Studies on Organophosphorus Insecticides

Part X. Chemical and Biological Properties of O,O-Dimethyl-O-(4-cyanophenyl) phosphorothioate and O-Ethyl-O-(4-cyanophenyl) phenylphosphonothioate

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In our previous paper¹⁾, it was reported that O,O-dimethyl-O-(4-cyanophenyl) phosphorothioate (S-4084) has only a low toxicity towards mammals though possessing a high activity towards rice stem borers. In this paper, the chemical and biological properties of S-4084 and its phenylphosphonothioate derivative (S-4087) will be reported in detail. It should especially be noted that S-4084 had a higher activity towards rice stem borers than S-4087 by topical or pot test in a room or in a green-house, but by field test S-4087 was stronger than S-4084.

It was first proved by G. Schrader²⁾ that phosphorothioate having 4-cyanophenyl group (I) possesses a high degree of insecticidal activity. However, the compound (I) had also a high toxicity towards warm blooded animals^{*1)} and moreover in insecticidal activity the compound (I) was inferior to E-605 (Parathion) which was discovered by him at the same time. So, no systematic study has been reported thereafter of this series except T. R. Fukuto's report³⁾, in which he reported the interesting fact that the infrared absorption band of P-O-Aromatic had some relationship with its inhibitory potency towards acetylcholinesterases. In the report, he prepared a 4-cyanophenyl phosphorate (II) and reported its properties.

Recently, however, studies in this field have

become active again. G. Schrader⁴⁾ published detailed results of his subsequent studies covering this field and Y. Sumiki and S. Tamura⁵⁾ also prepared and evaluated many phosphorothioates having cyanophenyl group.

Independently from these, O,O-dimethyl-O-(4-cyanophenyl) phosphorothioate (S-4084) was found in this laboratory and was already reported¹⁾ as a promising insecticide

1) Y. Nishizawa, *This Journal*, **24**, 744 (1960).

2) G. Schrader, "Die Entwicklung neuer Insektizide auf Grundlage Organischer Fluor- und Phosphor-Verbindungen," 1952, S. 59.

*1) Oral towards mice, LD₅₀ 7.0 mg/kg.

3) T.R. Fukuto, *Agr. Food Chem.*, **4**, 930 (1956).

4) G. Schrader, *Angew. Chem.*, **73**, 331 (1961).

5) S. Tamura et al., *This Journal*, **25**, 773 (1961).

just as Sumithion⁶⁾ which had been found during the course of a systematic investigation⁷⁾ of phosphorothioates having methylphenyl group, in that S-4084 had only a low toxicity towards mammals, whereas indicating a high degree of insecticidal activity by various laboratory tests.

Chemical Properties of S-4084.

S-4084 is prepared in good yield from 4-cyanophenol or its alkaline metal salt and O,O-dimethyl phosphorochloridothioate.

M is a hydrogen atom or an alkaline metal atom.

The distillation of S-4084 causes its isomerization even under reduced pressure and produces the mixture of S-4084 and O-methyl-S-methyl-O-(4-cyanophenyl) phosphorothioate, but does not cause explosion contrary to that of Methylparathion. Pure S-4084 is obtained by recrystallization from methyl alcohol and shows the melting point at 14~15°C, the refractive index n_D^{25} 1.5413 and the specific gravity d_4^{25} 1.260.

Pure S-4084, at room temperature, is a pale yellow oil, easily soluble in alcohols, ethers, ketones, esters or cyclic-hydrocarbons and hardly soluble in chain-hydrocarbons or water.

The stability of S-4084 in alkaline aqueous solution was studied by colorimetric determination^{*2)} of sodium salt of 4-cyanophenol liberated during incubation with sodium

hydroxide solution and was compared with those of Sumithion, Methylparathion, Oxo-S-4084^{*5)}, Sumioxon^{*6)} and Methylparaoxon under the same condition. The stabilities of S-4084 and Oxo-S-4084 are shown in Table I as the required time for decomposition of a quarter amount of them at 40°C. Table I

TABLE I. REQUIRED TIME FOR DECOMPOSITION OF A QUARTER AMOUNT^{*3)} OF S-4084 AND OXO-S-4084 IN ALKALINE AQUEOUS SOLUTION. (40% alcoholic, at 40°).

Compounds	t (min.)	Compounds	t (min.)
S-4084	106.4	Oxo-S-4084	15.6
Sumithion	94.5	Sumioxon	10.0
Me-parathion	61.0	Me-paraoxon	5.8

shows clearly that S-4084 is more stable than Methylparathion and Sumithion, and its oxo-analogues show the same tendency. All this coincides with the estimated results with the Hammett Constants of *p*-nitrophenol and *p*-cyanophenol or the dipole moments of nitrobenzene and cyanobenzene. Moreover, the above fact that the hydrolytic speed of Oxo-S-4084 is smaller than those of Sumioxon and Methylparaoxon suggests that the inhibitory potency of Oxo-S-4084 to acetylcholinesterase may also be weaker than those of Sumioxon and Methylparaoxon. The suggestion agrees well with the results shown in Table II.

It was expected from the results of Table I that S-4084 might have a longer residual effect than those of Sumithion and Methylparathion, but in reality, S-4084 proved to be inferior to them in the residual effect by field test. This shows that the residual effect of organophosphorus insecticides cannot be foretold only from any tendency of the hydrolysis of their P-O-Aromatic linkage in alkaline aqueous solution; that is to say that the properties of other linkages in the mole-

6) Y. Nishizawa *et al.*, This Journal, **25**, 605 (1961).

7) Y. Nishizawa *et al.*, *ibid.*, **25**, 597 (1961).

*2) 4-Cyanophenol and 4-nitrophenol were estimated by Shimadzu Q-B50 Spectrophotometer at 310 m μ and 460 m μ respectively.

*5) O,O-Dimethyl-O-(4-cyanophenyl) phosphorate.

*6) O,O-Dimethyl-O-(3-methyl-4-nitrophenyl) phosphorate.

*3) Experimental procedures are as same as the previous paper.⁶⁾

FIG. 1. The Infrared Absorption Spectra (by Shimadzu IR Infrared Spectrophotometer).

- (a) O,O-Dimethyl-O-(4-cyanophenyl) phosphorothioate (S-4084, liquid).
 (b) O,O-Dimethyl-O-(4-cyanophenyl) phosphorate (Oxo-S-4084, liquid).

FIG. 2. Ultraviolet Absorption Spectra (in Ethanol, by Shimadzu RS-27 Recording Spectrophotometer).

———— 4-Cyanophol. - - - - S-4084. - · - · S-4087.

cule have also to be considered in this connection.

The infrared spectrum and the ultraviolet spectrum of S-4084 are shown in Figs. 1 and 2, respectively. In the ultraviolet spectrum, $\lambda_{\max}$ ($\epsilon_{\max}$) of S-4084 is at $243 \text{ m}\mu$ (1.6×10^4).

Biological Activities of S-4084.

Though pure S-4084 has little inhibitory power towards acetylcholinesterase, its oxo-form has a strong power. We may conclude that S-4084 is changed to an oxo-form by enzymatic oxidation *in vivo*, which inhibits

TABLE II. INHIBITORY POTENCY*³⁾ OF OXO-S-4084 TOWARDS ACETYLCHOLINESTERASE. (Mol. concentration required to cause 50% inhibition).

Compounds	Fly head	Mouse brain
Oxo-S-4084	1.05×10^{-4}	4.16×10^{-4}
Sumioxon	6.31×10^{-6}	5.52×10^{-5}
Me-paraoxon	1.76×10^{-5}	1.20×10^{-5}

the acetylcholinesterase. The inhibitory potency of Oxo-S-4084 towards fly head and mouse brain acetylcholinesterase is given in Table II, which shows clearly that the inhibitory potency of Oxo-S-4084 is weaker than those of Sumioxon and Methylparaoxon, but different acetylcholinesterases show different sensitivities to the oxo-forms

of low toxic organophosphorus insecticides. The acetylcholinesterase of fly head shows the same degree of sensitivity as that of mouse brain to Methylparaoxon, but to Oxo-S-4084, fly head shows four times as great sensitivity as mouse brain; and furthermore, to Sumioxon, the former shows ten times as great sensitivity as the latter. From these results it will be clear that the selective toxic phosphorates react with acetylcholinesterase in different degrees according to the various species. It must be noted, however, that the inhibitory potency of oxo-form to acetylcholinesterase sometime is not directly related with the insecticidal activities and the toxicities of the corresponding thiono-forms; especially, in such low toxic compound as S-4084, the inhibitory potency of oxo-form has not any relationship with the activity of its thiono-form. The insecticidal activities of S-4084 proved to be stronger than the estimated power with the inhibitory potency of its oxo-form.

The toxicity of S-4084 towards mouse and rat was compared with that of Parathion, their administration routes varied (see Table III). The toxicity of S-4084 towards goldfish

TABLE III. TOXICITY*³⁾ OF S-4084 TOWARDS MAMMALS (LD₅₀ value)

Route	Mice		$\frac{\text{♂}}{\text{♀}}$	Rats	
	S-4084	Et-parathion		S-4084	Et-parathion
Oral administration	860 mg/Kg	5.8 mg/Kg	$\frac{215}{235}$	215 mg/Kg 235	8.5 mg/Kg 10.0
Subcutaneous administration	1080	14.2	—	—	—
Intraperitoneal administration	350	5.3	—	—	—
Dermal application	>50 mg/one mouse	0.48/mg/one mouse	—	—	—

TABLE IV. TOXICITY*³⁾ OF S-4084 TOWARDS GOLDFISHES

Compounds	after 24 hrs.				after 48 hrs.			
	12 ppm	8 ppm	6 ppm	4 ppm	12 ppm	8 ppm	6 ppm	4 ppm
S-4084	5/5* ⁴⁾	4/5	0/5	—	5/5	4/5	1/5	—
Me-parathion	—	—	5/5	1/5	—	—	5/5	1/5
Contrast	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

*4) A numerator shows the number of dead bodies of goldfish and a denominator shows the number of total bodies used in the test.

(*Carassicus auratus*) was also nearly equal to that of Sumithion (see Table IV).

Insecticidal Activities of S-4084.

Tables V, VI and VII show the comparative efficacies of S-4084 and of Parathion towards rice stem borers. S-4084 is shown to have a higher activity than Parathion in

room test. The effectiveness of S-4084 to-

TABLE VI. POT-TEST*³⁾ OF S-4084 TOWARDS THE FIRST GENERATED LARVAE OF RICE STEM BORERS (kill%)

(spray 3 days later from the encroachment)

Compounds	×4000	×8000	×16000
S-4084	100%	71.4%	58.4%
Et-parathion	94.6	97.1	46.5

TABLE V. TOPICAL APPLICATION*³⁾ OF S-4084 AND S-4087 TOWARDS HIBERNATING LARVAE OF RICE STEM BORERS (kill %)

Compounds	20 γ /larva	6.7 γ /larva	2 γ /larva	0.67 γ /larva	0.2 γ /larva
S-4084	100%	100%	100%	100%	80%
S-4087	100	100	100	60	40
Et-parathion	100	100	100	100	50

TABLE VII. POT-TEST*³⁾ OF S-4084 TOWARDS THE SECOND GENERATED LARVAE OF RICE STEM BORERS. (kill%)

	Compounds	×2000	×4000	×8000
Spray 3 days later from encroachment	(S-4084)	100%	100%	71.4%
	(Et-parathion)	100	94.6	67.1
Spray 7 days later from encroachment	(S-4084)	92.5	71.5	—
	(Et-parathion)	92.9	65.1	—

TABLE VIII. INSECTICIDAL ACTIVITY*³⁾ OF S-4084 TOWARDS HOUSE FLY AND MAGGOT
Spraying to generative place

Compounds	Conc. g/100 ml	Adult fly killed %	Maggot killed %	the day	after 1 days	after 2 days	after 3 days	after 4 days
S-4084	0.2	98.9	84.9	100	96.4	87.2	75.3	51.1
Marathion	0.5	86.6	65.7	98.8	46.1	16.8	5.2	—
untreated	—	2.0	3.2	0	0	3.2	0	0.4

TABLE IX. INSECTICIDAL ACTIVITY*³⁾ OF S-4084 TOWARDS MANY PESTS

Pests	Crops	Formulation	Conc. of the actual ingredient	Type of application	Effect
Chrysanthemum aphid	Chrysanthemum	50% E	1/10000	spray	100% killed
Green peach aphid	Radish	50 E	1/10000	spray	100 killed
Green leafhopper	Rice	50 E	1/2000	spray	95 killed
Green rice leafhopper	Rice	50 E	1/5000	spray	100 killed

TABLE X. FIELD-TEST OF S-4084 AND S-4087 TOWARDS THE FIRST GENERATION OF RICE STEM BORERS

Date of Spray;	July 7, 1961, Osaka, Japan.		
Date of Field Survey;	July 30~Aug. 5, 1961.		
Quantity of Spray;	72.6 cc/m ²		
Variety of Rice;	<i>O. sativa</i> L. var. <i>Hatsushimo</i> .		
Compounds	Formulation	Concentration of emulsion	Rate of damaged plants (%)
Ethylparathion	50% E	1/2000	2.71
		1/1000	1.39
EPN	45 E	1/1500	1.95
		1/750	2.14
S-4084	50 E	1/2000	0.89
		1/1000	0.58
S-4087	25 E	1/800	1.44
		1/400	0.68
untreated	—	—	7.88

TABLE XI. FIELD-TEST OF S-4084 AND S-4087 TOWARDS THE SECOND GENERATION OF RICE STEM BORERS

Date of Spray;	Aug. 30, 1961, Osaka, Japan.		
Date of Field Survey;	Oct. 3~9, 1961.		
Quantity of Spray;	151 cc/m ²		
Variety of Rice;	<i>O. sativa</i> L. var. <i>Hatsushimo</i> .		
Compounds	Formulation	Concentration of emulsion	Rate of damaged plants (%)
Ethylparathion	50% E	1/1000	29.5
		1/750	17.1
S-4084	50 E	1/1000	27.6
		1/500	19.8
S-4087	25 E	1/500	11.8
		1/250	6.1
untreated	—	—	28.4

wards such sanitary pests as house fly and its larvae (maggot) is shown in Table VIII. S-4084 proved more effective than Malathion as a low toxic insecticide. Table IX shows the insecticidal activity of S-4084 towards some other pests than the above; further tests on the effect of S-4084 towards various other pests are under way.

Here, the results of the field tests of S-4084 must be reported in this connection. Tables X and XI clearly show that S-4087, which is a phenylphosphonothioate derivative of S-4084, has a higher activity than S-4084, EPN or Parathion though it has a lower activity than them in room or in green house. The cause has not yet been made clear, but presumably S-4087 has a longer residual effect than S-4084, EPN or Parathion in field though it is less stable in alkaline aqueous solution than S-4084 or Parathion while almost as stable as EPN (see Table XII).

Chemical Properties of S-4087.

The present authors prepared S-4087⁵⁾ side by side with S-4084, considering the fact that EPN⁸⁾, which is a phenylphosphonothioate derivative of Parathion, has excellent properties as an insecticide. Moreover, S-4087 proved to have the best property in all its analogues⁹⁾.

S-4087 is prepared from 4-cyanophenol or its alkaline metal salt and O-ethyl phenylphosphonochloridothioate as already reported⁹⁾ can be led easily to Oxo-S-4087^{*7)} by

oxidation with nitric acid¹⁰⁾.

S-4087 is white crystalline which melts at 83°C and has generally a smaller solubility than S-4084 to various organic solvents. The stability of S-4087 in alkaline aqueous solution, as compared with those of EPN, Oxo-S-4087 and Oxo-EPN^{*8)}, is shown in Table XII as the required time for decomposition of a quarter amount of them at 30°C. EPN has been said to have a longer residual effect than Parathion in field test, but the stability of EPN in an alkaline aqueous solution is smaller than that of Parathion, and S-4087 is as stable as EPN although S-4087 is less stable than Sumithion, S-4084 or Methylparathion.

Figs. 2 and 3 show the ultraviolet spectrum and the infrared spectrum of S-4087 respectively; $\lambda_{\max}$ ($\epsilon_{\max}$) of S-4087 is 235 m μ (2.09×10^4).

TABLE XII. REQUIRED TIME^{*3)} FOR DECOMPOSITION OF A QUATER AMOUNT OF S-4087 AND Oxo-S-4087 IN ALKALINE AQUEOUS SOLUTION (40% alcoholic, at 30°C)

Compound	t (min.)	Compound	t (min.)
S-4087	29.7	Oxo-S-4087	2.1
EPN	30.5	Oxo-EPN	1.2

Biological Properties of S-4087.

S-4087, like other phosphorothioates, has no inhibitory potency to acetylcholinesterase in itself, but its oxo-form has a high potency. Though Oxo-S-4087 has a weak potency towards acetylcholinesterase of mouse brain

M is a hydrogen atom or an alkaline metal atom.

8) E.I. du Pont., U.S.P. 2,503,390 (1950).

9) H. Sakamoto *et al.*, This Journal, **26**, 252 (1962).

*7) O-Ethyl-O-(4-cyanophenyl) phenylphosphonate.

*8) O-Ethyl-O-(4-nitrophenyl) phenylphosphonate.

10) H. Sakamoto *et al.*, This Journal, in press.

FIG. 3. The Infrared Absorption Spectra (by Shimadzu IR Infrared Spectrophotometer).

- (a) O-Ethyl-O-(4-cyanophenyl) phenylphosphonothioate (S-4087, in Nujol).
 (b) O-Ethyl-O-(4-cyanophenyl) phenylphosphonate (Oxo-S-4087, liquid).

compared with the oxo-forms of other organophosphorus insecticides, it has a high potency towards fly head as shown in Table XIII. All this means that selective toxic phosphorus compounds react selectively with different

acetylcholinesterase, as has already been mentioned for S-4084. This property itself should be considered to make a condition in developing the selective toxicity in question.

The toxicity of S-4087 towards mouse and rat was compared with that of EPN, their administration routes varied and the results are shown in Table XIV. This Table shows clearly that S-4087 has a weaker toxicity towards mammals than EPN. To goldfish, S-4087 has a higher activity than Parathion as

TABLE XIII. INHIBITORY POTENCY*³⁾ OF OXO-S-4087 TOWARDS ACETYLCHOLINESTRASE (Mol. concentration required to cause 50% inhibition)

Compounds	Fly head	Mouse brain
Oxo-S-4087	3.06×10^{-6}	1.43×10^{-3}
Oxo-EPN	1.05×10^{-6}	3.98×10^{-5}

TABLE XIV. TOXICITY*³⁾ OF S-4087 TOWARDS MAMMALS (LD₅₀ values)

Routes	Mice			Rats	
	S-4087	EPN		S-4087	EPN
Oral administration	46 mg/Kg	12.2 mg/Kg	⊕ ⊖	60 mg/Kg 28.5	25.3 mg/Kg 10.8
Subcutaneous administration	145	22.5		—	—
Intraperitoneal administration	45	9.9		—	—
Dermal application	>15mg/one mouse	8.7 mg/one mouse		—	—

TABLE XV. TOXICITY*³⁾ OF S-4087 TOWARDS GOLDFISH

Compounds	after 24 hrs.				after 48 hrs.			
	4 ppm	2 ppm	1 ppm	0.5 ppm	4 ppm	2 ppm	1 ppm	0.5 ppm
S-4087	5/5* ⁴⁾	5/5	4/5	1/5	—	5/5	5/5	1/5
Et-parathion	5/5	3/5	0.5	—	5/5	3/5	0/5	—
contrast	0/5	0/5	0.5	0/5	0/5	0/5	0.5	0.5

shown in Table XV.

Insecticidal Activities of S-4087.

Insecticidal activities of S-4087 towards various generations of rice stem borers are shown in Table V and XVI, and the results of the field test are shown in Tables X and XI. The insecticidal activities of S-4087 to-

TABLE XVI. POT-TEST*³⁾ OF S-4087 TOWARDS THE FIRST GENERATED LARVAE OF RICE STEM BORERS (kill %)

(Spray 3 days later from the encroachment)

Compounds	×1000	×2000	×4000
S-4087	97.6	86.1	66.3
Et-parathion	—	99.0	92.1
EPN	—	94.8	88.4

TABLE XVII. INSECTICIDAL ACTIVITY*³⁾ OF S-4087 TOWARDS MANY PESTS

Pests	Crops	Formulation	Conc. of the actual ingredient	Type of application	Effect
Spider mite	Soy bean	25% E	1/2000	spray	95% killed
Chrysanthemum aphid	Chrysanthemum	25 E	1/2000	spray	95 killed
Green peach aphid	Radish	25 E	1/2000	spray	95 killed
Common Cabbage worm	Cabbage	25 E	1/1000	foliage spray	95 killed
Adult fly	—	25 E	1/1000	spray to generative place	95 killed

wards other pests are shown in Table XVII; as for limits of its activities, tests are under way.

From the above results, S-4087 may safely be credited to have good qualities of an effective insecticide.

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