

1,3,2-オキサアザホスホリン2-スルフィド類および1,3,2-ベンゾジオキサホスホリン2-スルフィド類の光学異性体の殺虫活性

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Original Article

Insecticidal Activity of Optically Active 1,3,2-Oxazaphospholidine 2-Sulfides and 1,3,2-Benzodioxaphosphorin 2-Sulfides

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Optical isomers of ten 1,3,2-oxazaphospholidine 2-sulfides and two 1,3,2-benzodioxaphosphorin 2-sulfides, including a commercial insecticide, salithion, were topically applied to an organophosphate-susceptible strain of housefly. Of all the oxazaphospholidines, $(S)_C(R)_P$ isomers showed the highest insecticidal activity, and the potency decreased with configuration in the order of $(S)_C(R)_P > (S)_C(S)_P > (R)_C(R)_P > (R)_C(S)_P$. In the benzodioxaphosphorins, the $(S)_P$ configuration was favorable for high insecticidal activity. Some optical isomers were also tested against the onion maggot, two species of planthopper and an organophosphate-resistant strain of housefly. The relationship between configuration and insecticidal activity was similar in the susceptible and resistant strains of housefly, but varied with species of insect.

INTRODUCTION

The insecticidal activity of some 1,3,2-oxazaphospholidine 2-sulfides (OS; 1) and 1,3,2-benzodioxaphosphorin 2-sulfides (BS; 2) was found from the studies on L-leucine's neuroactivity^{1,2)} and neurotoxic metabolites of tri-*o*-tolyl phosphate, respectively,^{3,4)} and numerous analogic compounds have been synthesized and bioassayed to optimize the chemical structure as insecticides.^{5,6)} Among the compounds, 2-methoxy-4*H*-1,3,2-benzodioxaphosphorin 2-sulfide (salithion) was developed into a commercial insecticide in 1968.⁶⁾ The cyclic phosphorus compounds which showed potent insecticidal activity do not have electron-withdrawing groups, indicating the ring strain is one of the essential factors for the activity.^{7,8)}

Because of the existence of an asymmetric phosphorus atom and, in some cases, an asymmetric carbon atom in the molecule of insecti-

cidal OS's and BS's, the insecticidal activities of these compounds reported so far are those of a mixture of stereoisomers, either enantiomers or diastereomers, or sometimes both.

There are many chiral organophosphorus pesticides whose stereoisomers show different potencies for each other in biological activities.⁹⁻¹¹⁾ But on this aspect little has been reported about cyclic phosphorus compounds having an asymmetric phosphorus atom as a ring member, probably owing to the rareness of commercialized insecticidal compounds of this type and the difficulty to obtain their stereoisomers. Cyclic organophosphorus compounds possess a rather fixed conformation compared with acyclic compounds, so that the examination of their biological activities provides accurate informations concerning the steric effects on the activities.

Stereoisomers of OS's and BS's of high optical purity have been synthesized by using optically active aryl methyl phosphorochlorido-

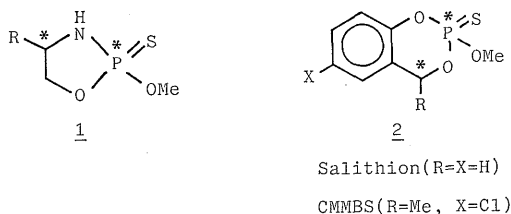


Fig. 1 Oxazaphospholidine 2-sulfides (OS, 1) and benzodioxaphosphorin 2-sulfides (BS, 2).

Asymmetric atoms are shown by an asterisk.

thionates as chiral two-step phosphorylating reagents to react with optically active amino-alcohols and hydroxybenzenealcohols, respectively.¹²⁻¹⁴⁾ This paper deals with the relationships between insecticidal activity and stereochemistry of some OS's and BS's. In all the OS's, the (*S*)_C(*R*)_P stereoisomer was found to be the most active among the four isomers against any insect species tested. In the BS series, however, the (*S*)_P configuration was favorable for the insecticidal activity.

MATERIALS AND METHODS

1. Chemicals

Nine OS compounds out of 11 homologs were different in substituents at the 4-position as listed in Table 1. Two homologs substituted at the 5-position were also used. Optical purity of stereoisomers was more than 97% based on ¹H- and ³¹P-NMR information. Among the BS's listed in Table 2, optical purity of (*S*)-(–)- and (*R*)-(+)-2-methoxy-4*H*-BS (salithion) was 84 and 79% e.e., respectively, and that of both *cis* (*R*)_C(*R*)_P- and (*S*)_C(*S*)_P-6-chloro-2-methoxy-4-methyl-BS (CMMBS) about 97% e.e. according to HPLC analysis using an optically active poly (triphenylmethylmethacrylate) column. Enantiomers of *trans* CMMBS were not separable using this column, so that their optical purity was not determined. Details of the asymmetric syntheses of all OS and BS compounds used in this paper, determination of their configuration and their optical purity will be described elsewhere.¹⁴⁾

2. Insects

Houseflies (*Musca domestica* LINNE, WHO's standard susceptible and 3rd Yumenoshima resistant strains) were left to oviposit on the

surface of absorbent cotton soaked with a suspension of skim milk (Yukijirushi Nyugyo Co., Ltd.). The larvae were raised with a mixture of commercial housefly feed and rat feed (Oriental Yeast Co., Ltd.) at a 6:1 ratio and the adults with sugar, skim milk and water; both on a 12-hr light and 12-hr dark life cycle, at 25–28°C. Three- to five-day-old female adults were used for the insecticidal test. Average weight was 19 mg/fly for the susceptible strain and 20 mg/fly for the resistant strain.

Onion maggots (*Hylemyia antiqua* MEIGEN) were left to oviposit on onions. The larvae were raised with onions and the adults with dry yeast (Ebiosu, Tanabe Seiyaku Co., Ltd.), skim milk, honey (Meijiseika Co., Ltd.) and water at 22–25°C. Male and female adults were used for the insecticidal test. Average weight was 9 mg/insect for males and 16 mg/insect for females.

Brown planthoppers (*Nilaparvata lugens* STÅL) were collected in Hatano, Kanagawa in 1966 and smaller brown planthoppers (*Laodelphax striatellus* FALLEN) in Nishigahara, Tokyo in 1968. Both were organophosphate susceptible strains. The successive generations were raised with seedlings of NIHONBARE, a variety of rice, on a life cycle of 16-hr light at 23°C and 8-hr dark at 20°C. Female adults were used for the toxicity test. They were a mixture of individuals who have either long wings or short wings. Their ratios were almost the same in both species. Average weight was 2.36 mg/insect for brown planthoppers and 0.86 mg/insect for smaller brown planthoppers.

3. Toxicity Test

A test sample in 1 μl of acetone was topically applied to a dorsal thoracic section of 10–20 houseflies anesthetized with ether for 1.5 min, and the treated flies were maintained at 25°C in a 2×9.5(φ) cm petri dish containing absorbent cotton soaked with 5% sugar solution. The mortality was counted at 24 hr after treatment. The same operation was duplicated at 5–6 different concentrations.

A test sample in 0.5 μl of acetone was topically applied to a dorsal thoracic section of 10 onion maggots anesthetized with carbon dioxide gas, and the treated insects were maintained at 22–23°C in a 4×8(φ) cm ice cream

cup containing absorbent cotton soaked with 10% sugar solution and covered with a sheet of gauze. The mortality was counted at 24 hr after treatment. The same operation was duplicated at 4 different concentrations.

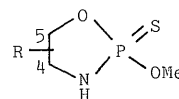
A test sample in 0.1 μ l of acetone was topically applied to a dorsal thoracic section of 10 planthoppers each anesthetized with carbon dioxide gas, and the treated insects were maintained at 26°C in a 20 \times 3(ϕ) cm test tube containing several rice seedlings whose roots were wrapped round with wet absorbent cotton. The mortality was counted at 24 hr after treatment. The same operation was repeated 2–4 times at 4–5 different concentrations.

Controls were prepared along with each test under the same conditions except that they were given acetone instead of a test sample solution. The LD₅₀ values and 95% confidence limits were calculated by a NEC PC-9801VM personal computer system using a program designed for probit analysis by adopting the average value of replications.

RESULTS AND DISCUSSION

The insecticidal activity of optically active OS's and BS's against the susceptible housefly is shown in Tables 1 and 2, respectively. For all the 4-substituted OS's tested, the (S)_C(R)_P or (+)-*cis* diastereomer among isomers showed the highest insecticidal activity. The insecticidal activity of the most potent isomer, (S)_C(R)_P, in each homolog varied with the substituent at the 4-position. The highest activity was obtained for the 4-isopropyl derivative (¹PMOS), and the activity decreased in the order of iso-Pr > iso-Bu > Et > *sec*-Bu > Bz > Ph > Me > *tert*-Bu \gg H. Compared with the insecticidal activity of the corresponding stereoisomers of 4-isopropyl (¹PMOS) and 4-*tert*-butyl derivatives, the difference was only 5 times in the most potent (S)_C(R)_P isomers but the difference reached 58 times in the weakest (R)_C(S)_P isomers. The insecticidal activity of optically active OS isomers varied most regularly with configuration and decreased in the order of (S)_C(R)_P > (S)_C(S)_P > (R)_C(R)_P > (R)_C(S)_P, as shown in Fig. 2. Most of respective isomers of 4-isopropyl and 4-isobutyl derivatives showed higher insecticidal activity

Table 1 Insecticidal activity of optical isomers of 1,3,2-oxazaphospholidine 2-sulfides (OS) against organophosphate-susceptible houseflies.



R	Config.		LD ₅₀ , μ g/fly (95% confidence limit)
	C	P	
H		R	29.97 (23.57–37.83)
		S	37.25 (32.80–42.60)
4-Me	S	R	0.43 (0.32–0.59)
	R	S	8.07 (6.35–10.32)
	S	S	0.72 (0.59–0.90)
	R	R	18.60 (15.76–22.01)
4-Et	S	R	0.14 (0.11–0.19)
	R	S	0.98 (0.77–1.25)
	S	S	0.25 (0.21–0.32)
	R	R	0.35 (0.27–0.48)
4- <i>i</i> -Pr	S	R	0.09 (0.08–0.10)
	R	S	0.22 (0.20–0.25)
	S	S	0.11 (0.09–0.14)
	R	R	0.20 (0.18–0.22)
4- <i>t</i> -Bu	S	R	0.46 (0.32–0.61)
	R	S	12.74 (10.47–15.61)
	S	S	0.73 (0.57–0.94)
	R	R	4.96 (4.20–5.31)
4- <i>i</i> -Bu	S	R	0.13 (0.11–0.15)
	R	S	0.36 (0.33–0.38)
	S	S	0.30 (0.25–0.34)
	R	R	0.31 (0.28–0.34)
4- <i>s</i> -Bu	S	R	0.16 (0.12–0.24)
	S	S	0.24 (0.19–0.40)
4-Ph	S	R	0.37 (0.30–0.45)
	R	S	2.72 (2.26–3.31)
	S	S	0.87 (0.75–1.02)
	R	R	1.80 (1.54–2.08)
4-Bz	S	R	0.21 (0.17–0.27)
	R	S	4.20 (3.61–4.89)
	S	S	0.42 (0.37–0.47)
	R	R	2.34 (2.12–2.52)
5-Me	S	R	(100. 30) ^{a)}
	R	S	(60. 0) ^{a)}
	S	S	(90. 0) ^{a)}
	R	R	(80. 0) ^{a)}
5-Et	RS	RS	6.00 (4.46–8.03)

^{a)} Mortality (%) at 10 and 1 μ g/fly, respectively.

Table 2 Insecticidal activity of optical isomers of benzodioxaphosphorins (BS) against organophosphate-susceptible houseflies.

Compounds & configuration	LD ₅₀ , μg/fly (95% confidence limit)
(<i>S</i>)-Salithion	0.05 (0.04–0.06)
(<i>R</i>)-Salithion	0.07 (0.06–0.08)
(<i>R</i>) _C (<i>S</i>) _P -CMMBS(<i>trans</i>)	0.06 (0.05–0.07)
(<i>S</i>) _C (<i>R</i>) _P -CMMBS(<i>trans</i>)	0.12 (0.10–0.14)
(<i>S</i>) _C (<i>S</i>) _P -CMMBS(<i>cis</i>)	0.14 (0.12–0.16)
(<i>R</i>) _C (<i>R</i>) _P -CMMBS(<i>cis</i>)	0.61 (0.55–0.74)

than those of other 4-substituted derivatives. The difference in the activity among the stereoisomers was only small in such potent

series as 4-isopropyl and 4-isobutyl derivatives, whereas the difference was more obvious when such bulky groups as *tert*-butyl and benzyl were introduced to C₄ atom. It is interesting to note that the 4-methyl derivative also showed a high degree of such stereoselectivity. Generally, the configuration of C₄ atom seemed to be more important for the insecticidal activity than that of phosphorus atom, and the (*S*)_C configuration derived from L-amino acids appeared to be very important for high insecticidal activity. This means that the compounds having the configuration of natural amino acids in this moiety are probably suitable for interaction with insect AChE which is expected to be the target. The effect of the configuration of phosphorus atom was also apparent and the

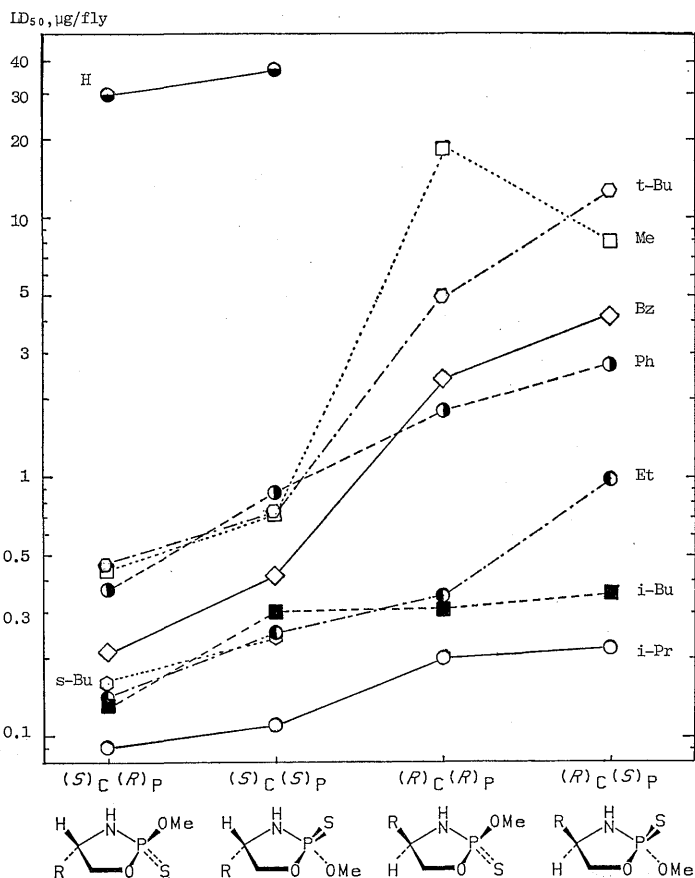


Fig. 2 Relationships between configuration of the 4-substituted 2-methoxy-1,3,2-oxazaphospholidine 2-sulfides (OS) and their insecticidal activity.

4-Substituents (R) are as follows: H (●), *s*-Bu (*sec*-butyl, ○), *i*-Pr (isopropyl, ○), *i*-Bu (isobutyl, ■), Et (●), Ph (○), Bz (◇), Me (□) and *t*-Bu (*tert*-butyl, ○).

(*R*)_P configuration was preferable. All results shown in Table 1 suggest that the (*S*)_c(*R*)_P configuration is the most appropriate for the insecticidal activity in the OS series.*

The insecticidal activity of optically active 5-methyl OS's is also given in Table 1. Again the (*S*)_c(*R*)_P isomer showed the most potent insecticidal activity and the order of the potency among the four isomers was the same as that of the 4-substituted analogs mentioned above. Compared with the ring non-substituted parent OS's, the introduction of a methyl group at C₅ atom apparently increased the insecticidal activity, but, unlike at C₄, the substitution of an ethyl group for the methyl group did not increase the insecticidal activity.

Among both enantiomers of salithion which show high insecticidal activity, the (*S*)_P configuration was slightly superior to the (*R*)_P configuration (Table 2). Because salithion used in this study was not optically pure, the difference in LD₅₀ values of the enantiomers would have been more obvious if optically pure samples had been used.

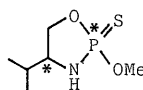
Optical isomers of CMMBS also showed clear stereoselectivity in insecticidal activity. Here, again, the diastereomeric effect on insecticidal activity was also important, the *trans* isomers being more potent than the *cis* isomers. The (*S*)_P configuration in both *cis* and *trans* isomers was favorable for the insecticidal activity, which is consistent with the results on salithion.

Four stereoisomers of 4-isopropyl OS(¹PMOS) and both enantiomers of salithion were applied to the resistant strain of housefly. In each set of compounds, the relationships between configuration and insecticidal activity were found to be very similar to those when they were applied to the susceptible strain as shown in Table 3. The resistant ratios (R/S) among stereoisomers of each set of compounds are, therefore, fairly close each other. The results suggest that the OP-resistant mechanisms of the 3rd Yumenoshima strain of housefly are poorly stereospecific for these cyclic phosphorus compounds. In contrast with these two types

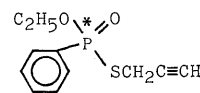
Table 3 Insecticidal activity of optically active OS and BS against susceptible and resistant houseflies (LD₅₀, μg/fly).

Compounds	Susceptible	Resistant	R/S
(<i>S</i>) _c (<i>R</i>) _P - ¹ PMOS(<i>cis</i>)	0.09	1.44	16.0
(<i>S</i>) _c (<i>S</i>) _P - ¹ PMOS(<i>trans</i>)	0.11	1.38	12.5
(<i>R</i>) _c (<i>R</i>) _P - ¹ PMOS(<i>trans</i>)	0.20	2.15	10.8
(<i>R</i>) _c (<i>S</i>) _P - ¹ PMOS(<i>cis</i>)	0.22	2.54	11.5
(<i>S</i>)-Salithion	0.05	0.30	6.0
(<i>R</i>)-Salithion	0.07	0.42	6.0
(<i>R</i>)-EPPP ^{a)}	0.05	0.56	11.2
(<i>S</i>)-EPPP ^{a)}	0.09	10.00	111
Fenitrothion	0.07	18.16	259
Malathion	0.26	135	519

^{a)} The data were taken from Ref. 10).



¹PMOS



EPPP

of cyclic phosphorus compounds, an acyclic asymmetric compound, *O*-ethyl *S*-propargyl phenylphosphonothiolate (EPPP), was very different in resistant ratios between enantiomers.¹⁰⁾ Moreover, compared with the R/S ratios of two commercial organophosphorus insecticides, fenitrothion (dimethyl 3-methyl-4-nitrophenyl phosphorothionate) and malathion (*S*-1,2-bis(ethoxycarbonyl)ethyl dimethyl phosphorothiolothionate), the ratios of the above described OS's and BS's were much lower (Table 3), being partly due to their high efficiency even to the resistant strain of insect.

To evaluate the insecticidal activity to agricultural pest insects, the onion maggot, the brown planthopper and the smaller brown planthopper were treated with four stereoisomers of ¹PMOS. The results are shown in Table 4 along with those of two agricultural insecticides, malathion and MTMC (*m*-tolyl methylcarbamate). The highest insecticidal activity against the planthoppers and the onion maggot was again found in the (*S*)_c(*R*)_P stereoisomer, followed by the decreasing order of (*R*)_c(*R*)_P > (*R*)_c(*S*)_P > (*S*)_c(*S*)_P and (*R*)_c(*R*)_P > (*S*)_c(*S*)_P > (*R*)_c(*S*)_P, respectively. In these three insect species, the (*R*)_P configuration

* The LD₅₀ values of 4-isobutyl OS in the previous paper (Ref. 12)) should be revised as those in Table 1.

Table 4 Insecticidal activity of optically active ¹PMOS, malathion and MTMC against some agricultural pest insects.

Compounds & configuration	LD ₅₀ , µg/insect (95% confidence limit)		
	Brown planthopper	Smaller brown planthopper	Onion maggot
¹ PMOS			
(S) _c (R) _F (<i>cis</i>)	0.0061 (0.0047-0.0078)	0.0053 (0.0040-0.0068)	0.070 (0.059-0.084)
(S) _c (S) _F (<i>trans</i>)	0.0205 (0.0174-0.0245)	0.0293 (0.0203-0.0411)	0.192 (0.162-0.228)
(R) _c (R) _F (<i>trans</i>)	0.0080 (0.0070-0.0093)	0.0077 (0.0054-0.0111)	0.129 (0.102-0.167)
(R) _c (S) _F (<i>cis</i>)	0.0171 (0.0148-0.0214)	0.0145 (0.0122-0.0172)	0.444 (0.365-0.534)
Malathion	0.0143 (0.0102-0.0200)	0.0103 (0.0078-0.0134)	
MTMC	0.0039 (0.0033-0.0049)	0.0086 (0.0071-0.0119)	

is more important for the insecticidal activity than the (S)_c configuration in contrast with the case against the housefly. Some stereoisomers are more potent than malathion against both species of planthopper and than MTMC against the smaller brown planthopper. The *cis* isomer is more potent than the *trans* isomer against both species of planthopper, provided the compounds have the (S)_c configuration. Thus, a target, AChE, and/or enzymes related to the metabolism of organophosphates of one insect species may be presumed to recognize a phosphate molecule in some delicately different way in terms of steric effects from the other insect species. On the other hand, the (S)_c(R)_F and (R)_c(S)_F isomers showed the strongest and the weakest insecticidal activity, respectively, against both strains of housefly as well as the onion maggot. The former is also the most toxic against both species of planthopper. The broad spectrum of ¹PMOS having the (S)_c(R)_F configuration in the insecticidal activity suggests the existence of a certain common stereochemical structure at the binding site of the target of these insect species used in this study.

In conclusion, five-membered cyclic phosphoramidate OS's and six-membered cyclic phosphate BS's showed obvious stereoselectivity in insecticidal activity. The stereoselectivity was not exactly the same among insect species tested, though some common tendency existed. All stereoisomers of ¹PMOS and sali-

thion showed high toxicity against not only the organophosphate-susceptible strain but also the resistant strain of housefly. The difference in insecticidal activity among stereoisomers was not very large in most cases, indicating that the stereoisomeric mixtures are suitable enough as economical and effective insecticides.

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要 約

1,3,2-オキサザホスホリジン 2-スルフィド類および 1,3,2-ベンゾジオキサホスホリン 2-スルフィド類の光学異性体の殺虫活性

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不斉合成で得られた 1,3,2-オキサザホスホリジン 2-スルフィド類と 1,3,2-ベンゾジオキサホスホリン 2-スルフィド類(殺虫剤サリチオンを含む)の光学異性体の有機リン剤感受性および抵抗性系統のイエバエに対する殺虫活性を局所用法によって調べた。4-位置換されたオキサザホスホリジン誘導体では(S)_c(R)_pの立体配置をもつ異性体が最も強い殺虫活性を示し、以下(S)_c(S)_p(R)_c(R)_p, (R)_c(S)_pの順に活性が低下した。ベンゾジオキサホスホリン類のリン原子については(S)体は(R)体より殺虫活性がやや強かった。オキサザホスホリジンの4-イソプロピル置換体の光学異性体はイエバエ, タマネギバエとウンカ類に対して、それぞれ異なった立体配置-殺虫活性相関を示した。