Studies on the Organophosphorus Insecticides

Part VII. Chemical and Biological Properties of New Low Toxic Organophosphorus Insecticide. O, O-Dimethyl-O-(3-methyl-4-nitrophenyl) Phosphorothioate

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In the previous paper¹⁾, it has been reported that O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate (commercial name, Sumithion) has not only an extremely low toxicity but also a superior activity towards insects, compared with any other isomers, homologues and analogues. In this paper, the chemical and the biological properties of Sumithion will be reported in detail.

It has been well known that phosphorothioates*¹⁾ having 4-nitrophenyl group therein possess a high degree of insecticidal activity and consequently are very useful as an active ingredient of agricultural chemicals. However, they have at the same time, a very high degree of toxicity towards mammals and this is, indeed, a serious defect of these compounds for practical application. Therefore, many attempts have been made to search for the compound having lower toxicity and higher insecticidal activity by the American and the German researchers and compounds such as Chlorthion²⁾ and Dicapton³⁾ have been found as the results of their efforts.

Although the so-called low toxic insecticides mentioned above really have comparatively low toxicity, they become also inferior in their

insecticidal activities, especially towards rice stem borer.

In this Laboratory, various studies have been made for the purpose of obtaining a compound which has a low toxicity and also a high degree of insecticidal activity towards rice stem borer, since such compound is anxiously wanted in rice producing districts. As the results, the present authors and their co-workers have succeeded, as already reported, in obtaining two new compounds which have not only the extremely low toxicities but also the superior activities towards insects, compared with any conventional insecticidal compounds or their isomers, homologues and analogues. In this paper, chemical and biological properties of Sumithion which is one of the new low toxic insecticides will be reported in detail.

Chemical Properties of Sumithion.

Sumithion is prepared in good yield from 3-methyl-4-nitrophenol or its alkaline metal salt and O,O-dimethyl phosphorochloridothio-

1) Y. Nishizawa et al., This Journal, **25**, 597 (1961).

*1) Chemical names of phosphorus compounds in this paper were based upon the Drake Committee's Report (*Chem. Eng. News*, **30**, 4515 (1952)).

2) G. Schrader, *Ang. Chem.*, **66**, 265 (1954).

3) American Cyanamid Co., Jap. Pat. Announced No. 6170 (1951), 3449 (1952).

ate as already reported in previous paper¹⁾.

M is hydrogen atom or alkaline metal atom.

The distillation of Sumithion causes its isomerization even under a reduced pressure and produces O-methyl-S-methyl-O- (3-methyl-4-nitrophenyl) phosphate, but does not cause explosion contrary to that of methylparathion.

Pure Sumithion is obtained by silicagel column chromatography in carbontetrachloride solution and shows the refractive index n_D^{25} 1.5528 and the specific gravity d_4^{25} 1.3227.

Sumithion is, in pure state, pale yellow in color and easily soluble in alcohols, ethers, ketones, esters or cyclichydrocarbons and hardly soluble in chain-hydrocarbons and water.

Stability of Sumithion in the alkaline aqueous solution was studied by colorimetric determination of 3-methyl-4-nitrophenol liberated during incubation with sodium hydroxide solution and was compared with that of methyl and ethylparathion.

The hydrolysis did not proceed proportionally with time, but the process was represented by the second order reaction, under the conditions tested, first order relative to the concentration of organophosphorus compound, and first order relative to the sodium hydroxide. The second order rate constant

and the half-life times of methyl-, ethyl-parathion and Sumithion are shown in Table I and II.

TABLE I. THE SECOND ORDER RATE CONST.*2) OF SUMITHION IN ALKALINE SOLUTION

Compound	Temp.	
	at 30°C	at 40°C
Me-parathion	0.375	0.895
Et-parathion	0.125	0.329
Sumithion	0.255	0.622

*2) $k_2 t = 2.303/b - 2a \cdot \log(b - 2x) a / (a - x) b$

k_2 is second order rate constant min⁻¹, mol⁻¹.

a is initial concentration of organophosphorus compound; $1 \sim 3 \times 10^{-3}$ mol.

b is initial concentration of sodium hydroxide; 0.01N as 40% ethanolic solution.

TABLE II. THE HALF-LIFE TIMES OF SUMITHION IN ALKALINE SOLUTION

Compound	Temp.	
	at 30°C	at 40°C
Me-parathion	210 min	84 min
Et-parathion	490	250
Sumithion	272	123

It is clear from Table I and II that Sumithion is one half time more stable than methylparathion.

The infrared spectrum and the ultraviolet spectrum of Sumithion are shown in Fig. 1 and 2, respectively. Frequency of P-O-C (arom.) stretching vibration of Sumithion is 1233 cm⁻¹ although that of methylparathion is 1215 cm⁻¹, therefore, it may be considered that the distance of P-O-C (arom.) linkage of Sumithion is shorter than that of methylparathion (see Fig. 1).

In ultra-violet spectra, $\lambda_{\max}$ ($\epsilon_{\max}$) of methylparathion is at 273 (9474) but that of Sumithion is at 268.5 (6756). This difference in the wavelength and the molecular extinction coefficient of absorption maximum shows clearly that the steric hindrance between methyl group and nitro group in phenyl group of Sumithion is relatively larger (see Fig. 2).

FIG. 1. The Infrared Absorption Spectra of Sumithion and Methyl Parathion.
(in Nujol, by Shimadzu IR Infrared Spectrophotometer.)

A: Sumithion B: Me-parathion

FIG. 2. The Ultra-violet Absorption Spectra of Sumithion and Methyl Parathion.
(in Ethanol, by Shimadzu RS-27 Recording Spectrophotometer.)

———— Sumithion
----- Me-parathion

Biological Activities of Sumithion.

Pure Sumithion has not inhibitory power towards acetylcholinesterase but is converted easily to the oxo-form by enzymatic oxidation in vivo and this oxo-Sumithion has high inhibitory power.

The inhibitory potency of oxo-Sumithion

TABLE III. INHIBITORY POTENCY OF OXO-SUMITHION TOWARDS ACETYLCHOLINESTERASE*³⁾

Compound	Fly head	Mouse brain
Me-paraoxon	2.2×10^{-5} * ⁴⁾	1.0×10^{-5}
Et-paraoxon	3.2×10^{-6}	2.6×10^{-6}
Oxo-Sumithion	5.0×10^{-6}	2.8×10^{-5}

*³⁾ Inhibition of fly head and mouse brain acetylcholinesterase by these compounds was investigated manometrically at pH 7.6, 37.5°C by using a homogenate of mouse brain and a supernatant from the homogenate of the adult house fly heads by centrifugation at 6,000 rpm for 7 mins. Acetylcholine perchlorate (5×10^{-3} mol.) was used as substrate. The inhibitor and the substrate were added simultaneously to the enzyme solution and the initial velocity observed was compared with that of the control. The inhibitory potency was determined by plotting the percent inhibition versus—log of the inhibitor concentration.

*⁴⁾ Mol. concentration required to cause 50% inhibition.

towards fly head and mouse brain acetylcholinesterase is shown in Table III.

The toxicity of Sumithion towards mouse is compared with that of parathion, varying

TABLE IV. TOXICITY OF SUMITHION TOWARDS MOUSE*⁵⁾ (LD₅₀ value)

Route	Sumithion	Parathion
Oral administration* ⁶⁾	870 mg/kg	9.5 mg/kg
Subcutaneous administration* ⁷⁾	1000 mg/kg	11.5 mg/kg
Intraperitoneal administration* ⁸⁾	280 mg/kg	6.0 mg/kg
Dermal application* ⁹⁾	50 mg/one mouse	0.81 mg/one mouse

*⁵⁾ The test was applied to each group of 10~15 bodies of mice which had almost the same body weights ranging from 13 to 18 grams and were abstained from food for 4 hours before beginning the test.

*⁶⁾ The test of the oral toxicity was conducted by the same method as in the previous paper⁴⁾

*⁷⁾ The test by subcutaneous administration route was conducted as follows. Each test compound was emulsified by dilution of 50% emulsifiable concentrate composition with water. Each concentration of the above solutions was injected subcutaneously. The volume of instilment was 50 ml per 1 kg of body weight of mice. After keeping it for 5 days, the symptoms of poisoning were observed and LD₅₀ (mg/kg) value was calculated according to the Litchfield and Wilcoxon⁵⁾ or Behrens-Käber⁶⁾ methods.

*⁸⁾ The test by the intraperitoneal administration route was conducted by almost the same process as that of subcutaneous route. But in this case, the test emulsion was injected into peritoneum and the symptoms of poisoning was observed for 3 days.

*⁹⁾ The test of dermal application was conducted as follows. Each concentration of the aforesaid emulsion was spread on the skin of the back of a mouse, cutting the hair in 1 cm² area and the spread part was covered with a piece of cellophane⁷⁾. The volume of the emulsion applied was 0.05 ml per one mouse. The symptoms of poisoning was observed for 7 days and LD₅₀ value was calculated by the aforesaid method.

TABLE V. ORAL TOXICITY OF SUMITHION TOWARDS VARIOUS ANIMALS*¹⁰⁾

Animals	Sumithion	Parathion
Rats $\frac{\odot}{\ominus}$	242	8.5
	433	10.0
Guinea pigs	1833	12.5
Cats	142.3	0.93

*¹⁰⁾ A suspension of the test compound was prepared by diluting the 5% gum arabic composition with water and was applied to each group of 3~5 bodies of rats, guinea pigs or cats which had almost the same body weights ranging 130~170g in rats, 200~250g in guinea pigs and 2.0~3.0 kg in cats and had been abstained from food for 4 hours before beginning the test.

their administration routes and the results are shown in Table IV.

Furthermore an outstanding characteristic of low toxicities of Sumithion will be apparent from the following Table VI.

The following Table VII shows the toxicity

4) Y. Nishizawa et al., *Bōtzu-Kagaku*, **26**, 4 (1961).

5) J. T. Litchfield et al., *J. pharmacol.*, **96**, 99 (1949).

6) B. Behrens et al., *Arch. exp. path. und pharmacol.*, **177**, 379 (1935).

7) T. Seki, *Yakugaku Kenkyū*, **23**, 138 (1951).

TABLE VI. THE ORAL TOXICITIES OF SUMITHION AND COMMERCIAL INSECTICIDES

Name	Formula	Oral Toxicities towards Mouse LD ₅₀ mg/kg
Sumithion		870
Parathion		9.8
Me-Parathion		26.0
EPN		14.5
Dipterex* ¹¹⁾		480
Chlorthion		337
Dicaptan		320

*¹¹⁾ Dipterex was produced to aqueous solution and used.

TABLE VII. THE TOXICITY*¹²⁾ OF SUMITHION TOWARDS GOLDFISH

Compound	10 ppm.	5 ppm.	2 ppm.	1 ppm.
Sumithion	6/6* ¹⁴⁾	5/6	0/6	0/6
Parathion	6/6	6/6	1/6	0/6
Contrast* ¹³⁾	0/6	0/6	0/6	0/6

*¹²⁾ It this test, an emulsion of the the test compound was made by dilution of 50% emulsifiable concentrate composition (comprised of 50 parts active ingredient, 30 parts Triton X-100 and 20 parts Xylene, by weight) with water. An emulsion was applied to 6 goldfish in 10 l of water. After keeping it for 70 hours, its death was recorded. All the goldfish used in this test had nearly the same body length of 4~5 cm.

*¹³⁾ Contrast means a solution without the test compound.

*¹⁴⁾ A numerator shows the number of dead bodies of goldfish and a denominator shows the number of total bodies used in this test.

towards goldfish (*Carassius auratus*). It will be evident from Table VII that Sumithion has almost the same toxicity towards goldfish as parathion.

TABLE VIII. TOPICAL APPLICATION*¹⁵⁾ OF SUMITHION TOWARDS HIBERNATING LARVAE OF RICE STEM BORER (kill %)

Compound	20 γ/ larva	6.7 γ/ larva	2 γ/ larva	0.67 γ/ larva	0.2 γ/ larva
Sumithion	100%	100%	100%	100%	60%
Parathion	100%	100%	100%	90%	50%

*¹⁵⁾ Topical test was conducted as follows. Each compound was dissolved in acetone to prepare a series of acetone solutions containing from 0.067 to 2% of the each compound (g/ml).

Each 1/1000 ml of the said solution was applied to the body of the larva by means of micrometer syringe and, after keeping it for 3 days at 25°C, its death or survival condition was observed. The larvae utilized in this test had almost the same body weights ranging 80~90 mg and each solution was applied to a group of 20 larvae in order to calculate the mean fatal per cent.

TABLE IX. POT-TEST*¹⁶⁾ OF SUMITHION TOWARDS THE FIRST GENERATED LARVAE OF RICE STEM BORER (kill %)
(Spray 3 days later from the encroachment)

Compound	×2000	×4000	×8000	×16000
Sumithion	97.7%	81.4%	60.4%	56.9%
Parathion	80.2%	75.6%	52.1%	49.9%

*¹⁶⁾ The pot-test towards the first generated larvae was conducted by the same method as the previous paper⁹⁾, but the rice plants elapsing further 15~25 days after transplanting into a porcelain pot, were used.

TABLE X. POT-TEST*¹⁷⁾ OF SUMITHION TOWARDS THE SECOND GENERATED LARVAE OF RICE STEM BORER (kill %)

	Compound	×1,000	×2,000	×4,000
Spray 2 days later	Sumithion	99.7%	99.8%	95.2%
from encroachment	Parathion	99.5	87.0	86.4
Spray 3 days later	Sumithion	98.6	96.5	86.5
from encroachment	Parathion	100	100	95.4
Spray 4 days later	Sumithion	100	100	—
from encroachment	Parathion	91.5	55.6	—

*¹⁷⁾ The pot-test towards the second generated larvae was conducted by the same method as the previous paper⁹⁾.

Insecticidal Activities of Sumithion.

The following Table VIII, IX and X show the comparative efficacies towards rice stem borer (*Chilo suppressalis* WALKER) of Sumithion and of parathion which is the typical insecticidal compound utilized in these days.

In order to make clear the effectiveness of Sumithion towards sanitary pests, Table XI shows the comparative efficacies towards house fly and its larva (maggot), of Sumithion and

TABLE XI. INSECTICIDAL ACTIVITY OF SUMITHION TOWARDS HOUSE FLY AND MAGGOT.*¹⁸⁾

Compound	Conc.* ¹⁹⁾ g/100 ml.	Adult fly* ²⁰⁾ kill %	Maggot* ²¹⁾ kill %	Spraying to generative place* ²²⁾					
				the day	after 1 day	after 2 days	after 3 days	after 4 days	after 7 days
Sumithion	0.25	99.5	98.4	100	89.2	85.2	81.3	73.0	15.1
Malathion	1.00	99.5	95.0	100	78.5	59.2	43.0	36.9	12.8
Diazinon	0.125	99.5	99.0	100	93.6	84.0	91.3	87.2	50.0
Dipterex	0.5	67.6	97.4	100	97.0	51.3	25.2	8.2	—
DDVP	0.5	99.5	94.7	98.8	85.2	18.1	8.2	—	—
Dicaptan	0.25	100	93.8	100	93.5	61.8	52.6	44.6	12.3
Dimethoate	0.0312	63.9	98.2	96.7	96.3	95.5	100	98.5	65.3
untreated	—	8.6	3.5	1.7	0.6	0.8	2.0	2.3	2.1

*¹⁸⁾ In this test, an emulsifiable concentrate was prepared by mixing 50 parts of an active ingredient, 30 parts of Triton X-100 and 20 parts of xylene by weight, and the concentrate was diluted with water to make a test emulsion.

*¹⁹⁾ The concentration, g/100 ml, shows the amounts (g) of the active ingredient present in 100 ml of the test emulsion, and these values shown in this item were adequately chosen so that the kill % of maggot came to more than 90%.

*²⁰⁾ By means of settling tower method (cf. *Bull. Ent. Res.*, 14, 223 (1924)).

*²¹⁾ By means of the Beaker method. That is, a culture ground of house fly was settled into a beaker having an inner diameter of about 9 cm and a height of about 10 cm, and eggs of house fly were blowed thereon. Two days later, 1 ml of the test emulsion was sprayed on the surface of the ground, and after 7 days the dead and the surviving numbers of the larvae (maggots) were calculated.

*²²⁾ About 300g of matured culture medium for the larva (maggot) of house fly were taken in a dish having an inner diameter of about 15 cm and a height of about 8 cm, and 5 g of sugar was added thereto and mixed well. To the dish, which was regarded as an artificial generative place, 1 ml of the test emulsion was sprayed. After that, adult flies were put therein every day and their vital states were observed. This experiment is concerned with the residual effectiveness of the test compound.

TABLE XII. INSECTICIDAL ACTIVITY OF SUMITHION TOWARDS MANY PESTS

Pest	Crop	Formulation	Conc. of the actual ingredient	Type of application	Effect
Chrysanthemum aphid	Chrysanthemum	50 E* ²³⁾	1/16,000	spray	100% kill
Green peach aphid	Radish	50 E	1/16,000	spray	100% kill
Green leafhopper	Rice	50 E	1/2,000	spray	100% kill
Green rice leavhopper	Rice	50 E	1/6,000	spray	100% kill
Black rice bug	Rice	50 E	1/2,000	spray	100% kill
Common Cabbage Worm	cabbage	50 E	1/4,000	foliaye spray	effective 10 days later

*²³⁾ 50 E means an emulsifiable concentrate containing 50 parts of Sumithion, 20 parts of xylene and 30 parts of Triton x-100 by weight.

of the conventionally utilized low toxic insecticides.

The following Table XII shows the insecticidal activity of Sumithion towards other pests than the above described injurious insects.

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