin 2-oxide. ¹H-NMR: 1.8~2.4 (4H, m, $\text{NCCH}_2\text{CH}_2\text{C}$), 3.27 (2H, 2t, NCH_2), 3.69 (3H, s, CH_3), 4.20~4.44 (1H, m, NCH), 4.84~5.64 (2H, m, CH_2O), 6.8~7.4 (4H, m, aromatic). This compound was subjected to methanolysis in a methanol (20 ml) solution containing 2 g of conc. sulfuric acid. After stirring at room temperature for 12 hr, the methanol was evaporated. The residue was neutralized, extracted with methylene chloride, and dried. The concentrated material was further purified by TLC as already mentioned, yielding 8 mg (24%) of the desired product.

¹H-NMR features of the a) and b) salioxon preparations were identical with those shown by the authentic sample.⁵⁾

Bioassay.

a) *Feeding method.* The dietary effect of the test compounds on larval and pupal mortality was measured according to the previously published method.⁶⁾ A house fly diet (10 g, Oriental Kobo K. K., Tokyo, Japan) was mixed with 30 ml of an acetone solution of the test compound, or with 30 ml of an acetone alone as a control. After the solvent had been evaporated under a hood for 2 days at room temperature, 12 g of water was added to the diet and the whole mixed well. The diet was distributed in 2 g portions in 13×150 mm test tubes. Three larvae of susceptible (SRS) house fly, 1~2 days after hatching and weighing 2 ± 1 mg each, were introduced to each test tube along with a thin piece of cotton placed over the diet to permit pupation. The tubes were kept at 28°C until pupation and adult emergence were complete.

b) *Topical Application Method.* A test sample in 1 μl of acetone was topically applied to susceptible (SRS) and fenitrothion resistant (3-Y_F) female house flies, *Musca domestica*, the mortality being measured after 24 hr. The resistant strain 3-(Y_F) was obtained by rearing the 3rd Yumenoshima strain (3-Y) under fenitrothion pressure for ten successive generations.

c) *Acetylcholinesterase Inhibition Assay.* Susceptible (SRS) house fly AChE was prepared by homogenizing 10 fly heads/ml of phosphate buffer (0.1 M, PH 8.0) and

centrifuging for 10 min at $2,500 \times g$ and $10,000 \times g$, respectively. Assays with 0.25 ml of the supernatant diluted to 2.5 ml with the buffer and various concentrations of the test compound in 50 μl of acetone were run by incubating for 15 min at 25°C . Aliquots of 1 ml were tested for residual AChE activity according to the Ellman procedure.⁷⁾

Results and Discussion

Synthesis

Salioxon could be obtained from the proline derivative (PS) by the two different routes shown in Fig. 1. Both routes consist of oxidative desulfurization and methanolysis, but the

Fig. 1. Synthetic Routes for Salioxon.

Table I. EFFECT OF DIETARY SALITHION ENANTIOMERS ON SUSCEPTIBLE (SRS) HOUSE FLY PUPATION AND EMERGENCE

Enantiomer and dietary conc. (ppm)	Relative to control (%) ^a	
	Pupation	Emergence
(S)(–)-salithion		
0.75	96 ± 10 ^b	105 ± 11 ^b
1.0	65 ± 14 ^c	47 ± 18 ^{cd}
1.5	12 ± 6 ^e	5 ± 5 ^e
2.0	0 ^e	0 ^e
(R)(+)-salithion		
1.0	93 ± 7 ^b	105 ± 11 ^b
1.5	66 ± 6 ^c	63 ± 10 ^c
2.0	65 ± 12 ^c	78 ± 14 ^{bc}
2.5	35 ± 9 ^d	36 ± 11 ^d
3.0	4 ± 4 ^e	0 ^e

^a Data are the average of 10 replicates of 3 larvae each. Larvae of 1~2 days old (2 ± 1 mg) were introduced into the diet and kept until adult emergence was complete. The average pupation and emergence of the untreated control was 86 ± 7 and $63 \pm 9\%$, respectively.

^{b,c,d,e} Represent data which differ significantly from each other at $p=0.05$ within the same column.

reaction order occurs in a reversed way. These conversions were found to be feasible by using a diastereomeric mixture of PS and racemic salithion. Such oxidative desulfurization and acidic methanolysis have been proved by several researchers in other organophosphorus compounds to proceed stereoselectively.⁸⁻¹¹⁾ In fact, we found that a salithion analog (6-chloro-2-methoxy-4-methyl-1,3,2-benzodioxaphosphorin 2-sulfide) was stereospecifically converted to the corresponding salioxon analog by MCPBA oxidation with retention of the configuration at the phosphorus atom.¹²⁾ The (–)- and (+)-salioxons were derived from (R)(+)– and (S)(–)-salithions by MCPBA oxidation, respectively. The absolute configurations of the (–)- and (+)-salioxons were assigned as (S) and (R), respectively, based on the configuration of the starting salithion and the reaction mechanism.

Biological activity

The (S)(–)- and (R)(+)-salithion enantiomers applied to the diet showed strong larvicidal activity resulting in 100% mortality at 2 and 3 ppm, respectively (Table I), and strong insecticidal activity against susceptible (SRS) house fly adults when applied topically (Table III). The LC₅₀ values of dietary salithion carried out with larvae and the LD₅₀ values for topical application carried out with adults revealed that (S)(–)-salithion was about two-fold more potent than the (R)(+)-enantiomer (Tables II and III). In a previous paper,¹²⁾ we found that (S)(–)-salithion (84% *e.e.*) and (R)(+)-salithion (79% *e.e.*) obtained by the

two-step phosphorylating method²⁾ resulted in LD₅₀ values of 0.05 and 0.07 µg/fly, respectively. Hence, the difference in LD₅₀ values of the enantiomers was more obvious when optically purer samples were used (Table III).

As in the case of the five-membered cyclic organophosphorus compounds, 4-isopropyl-2-methoxy-1,3,2-oxazaphospholidine 2-sulfide (*i*PMOS)¹³⁾ and 2-methoxy-5-phenyl-1,3,2-oxazaphospholidine 2-sulfide (5-PMOS),¹⁴⁾ the resistant ratios (*R/S*) of the enantiomers were fairly close and much lower than those of the commercial acyclic organophosphorus insecticides, fenitrothion (dimethyl 3-methyl-4-nitrophenyl phosphorothionate) and malathion [*S*-1,2-bis(ethoxycarbonyl)ethyl dimethyl phosphorothiolothionate], indicating their possible use for controlling house flies resistant to the latter compounds (Table III).

Salithion (0.085 µg) showed a 50% inhi-

Table III. LD₅₀ VALUES OF SALITHION ENANTIOMERS APPLIED TOPICALLY TO SUSCEPTIBLE (SRS) AND RESISTANT (3-Y_F) HOUSE FLY ADULTS

Compound	LD ₅₀ , µg/fly (95% confidence limits)		
	SRS (S)	3-Y _F (R)	<i>R/S</i> ratio
(S)(–)-Salithion	0.05	0.40	8.0
98% <i>e.e.</i>	(0.03~0.06)	(0.32~0.56)	
(R)(+)-Salithion	0.12	0.91	7.6
99% <i>e.e.</i>	(0.10~0.16)	(0.83~1.05)	
Fenitrothion	0.073	> 200	> 2740
	(0.063~0.084)		
Malathion	0.67	> 200	> 299
	(0.57~0.80)		

Table II. LC₅₀ VALUES OF DIETARY SALITHION ENANTIOMERS FOR CUMULATIVE MORTALITY OBTAINED UNTIL PUPATION OR EMERGENCE

Enantiomer	Cumulative mortality ^a	LC ₅₀ , ppm (95% confidence limits)
(S)(–)	Until pupation	1.1(1.0~1.2)
	Until emergence	1.0(0.9~1.1)
(R)(+)	Until pupation	2.1(1.8~2.5)
	Until emergence	2.4(2.0~2.9)

^a See Table I for the procedure and assay.

Fig. 2. Effect of Salithion Applied Topically to Susceptible (SRS) House Flies at the LD₅₀ Value (0.085 µg/fly) on the Mortality and AChE Activity at Various Periods after Application.

bition of acetylcholinesterase in the susceptible (SRS) house flies at 80 min after topical application, while a 50% mortality occurred only after about 20 hr (Fig. 2). (*R*)(+)-Salioxon, obtained by MCPBA oxidation from (*S*)(-)-salithion, showed stronger anti-cholinesterase activity ($I_{50}=0.170\text{ }\mu\text{M}$) than (*S*)(-)-salioxon ($I_{50}=0.534\text{ }\mu\text{M}$) against the susceptible (SRS) house fly head enzyme. These results agree with the concept that the high toxicity of (*S*)(-)-salithion to both house fly larvae and adults results from its biological conversion to (*R*)(+)-salioxon, exhibiting higher inhibitory activity on acetylcholinesterase.

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