原　著

Studies on Saligenin Cyclic Phosphorus Esters with Insecticidal Activity. Part XII. Insecticidal Activity of Ring-substituted Derivatives. Morifusa Eto, Ken Kobayashi,*1 Tadao Sasamoto,*1 Hong-Ming Cheng,*2 Takatoshi Aikawa,*3 Toyohiko Kume, and Yasuyoshi Oshima*4 (Department of Agricultural Chemistry, Kyushu University, Fukuoka) Received July 3, 1968, *Botyu-Kagaku* 33, 73, 1968.

10. 殺虫性サリゲニン環状リン酸エステルの研究（第12報） 核置換誘導体の殺虫性　江藤守総，小林健*1，笹本忠夫*1，鄭　弘命*2，相川高利*3，久米豊彦，大島康義*4（九州大学農学部農芸化学科，福岡市）43.7.3 受理

約50種の核置換サリゲニン環状リン酸エステルを合成しイエバエに対する殺虫力を調べた．置換基の種類および位置によって程度は異なるが，一般に核置換によって殺虫性は減少する．従って核置換基を有しないサリチオン，サリオキソンより殺虫力のすぐれたものは見出されなかった．フェノールエステル基に対しパラ位の置換基の電子吸引性の増加と共に殺虫性は減退する．電子供与基を導入しても殺虫性が減退した．

In these several years, we have prepared a lot of saligenin cyclic phosphorus esters and investigated on the relationship between chemical structure and biological activities.[1-7] The variety in the structure was mainly due to the substituents on phosphorus. It has been demonstrated that the size of the substituent on phosphorus is important to decide the specificity in the biological activity: small alkyl derivatives are insecticidal[3], whereas aryl derivatives are not insecticidal but are synergistic with malathion[6] and ataxic to chicken.[2]

In this paper, the effect of ring-substituent of saligenin on insecticidal activity is described. It appears that the electronic character of substituent at para-position to phenolic ester linkage is not correlate with the insecticidal activity.

Experimental

Ring-substituted saligenins.

Some of them were prepared by the condensation of corresponding phenols with aqueous formaldehyde by the catalytic action of alkaline (Lederer-Manasse method) or zink acetate.[8] They include 4-methyl-, 3,5-dichloro-, 3-phenyl-5-chloro- and 3-chloro-5-phenyl-2-hydroxybenzyl alcohols. Others were prepared by the reaction of trioxymethylene with metaborate of corresponding phenols followed by hydrolysis.[9] They include 3-methyl-, 5-methyl, 3-chloro-, 5-chloro- and 5-phenyl-2-hydroxybenzyl alcohols. In certain cases, Arct's method[10] was applied. *o*-Dimethylaminomethylphenols prepared by Mannich condensation of the corresponding phenols with dimethylamine and formaldehyde were refluxed with acetic anhydride to give diacetates of corresponding hydroxybenzyl alcohols and were followed by hydrolysis.

These procedures are illustrated by the following examples.

2-Hydroxy-5-phenylbenzyl alcohol by borate method.

A mixture of *p*-phenylphenol (17.2 g) and boric acid (6 g) in toluene (100 ml) was refluxed for six hours. After complete removal of the theoretical amount of water, the solution was gradually added with a suspension of trioxymethylene (3.5 g) in dry toluene (50 ml) at 90°C and kept for six hours at that temperature. The reaction product was hydrolyzed with concentrated potassium hydroxide solution. The alkaline solution was neutralized with acetic acid

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to give crude 2-hydroxy-5-phenylbenzyl alcohol in 72% yield. It was recrystallized from benzene to give leaf-plates, m. p. 154-155°C. *Anal.* Calcd. for $C_{13}H_{12}O_2$: C, 77.98; H, 6.04%. Found: C, 77.95; H, 6.15%.

2-Hydroxy-5-phenylbenzyl alcohol by Lederer-Manasse reaction.

4-Phenylphenol (17.2 g) was dissolved in 10% aqueous solution of potassium hydroxide (50ml) and equimole of 37% formalin was added. This mixture was kept at 40°C for four days and then poured into 200ml of ice water and acidified with acetic acid. The precipitation was submitted to a silicic acid column and eluted with acetone-benzene mixture (1:5) to give crystals in 25% yield which melted at 154-155°C and was identical with the product obtained by the above-mentioned borate method.

2-Hydroxy-5-phenylbenzyl alcohol by Arct's method.

To a mixture of *p*-phenylphenol (17.2 g) and equimole of dimethylamine was dropwise added formalin (8.5 g of 37% solution) below 10°C with stirring. After standing overnight, the reaction mixture was poured into 100ml of cold water and extracted with ether. The ether solution was extracted with 10% hydrochloric acid. The acid solution was made alkaline with 5% potassium hydroxide to precipitate 2-dimethylaminomethylphenylphenol which was recrystallized from ethanol to give leaf-plates, m. p. 90-91°C. Yield was 55%. *Anal.* Calcd. for $C_{15}H_{17}ON$: C, 79.26; H, 7.54; N, 6.16%. Found: C, 79.01; H, 7.68; N, 6.07%.

The mixture of 2-dimethylaminomethyl-4-phenylphenol (5 g) and acetic anhydride (25ml) was refluxed for twelve hours. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol to give colorless crystals of 2-acetoxymethyl-4-phenylphenyl acetate, which melted at 63-64°C. *Anal.* Calcd. for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67%. Found: C, 71.72; H, 5.83%.

A mixture of 2-acetoxymethyl-4-phenylphenyl acetate (5 g), 20% potassium hydroxide solution (40ml) and methanol (110ml) was warmed on a water-bath at 50-60°C for thirty minutes. The reaction mixture was neutrallized with alcoholic hydrochloric acid and treated with Mallinckrodt silicic acid (5 g) and filtered to remove insoluble materials. The filtrate was evaporated in vacuo. The residue was recrystallized from acetone-benzene (1:7) to give 0.5g of colorless crystals which melted at 152-153°C and were identical with the product obtained by the borate method.

Saligenin cyclic phosphorus esters.

Ring-substituted saligenin and appropriate phosphorus dichloride were condensed by the catalysis of base. Pyridine was used as the base for the preparation of all oxo esters.[11] However, caustic alkaline solution was applied for thiono esters with few exceptions.[7] The products were purified through distillation, recrystallization or column chromatography and characterized by elemental analysis of phosphorus and infrared spectrum.

Insecticidal test.

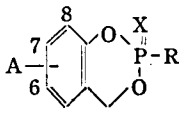
Three to four days old 25 female houseflies, *Musca domestica vicina* Macquart Takatsuki strain, were topically treated with the acetone solution of the test chemicals and kept at 25°C. The mortality account was made after twenty-four hours.

Results and Discussion

Twelve ring-substituted saligenins were prepared according to known methods. 2-Hydroxy-5-phenylbenzyl alcohol prepared from *p*-phenylphenol by the authors melted at 154-155°C, while Arct and his coworkers[10] have reported that the melting point of this compound is 64-65°C. In order to clarify this discrepancy, it was tried to prepare the compound by applying three different methods including Lederer-Manasse method, borate method[9] and Arct's method.[10] All samples prepared by these methods were identical with each other and melted at higher temperature than 150°C. No compound which melted at 64°C was obtained.

About fifty cyclic phosphorus esters of ring-substituted saligenins were prepared by the methods developed by the authors[7,11] and their physical properties are shown in Table 1. Table 2 to 5 show their insecticidal activity to housefly. In these tables the activity of unsubstituted saligenin cyclic esters is also listed for comparison.

Table 1. Structure and physical properties of ring-substituted saligenin cyclic phosphorus esters



X	A	R	Procedure*	B. p. °C/mmHg (m. p. °C)
O	6-CH_3	OCH_3	p	139-140/0.3
		OC_2H_5	p	152-156/0.3
	7-CH_3	OCH_3	p	109/0.05
		OC_2H_5	p	112-118/0.05
		O-*n*-C_3H_7	p	141-147/0.1
		C_6H_5	p	(93-95)
		$NHCH_3$	p	(145-146)
	8-CH_3	OCH_3	p	118-120/0.5
		OC_2H_5	p	165/0.6
		OC_6H_5	p	135-140/0.6
	6-Cl	OCH_3	p	145-152/0.2
		OC_2H_5	p	160/0.2
		O-*n*-C_3H_7	p	167-169/0.15
		O-*n*-C_4H_9	p	187/0.18
		OC_6H_5	p	(89)
		$NHCH_3$	p	(148)
	8-Cl	OCH_3	p	170-171/0.15
		OC_2H_5	p	151/0.18
		O-*n*-C_3H_7	p	183/0.18
		O-*i*-C_3H_7	p	137/0.04
		OC_6H_5	p	203/0.52(54)
		$NHCH_3$	p	(128-129)
S	6-CH_3	OCH_3	s	(34-35)
		OC_2H_5	s	(71-72)
		O-*n*-C_3H_7	s	158-160/0.2
	7-CH_3	OCH_3	s	110-115/0.65
		OC_2H_5	s	125-130/0.65
		O-*n*-C_3H_7	s	140-142/0.65
	8-CH_3	OCH_3	s	68-70/0.15
		OC_2H_5	s	108-109/0.15
		O-*n*-C_3H_7	s	120-124/0.15
		$NHCH_3$	s	(30)
	6-C_6H_5	OCH_3	s	oil‡
		OC_2H_5	s	oil‡
		O-*n*-C_3H_7	s	oil‡
	6-CH_3O	OCH_3	s	paste‡
	6-$COCH_3$	OCH_3	s	paste‡
	6-Cl	OCH_3	p	170-178/0.2
		$NHCH_3$	p	175-180/0.25
		SCH_3	s	160-170/0.2
	8-Cl	OCH_3	s, p	(72-73)
		$NHCH_3$	p	(46-47)
		SCH_3	s	oil‡
	6-NO_2	OCH_3	s	paste‡

continued

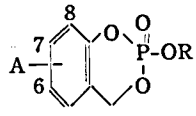
Table 1.- continued

X	A	R	Procedure*	B. p. °C/mmHg (m. p. °C)
S	6-Cl, 8-C_6H_5	OCH_3	s	paste‡
		OC_2H_5	s	paste‡
		O-*n*-C_3H_7	s	paste‡
	6-C_6H_5, 8-Cl	OCH_3	s	paste‡
		OC_2H_5	s	paste‡
		O-*n*-C_3H_7	s	paste‡
	6,8-Cl_2	OCH_3	s	(57-58)
		OC_2H_5	s	oil‡
		$NHCH_3$	s	oil‡

* Pyridine (p) or aqueous sodium hydroxide solution (s) was used as de-hydrogen chloride agent.

‡ These compounds were purified through silicic acid column chromatography.

Table 2. Insecticidal activity (LD_{50}/μg/housefly) of ring-substituted saligenin cyclic phosphates.



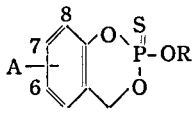
R\A	H	6-CH_3	7-CH_3	8-CH_3	6-Cl	8-Cl
CH_3	0.035	0.1	0.43	2.0	0.09	0.23
C_2H_5	0.33	0.4	0.7	2.1	0.13	0.15
n-C_3H_7	7.1	-	7.2	-	0.70	0.30
n-C_4H_9	>10(40%)‡	-	-	-	2.5	-
C_6H_5	>10(3%)‡	-	>10*	>10	>10	>10

* Phenylphosphonate

‡ Numbers in parentheses are mortality percentage at 10μg dose per female housefly.

As already shown in some previous papers[3-5], the insecticidal activity is influenced by the substituent on phosphorus: When the size of substituent on phosphorus increases, the insecticidal activity decreases (Tables 2 and 3). Thus, methyl ester is most active in each series except 8-chloro-benzodioxaphosphorin-2-oxide. The activity of methyl ester decreases by the introduction of any substituent tested on the benzene ring (Tables 2, 3 and 5). The effect diminishing insecticidal activity is remarkable in the introduction of substituent, especially of methyl group, at 8-position of benzodioxaphosphorin oxide (Table 2). However, some higher alkyl phosphate esters in ring-substituted series are rather superior to corresponding unsubstituted

Table 3. Insecticidal activity ($LD_{50}\mu g$/housefly) of ring-substituted saligenin cyclic phosphorothionates.



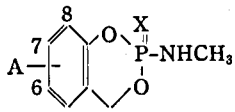
R\A	H	6-CH_3	7-CH_3	8-CH_3	6-C_6H_5	6-Cl	8-Cl	6-Cl, 8-C_6H_5	6,8-Cl_2
CH_3	0.05	2.0	0.23	1.3	0.4	1.75	0.13	1.2	0.3
C_2H_5	0.3	>10	3.0	3.0	0.5	-	-	3.0	4.0
n-C_3H_7	-	>10	7.5	7.5	1.0	-	-	>10	-

ester (Table 2). The effect of substituent on phosphorus is generally not so remarkable in ring-substituted saligenin cyclic phosphorus esters as in unsubstituted esters. This is significant in 8-substituted oxides.

Chloro-substituted derivatives are more active than methyl-substituted ones. In oxide series, the activity of positional isomers are, generally, in the order of 6>7>8 (Table 2). It is very interesting to note that, modifying oxides to sulfides, 6-substituted derivatives became almost inactive, while 8-substituted ones were rather active than oxides. Thus, the order of the insecticidal activity in sulfide series is 7>8>6 (Table 3).

Table 4 shows the insecticidal activity of N-methylphoshoramidates. In oxide series 6-chloro derivative is much more active than its 8-substituted isomer like as in the case of methyl esters. In sulfide series, however, 6-chloro derivative does not lose the activity but becomes more active, contrasting to the case of methyl esters. 8-Chloro derivative becomes highly active by modification of oxide to sulfide. Introduction of two chlorine atoms or methyl group resulted in loss of activity.

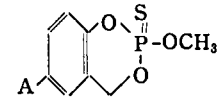
Table 4. Insecticidal activity ($LD_{50}\mu g$/housefly) of ring-substituted saligenin cyclic methylphosphoramidates.



A	X	LD_{50}
H	O	0.05
7-CH_3	O	0.14
6-Cl	O	0.09
8-Cl	O	0.30
H	S	0.04
8-CH_3	S	3.60
6-Cl	S	0.06
8-Cl	S	0.09
6,8-Cl_2	S	3.0

Table 5 shows the effect on insecticidal activity of the electronic character of substituents in the para-position of phenolic ester group.

Table 5. Effect of para-substitution on the insecticidal activity of saligenin cyclic methyl phosphorothionates.



A	σ	pKa of saligenin	$LD_{50}\mu g$/fly
CH_3O	−0.268	11.43	0.55
CH_3	−0.170	11.12	2.0
H	0.000	10.99	0.05
C_6H_5	+0.009	10.65	0.4
Cl	+0.226	10.36	1.75
CH_3CO	+0.87	-	2.5
NO_2	+1.27	7.91	3.0

Any substituents, which are of either electron-withdrawing or electron-releasing, decrease the insecticidal activity. In the series of diethyl phenyl phosphorothionates the toxicity to insects are progressively increased by the para-substitution of the phenyl ring in the order of increasing electron-withdrawing property.[12] An outstanding contrast in the effect of para-substitution between saligenin cyclic phosphorothionates and dialkyl phenyl phosphorothionates is noteworthy.

The mode of action of saligenin cyclic phosphorus esters should be different from that of ordinary organo phosphorus insecticides.

Summary

About 50 ring-substituted derivatives of saligenin cyclic phosphorus esters were prepared and examined for insecticidal activity. No compound which was superior in the activity than unsubstituted salithion (2-methoxy-4H-1, 3, 2-benzodioxaphosphorin-2-sulfide) was found. It appears that the electronic character of substituent at para-position to phenolic ester linkage is not correlate with the insecticidal activity.

Acknowledgement

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Substrate Specificity of Cholinesterases in Mites. Naoki Motoyama and Tetsuo Saito (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya Universty, Nagoya) Received May 8, 1968, *Botyu-Kagaku* 33, 77, 1968

11. ハダニのコリンエステラーゼの基質特異性

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ナミハダニ，カンザワハダニ，ミカンハダニおよびイエバエのコリンエステラーゼの数種コリンエステル類に対する特異性を，Hestrin の比色法を用いて比較した．

イエバエでは，アセチルコリンおよびプロピオニルコリンに対して鐘状型の活性度—pS曲線が示された．またブチリルコリンに対しては，過剰基質による阻害がおこらなかった．一方ハダニでは供試した3種類とも，プロピオニルコリンに対してのみ鐘状型の活性度—pS 曲線を示し，ブチリルコリンおよびアセチルコリンに対しては過剰基質による阻害がみとめられなかった．従って少なくともアセチルコリンに対する反応に関して，ハダニと昆虫の間にコリンエステラーゼの性質の差異が想像される．

Introduction

It is generally accepted that two types of cholinesterase, true cholinesterase and pseudo cholinesterase, exist in vertebrates[1]. In order to distinguish the two types of cholinesterase various methods have been examined. Augustinsson[2] demonstrated that the typical bell-shaped activity-pS curve of true cholinesterase was found in an electric eel, and the typical S-shaped activity-pS curve of pseudo cholinesterase in serum of blood. Cholinesterases in most insect species have been considered to be analogous to true cholinesterase in vertebrates, owing to the bell-shaped activity-pS curve for acetylcholine (ACh)[3)4)5)]. Aphids[5)6)] were the exception which showed