this new pyrethroidal compound showed insecticidal activity superior to other pyrethroids, characterized by the higher knockdown-mortality activity, although it was inferior to pyrethrins or phthalthrin in initial knock-down activity. Furthermore, Chrysron had better residual contact effectiveness against cockroaches and houseflies. With the above-mentioned properties, Chrysron can replace in practical applications of various kinds of synergists now available in enhancing the efficacy of other pyrethroids.

Synthesis and Biological Activities as Insecticides and Fungicides of Saligenin Cyclic Phosphorothiolates. Ken KOBAYASHI, Morifusa ETO*, Yasuyoshi OSHIMA**, Tadayoshi HIRANO, Toshiharu HOSOI and Shigeki WAKAMORI (Kumiai Chemical Co., Shimizu, *Department of Agricultural Chemistry, Kyushu University, Fukuoka and **Faculty of Agriculture, Meiji University, Kawasaki) Received August 24, 1969. *Botyu-Kagaku*, 34, 165, 1969.

21. サリゲニン環状チオールリン酸エステルの合成とその殺虫ならびに殺菌性

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数種のサリゲニン環状チオールリン酸エステルを合成し，その生物活性について検討した．これらは殺虫性と共に殺菌性も有している．殺虫力は *S*-アルキル基が小さいほど強く，メチル誘導体 (2-methylthio-4H-1, 3, 2-benzodioxaphosphorin-2-oxide) が最も強い．一方，イモチ病に対してメチル，エチルおよび *n*-ブチル誘導体が顕著な予防効果を示した．しかし，治療効果については，エチルおよび *n*-ブチル体は優れているが，メチル体にはほとんど活性がなかった．これらチオールエステル類はコリンエステラーゼのほかSH酵素も阻害することから作用機構について考察した．

A number of saligenin cyclic esters derived from phosphorus acids have been synthesized and evaluated for their biological activities by Eto and his coworkers[1~8], since they[9,10] found saligenin cyclic *o*-tolyl phosphate as the active metabolite of tri-*o*-tolyl phosphate (TOCP), a neurotoxic substance. The cyclic esters show interesting variety in toxicity, which appear to be decided by an exocyclic substituent on phosphorus[11]. The esters having a big substituent are active to cause ataxia[12] in hen and are synergistic with malathion[13] but are not insecticidal. On the other hand, the esters having a small alkyl group are not ataxic but highly insecticidal[3].

Thus, methyl phosphorothionate (2-methoxy-4H-1, 3, 2-benzodioxaphosphorin-2-sulfide; salithion[14]) is now practically used as insecticide. We undertook to prepare the isomeric phosphorothiolate and its homologs for the evaluation of biological activities and found that some of them had fungicidal activity as well as insecticidal activity.

Experimental

Chemical

Phosphorodichloridothiolates. To a mixture of three equivalents of phosphorus oxychloride and one equivalent of pyridine was added in dropwise one equivalent of appropriate mercaptan with stirring at 20 to 50°C. After stirring for four hours the precipitation of pyridinium chloride was separated by filtration. Unreacted phosphorus oxychloride was distilled off and the residue was fractionally distilled.

Saligenin cyclic phosphorothiolates. Phosphorodichloridothiolates reacted with saligenin in the presence of solvent and pyridine or other tertiary amines at room temperature or at about 50°C. The products were purified by distillation *in vacuo* or recrystallization. The following example is shown as a typical procedure.

2-Methylthio-4H-1, 3, 2-benzodioxaphosphorin-2-oxide. To a mixture of saligenin (6.2 g), pyridine (8 g) and chloroform (100 m*l*) was added dropwise methyl phosphorodichloridothiolate (8 g) with stirring at 20°C. After stirring three hours, the reaction mixture was washed in sequence

with water, dilute alkali, dilute hydrochloric acid and water and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue was distilled *in vacuo* to give 3.2g of oil at b. p. 144~145°C (0.1 mmHg). The oil solidified slowly at room temperature.

Biological

Insecticidal evaluation test. Insecticidal activity was determined against 4- to 5-days old female *Musca domestica vicina* Macquart of Sapporo strain by topical application of acetone solutions. They were kept at 25°C for 24 hours and then mortality was counted. The test was duplicated. Fungicidal evaluation tests. When the second leaf had been developed, rice plants in a pot were sprayed with 20ml of an aqueous emulsion of test chemicals. The plants were then inoculated with *Piricularia oryzae* by spraying of spore suspension. They were kept in a green house for 4 days and symptom was assessed for protective value.

In another experiment, the chemicals were sprayed at 24 hours after the inoculation in order to determine therapeutic value. All the tests were triplicated and ten plants in a pot were used for counting.

The protective value (PV) and the therapeutic value (TV) were calculated by the following equation. PV or TV=

$$\left(1-\frac{\text{average number of spots in a treated leaf}}{\text{average number of spots in an untreated leaf}}\right)\times 100$$

Other methods

Anticholinesterase activity was determined by incubating each compound with housefly homogenate for 30 minutes at room temperature and assaying residual cholinesterase activity by Warburg-manometric method[15]. Infrared absorption spectra were recorded from 10% chloroform solutions with a Shimazu IR-27G spectrophotometer with a grating.

Results and Discussion

Synthesis of saligenin cyclic phosphorothiolates

Only few reports have been presented for the preparation of thiol esters of phosphorodichloridothioic acid. Petrov[16] made them by the reaction of phosphorus trichloride and sulfenyl chlorides in liquid sulfur dioxide. Bliznyuk[17] prepared them from alkyl phosphorodichloridite and phosphorus thiochloride by heating. We tried to prepare them from phosphorus oxychloride and mercaptans. Any attempts such as heating the mixture with or without sodium metal and using sodium salts of mercaptans were unsuccessful. However, when pyridine was used as a catalyst, the reaction took place smoothly under a mild condition with a moderate yield. The yield and boiling point of the phosphorodichloridothiolate esters are shown in Table 1. Phenyl ester was not distilled but the crude product could be used for the synthesis of saligenin cyclic ester.

Table 1. Yield and boiling point of phosphorodichloridothiolate esters prepared by the following reaction.

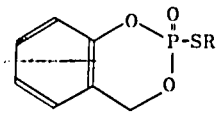
$$POCl_3+HSR \xrightarrow{\text{pyridine}} RSPOCl_2$$

R	Yield (%)	B.p. °C/mmHg
CH_3	30~45	95~108/15
C_2H_5	55~60	95~105/20
n-C_3H_7	60	105~110/15
i-C_3H_7	40~70	70~80/5
n-C_4H_9	60~75	115~120/17
C_6H_5	—	undistillable liquid

All kinds of saligenin cyclic esters derived from phosphorus acids have been synthesized by the condensation of saligenin with mono-substituted phosphorus oxychloride or thiochloride. Saligenin cyclic phosphorothiolates were also prepared from saligenin and appropriate thiol esters of phosphorodichloridothioic acid according to this general method. The synthesized cyclic phosphorothiolates are listed in Table 2 with physical and analytical data.

In other experiments, some attempts were tried to prepare the cyclic phosphorothiolates from their isomeric phosphorothionates, because many saligenin cyclic phosphorothionates have been prepared and particularly the most important methyl ester, salithion, is now available in a great quantity. Although the isomerization reaction of phosphorothionates to phosphorothiolates with heat[18] or dimethylformamide[19] is

Table 2. Physical and analytical data of saligenin cyclic phosphorothiolates.



No.	R	Yield (%)	b. p. °C/mmHg (m. p. °C)	Formula	Phosphorus (%)	
					Calcd.	Found
I	CH_3	48	144-5/0.1	$C_8H_9O_3SP$	14.33	14.45
II	C_2H_5	68	140-5/0.04	$C_9H_{11}O_3SP$	13.45	13.04
III	n-C_3H_7	53	145-7/0.07	$C_{10}H_{13}O_3SP$	12.68	13.21
IV	i-C_3H_7	63	155-8/0.1	$C_{10}H_{13}O_3SP$	12.68	13.10
V	n-C_4H_9	71	157-60/0.02	$C_{11}H_{15}O_3SP$	11.99	12.06
VI	C_6H_5	59	(88-9)	$C_{13}H_{11}O_3SP$	11.15	10.82

known, all attempts applied to the cyclic esters were unsuccessful. The application of boron fluoride etherate known as a catalyst for the rearrangement of thionocarbamates[20] resulted in fail again.

The infrared spectra of the cyclic esters prepared from saligenin and phosphorodichloridothiolates are almost identical with those of corresponding cyclic phosphates except a few small differences. They show characteristic bands at 1280~1285cm^{-1} (P=O), 1190~1192cm^{-1} (P-O-aryl), 1023~1026cm^{-1} (P-O-CH_2-) and 820 cm^{-1}. It is interesting to note that the P=O stretching vibration of the phosphorothiolates are lower in frequency than that of the cyclic phosphate esters, which have the band at about 1305cm^{-1}. The band at 820cm^{-1} is also observed in the cyclic phosphorodithioates, whereas it shifts to 840cm^{-1} and to the region between 850 and 870cm^{-1} for the cyclic phosphorothionates and phosphates respectively.

Insecticidal activity

The toxicity to houseflies of the saligenin cyclic phosphorothiolates and some other related phosphorus compounds is shown in Table 3. The methyl ester (I) is most toxic to houseflies in the cyclic thiolate series. Its insecticidal activity is about a half of its thionate isomer, salithion, and is almost comparable to sumithion (*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate).

When the size of an exocyclic substituent on phosphorus increases, the insecticidal activity decreases. This is agreeable with observations in other series of saligenin cyclic esters, including phosphates[3,14], phosphoramidates[4] and their thiono analogs. However, the effect of the substituent is smaller than in the phosphate ester series. The methyl thiolate is only 70 times more toxic than butyl homolog, whereas the corresponding difference is over 300 times in the phosphate series[3]. Murdock[21] reported that the range of activity between the least and most active compound in a series of *O,O*-dialkyl *S*-aryl phosphorothiolates was much small in

Table 3. Toxicity to oriental housefly of saligenin cyclic phosphorothiolates and some organophosphorus esters.

Compound	LD_{50}/γg/g
I	3.00
II	11.21
III	94.50
IV	17.23
V	211.8
VI	73.61
Salithion[a]	1.60
Sumithion[b]	2.41
Kitazin[c]	375

a) 2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulfide; thiono isomer of I
b) *O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate
c) *O,O*-diethyl *S*-benzyl phosphorothiolate

comparison to their phosphate analogs.

Fungicidal activity

Recently some phosphorothiolate esters were found to have activity to protect the rice plant from rice blast disease caused by the infection of *Piricularia oryzae*[22]. Saligenin cyclic phosphorothiolates also have activity to control the rice blast. The protective values against *Piricularia oryzae* of the cyclic phosphorothiolates and related compounds are shown in Table 4. The data of some commercial fungicides including an organophosphorus compound, Hinosan (*O*-ethyl *S*, *S*-diphenyl phosphorodithioate), are also shown in the Table for comparison. The methyl (I), ethyl (II) and *n*-butyl (V) phosphorothiolates have high fungitoxicity comparable to other commercial fungicides. The normal (III) and isopropyl (IV) derivatives are less effective

Table 4. Protective value (%) against *Piricularia oryzae* of saligenin cyclic phosphorothiolates and some related compounds in comparison with some commercial fungicides.

Compound	500ppm	250ppm	100ppm	50ppm	25ppm
I	—	100	100	100	84.8
II	—	100	93.7	92.5	81.5
III	—	100	57.1	34	—
IV	—	—	68.7	34.4	—
V	—	100	91.7	93.3	75.6
VI	—	50.2	—	—	—
Salioxon[a]	65	—	—	—	—
Salithion[b]	52	—	—	—	—
Hinosan[c]	—	100	—	86.2	—
Pentachloro-benzyl alcohol	—	—	98.8	93.5	—
Blasticidin S	—	98.5	—	86.3	—

a) 2-methoxy-4H-1, 3, 2-benzodioxaphosphorin-2-oxide; phosphate analog of I
b) see the footnote of Table 3.
c) *O*-ethyl *S*, *S*-diphenyl phosphorodithioate.

Therapeutic values obtained by spraying chemicals 24 hours after the inoculation are shown in Table 5. The cyclic methyl phosphorothiolate (I) is almost ineffective as pentachlorobenzylalcohol (PCBA) is. The ethyl (II) and *n*-butyl (V) thiolates are still effective as well as blasticidin-*S* and Hinosan.

Table 5. Therapeutic value to *Piricularia oryzae* of saligenin cyclic phosphorothiolates and some commercial fungicides.

Compound	Therapeutic value (%) at 200 ppm
I	7.1
II	100
V	97.6
Hinosan	95.2*
Pentachloro benzyl alcohol	0
Blasticidin S	97.6

* data at 250 ppm

Saligenin cyclic methyl phosphate and phosphorothionate (salithion) are highly active as insecticide (Table 3) but are almost inactive as fungicide (Table 4). It has been observed that, in the series of dialkyl benzyl esters of phosphorus acids, only *S*-benzyl phosphorothiolates are highly active as fungicide but phosphates, phosphorothionates and phosphorodithioates are inactive[22].

It is interesting to note that some cyclic phosphorothiolates have high activities both as insecticide and fungicide. An organophosphorus fungicide, *O*, *O*-diethyl *S*-benzyl phosphorothiolate (Kitazin) has only weak insecticidal activity (Table 3).

Mode of action

It is generally accepted that the insecticidal action of organophosphorus insecticides is due to the inhibition of cholinesterase by phosphorylation. The saligenin cyclic phosphorothiolates are potent inhibitors of housefly cholinesterase (Table 6). They are almost same with or rather more than corresponding phosphates in anticholinesterase activity. The methyl phosphorothiolate is 3.6 times as active as the corresponding

Table 6. Inhibition of enzymes by saligenin cyclic alkyl phosphates and phosphorothiolates.

	I_{50} (M)			
	Housefly cholinesterase		Yeast alcohol[24] dehydrogenase	
Alkyl group	Phosphate	Phospho-rothiolate	Phosphate	Phospho-rothiolate
CH_3	7.6×10^{-8}	2.1×10^{-8}	6.2×10^{-4}	4.5×10^{-5}
C_2H_5	1.3×10^{-7}	1.4×10^{-7}	—	4.4×10^{-5}

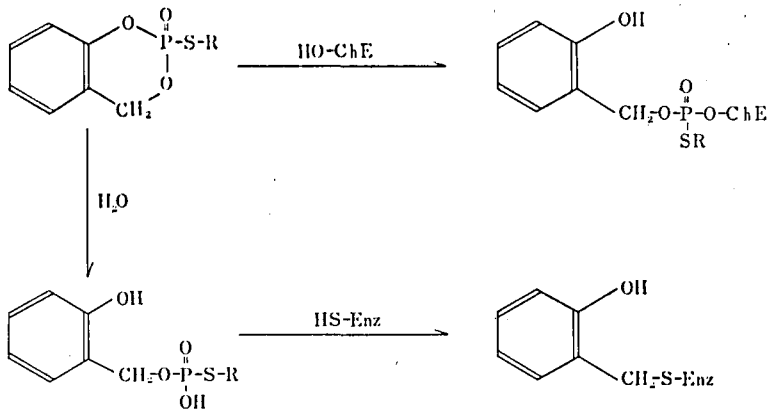


Fig. 1. Proposed mode of action of saligenin cyclic phosphorothiolates.

phosphate. The ethyl thiolate is one-seventh as active as the methyl homologue and almost same with the ethyl phosphate.

On the other hand, it was recently found that the cyclic phosphorothiolates have high activities to alkylate (salicylate) mercaptans and to inhibit "SH enzymes"[23,24]. The activities seem to be related with fungicidal property but not with insecticidal activity.

Saligenin cyclic phosphorothiolates are hydrolyzed more rapidly than corresponding phosphate esters in phosphate buffer solution. The hydrolysis occurs at heterocyclic P-O-C (aryl) bond. It was demonstrated that the hydrolyzate of saligenin cyclic methyl phosphorothiolate, *i.e.* *O-o*-hydroxybenzyl *S*-methyl hydrogen phosphorothiolate, reacted with mercaptans to give salicyl thioethers[24]. These findings indicate that the saligenin cyclic phosphorothiolates may react with cholinesterase to phosphorylate its serine hydroxyl group and may, on the other hand, be hydrolyzed to the *o*-hydroxybenzyl esters which react with "SH enzymes" to alkylate their thiol group (Fig. 1). Cholinesterase is essential for the life of insects and "SH enzymes" may be for fungi.

Summary

Several saligenin cyclic phosphorothiolates were synthesized and evaluated for biological activities. They showed fungicidal activity as well as insecticidal activity. The smaller the *S*-alkyl group is, the higher the insecticidal activity is. Thus, the *S*-methyl thiolate is the most active insecticide. The methyl, ethyl and *n*-butyl homologues were very effective to protect rice plants against *Piricularia oryzae*. The latter two compounds showed also a high effectiveness to the plants inoculated previously.

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Toxicity of *p,p'*-DDT, *o,p'*-DDT and Their Mixtures Against Mosquitoes. R.L. KALRA (National Malaria Eradication Programme, Delhi, India) Received September 25, 1969. *Botyu-Kagaku*, 34, 170, (1969).

22. カに対する *p,p'*-DDT, *o,p'*-DDT およびその混合物の毒性

R.L. KALRA (National Malaria Eradication Programme, Delhi). 44. 9. 25 受理

Culex pipiens fatigans, *Aedes aegypti*, *Anopheles subpictus* に対する *p,p'*-DDT, *o,p'*-DDT の殺虫力を dry film, oil solution, topical application 法で検討した．その結果 dry film 法では *o,p'*-DDT は *C.p.fatigans* に対し *p,p'*-DDT より殺虫力が強く，oil solution および topical application 法では，両化合物はほぼ同じ殺虫力を示した．*Aedes aegypti* に対しては，すべての施用法で *p,p'*-DDT は *o,p'*-DDT より殺虫力が強く，*Anopheles subpictus* に対してほぼ同じ殺虫力であった．

p,p'-DDT と *o,p'*-DDT との混合はカの成虫に対して共力作用が認められない．*Aedes aegypti* および *Culex p.fatigans* の *p,p'*-DDT, *o,p'*-DDT に対する感受性を比較すると，*A.aegypti* は *p,p'*-DDT に対して感受性が高く，*o,p'*-DDT に対しては，両種にあまり感受性の差がない．

Yasutomi[22] observed that the joint action of *p,p'*-DDT and *o,p'*-DDT was synergistic against houseflies and body lice. Furthermore, technical DDT has been found to be more toxic than pure *p,p'*-DDT, thereby suggesting the interaction of the isomers[1,16,22]. Kalra and Joshi[10] studied the toxicity of the mixture of *p,p'*-DDT and *o,p'*-DDT against the various species of mosquito larvae. The joint action of the isomers was found to be simple similar against *Culex pipiens fatigans* and synergistic against *Aedes aegypti*. The present communication summarizes the results of the investigation on the relative toxicity of *p,p'*-DDT, *o,p'*-DDT and their mixtures against the adults of various species of mosquitoes and the larvae of *Anopheles subpictus*.

Insect Material

Three to four days old laboratory reared females of *C.p.fatigans* and *Aedes aegypti* were used. Pupae of *Anopheles subpictus* were collected from the areas around Delhi and hatched in the laboratory. The female adult mosquitoes thus obtained were used when 3-4 days old. All the tests were done on glucose fed female mosquitoes. Larvae of *Anopheles subpictus* collected from the field were used as such.

Methods

Dry film method

The insecticides were applied in standard w/v acetone solution to whatman filter papers No. 1 (15cm×12cm), 1.5ml. of the different concentrations of insecticidal solutions were pipetted on the papers and allowed to evaporate com-