PART - II

REVIEW ON SALIGENIN CYCLIC PHOSPHORUS COMPOUNDS.

1. Discovery of Salithion:

In 1930 about ten thousand people in U.S.A. suffered by a flaccid paralysis of the lower limbs about 10 days after drinking an adulterated fluid extract of ginger (ginger jake)[1]. This was due to the phosphate triester of o-cresol, so called TOCP, which contaminated the ginger extract. The phosphate triesters of cresols have been widely used in industries as plasticizers, lubricants, solvents, oil additives and fire-retardants. The outbreaks of TOCP, a poisoning have occurred by the ortho isomers in technical products. In Morocco a similar big outbreak took place in 1959 from cooking oil contaminated with lubricating oil of turbo-jet air craft engines[2].

Results on hens show that neurotoxic triaryl phosphates, except tri-p-ethyl phenyl phosphate, have at least one alkyl group carrying the α-hydrogen atom on the ortho position[3,4]. This structure-neurotoxicity relationship of triaryl phosphates became clearly understandable by the isolation and characterisation of the active metabolites of TOCP in 1961[5,6]. The principal metabolite (A) was o-tolyl Saligenin cyclic phosphate (2-o-tolyloxy-4H-1,3,2-benzodioxaphosphorin 2-oxide). It is extra-ordinarily active in all the biological properties shown by TOCP : (A) was about 100 times more potent to cause ataxia in hens than TOCP; (A) was ten million times more active than TOCP in the in vitro inhibition of plasma cholinesterase[7].

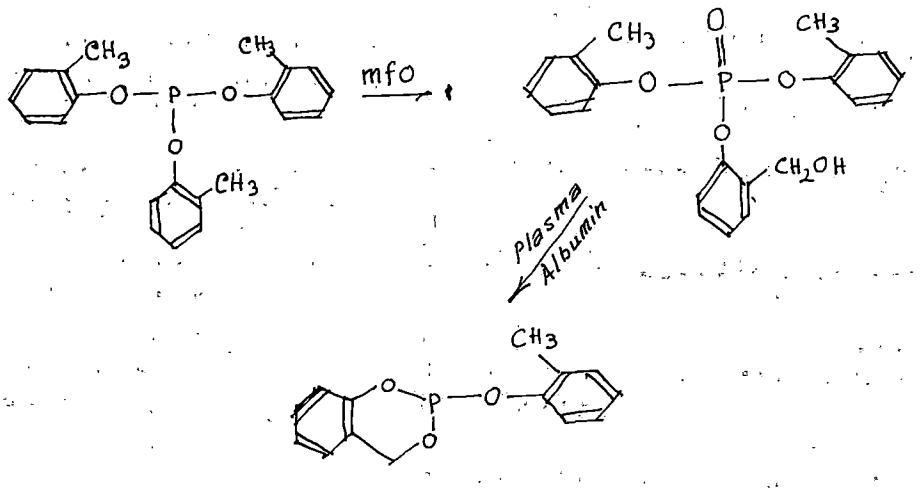
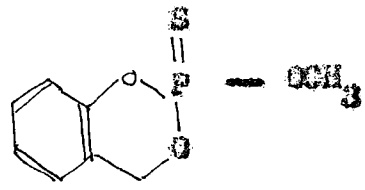


Fig. 1 : Metabolic activation of TOCP.

The conversion of TOCP into the cyclic phosphate[6] via two steps is shown in Fig. 1. The hydroxylation of the methyl group of TOCP is affected by the microsomal monoxygenase and then cyclisation is followed by intramolecular transphosphorylation of the intermediate, di-o-tolyl o-(α -hydorxy) tolyl phosphate, eliminating one molecule of cresol. Ordinarily the latter reaction is a slow one but greatly accelerated by the presence of plasma albumin[8].

Thus it looked rational to presume that the triaryl phosphates having an o-alkyl group with the α-hydrogen atom may be similarly metabolized to give the corresponding active cyclic esters. In the cyclization reaction, no alkyl ester group participates as the leaving group[9]. Actually no aryl but alkyl Saligenin cyclic phosphate was formed *in vivo* from alkyl di-o-tolyl phosphates. Such metabolic activation of TOCP or its analogs was observed in rats[6], hens[6], cats[10] and insects[11].

As a result of the aforesaid research SALITHION (2-methoxy-4H-1,3,2-benzodioxaphosphorin-2-sulphide), an organophosphorus insecticide having a unique cyclic ester structure was discovered by the pesticide research - group of Kyushu University[12] in 1963. Salithion was developed into a commercial insecticide in 1968 by Sumitomo chemical Co. of Japan.



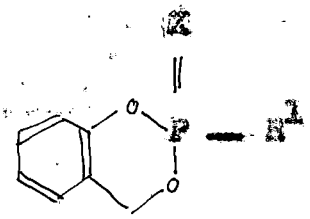
Salithion

This review is aimed at presenting an account of Salithion and related compounds as pesticides as well as their chemistry and biochemistry.

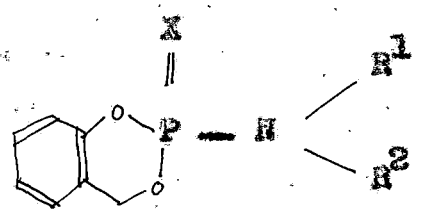
2. Synthesis of Saligenin cyclic phosphorus esters:

The cyclic phosphate and phosphonate esters of Saligenin are readily synthesised by condensation of Saligenin and substituted phosphoryldichlorides in the presence of a dehydrogenchloride agent such as tertiary amine in a dry solvent like chloroform or toluene at low temperature[13]. In some cases, where the reaction is effected difficultly by using the tertiary amine, the reaction is effected by heating the reaction mixture for 10 to 20 hours in the presence of anhydrous potassium carbonate together with copper powder[14] instead of a tertiary amine.

Such compounds, which are difficultly produced by the method employing a tertiary amine, include the compounds having meanings of X = S and R^1 = methoxy in formula I and X = S, R^1 = H and R^2 = alkyl containing more than one carbon atom or R^1 = R^2 = alkyl in the Formula II.



I



II

The process employing potassium carbonate is made to proceed by a reaction between liquid and solid phases. Therefore, even if Potassium carbonate is employed as finely divided powder often it causes a remarkable lowering and fluctuation of the yield[14]. Thus Salithion was first prepared with inconsistent and, often, very low yield by heating (90°C) Saligenin and methyl phosphorodichloridothionate in toluene for a long period (more than 15 hours) in the presence of anhydrous Potassium carbonate together with copper powder as catalyst[15]. This difficulty was, however, overcome later by applying the well-known Schotten-Baumann acylation procedure using an aqueous solution of Sodium hydroxide (Fig. 2).

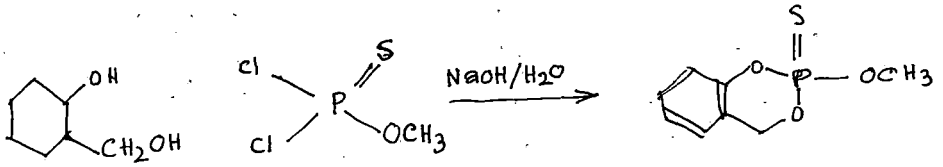


Fig. 2 : Synthesis of Salithion.

The present process is superior to the known process in view of the following considerations.

The improved process is carried out in an aqueous solution at a lower temperature and in a shorter reaction time period than the known process. Also, it gives an objective product of better yield having higher purity than the known process. Salithion was obtained smoothly in a crystalline form (m.p. 49-53°C) under mild conditions (20°C, 2 hrs.) with a consistently high yield (70-80%)[16]. This method can be applied also to synthesise other thiono analogs of saligenin Cyclic phosphorus esters from relatively less reactive dichlorides such as dialkyl phosphoramidodichloridothionates (Formula II).

Further, it is possible in the Schotten-Baumann procedure to produce cyclic dithiophosphate esters such as S-alkyl cyclic phosphorothiolothionates of saligenin (i.e. X = S, R^1 = S-alkyl in formula-I) which cannot be produced at all by the known process.

3. Properties of Salithion:

Referring back to Salithion, we pinpoint our discussion to its important properties[17] relating to its structure, degradation, isomerization, etc.

Pure salithion is a colourless crystalline powder : m.p., 55-56°C; practically insoluble in water, easily soluble in acetone and benzene, moderately soluble in cyclohexane, toluene and xylene; vapour pressure 1.5×10^{-6} mm Hg at 25°C; UV λ_{max}^{nm} (ϵ) 274 (860), 267 (860). Salithion has a characteristic IR band at 1020 cm^{-1} for

P-O-CH_2 in hetero ring. NMR δ (CS_2) ppm: 3.76 (3H, doublet, J_{PH} = 14 Hz, CH_3), 5.21 (2H, doublet, J_{PH} = 15 Hz, CH_2), 6.8 - 7.2 (4H, multiplet, benzene ring).

The signal at the upper field of the doublet at 5.21 ppm slightly splits further (1.5 Hz). This becomes much significant at -30°C, suggesting that the methylene protons (H_A, H_B) are not equivalent to each other, but the dioxaphosphorin ring is conformationally mobile in a solution (Fig. 3). X-ray crystallographic analysis shows that the hetero ring of salithion is

Fig. 3 Conformational change of salithion hetero-ring.

a half - chair form in which the sulphide group is in equatorial position (III). The strain in the ring appears little; the endocyclic O-P-O angle is 104°.

Salithion gives a characteristic fragmentation pattern in mass spectrometry. It gives an intense peak of $(M - CH_3)^+$ (m/e 201) by a β-cleavage occuring at the exocyclic ester group. Another characteristic fragmentation process is the direct loss of SH followed

by the elimination of formaldehyde (Fig. 4).

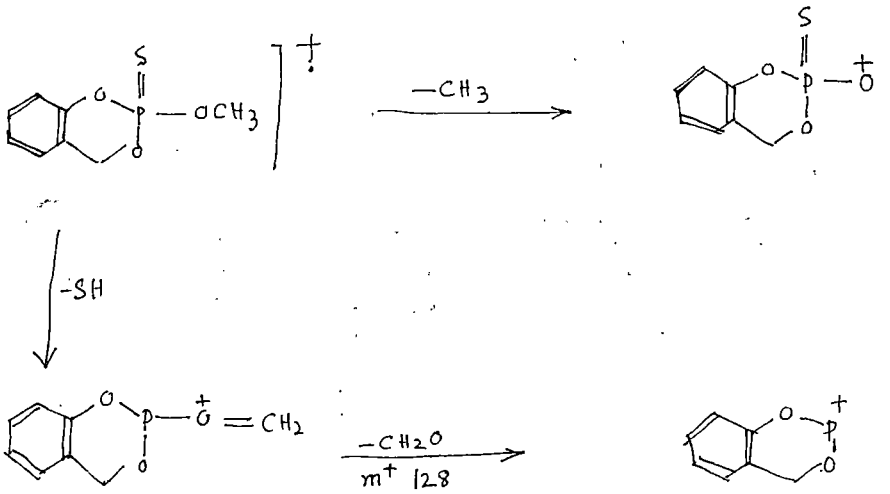


Fig. 4: Fragmentation of Salithion in Mass spectrometry.

Salithion is relatively unstable in storage. Some secondary amines, such as carbazole and N-phenyl- α -napthyl amine, stabilize the formulation[13]. In a phosphate buffer (pH 7.7), salithion is hydrolysed slowly through opening of the hetero ring by the P-O-(aryl) bond cleavage:

the hydrolysis rate constant (25°) $K = 2.4 \times 10^{-4}$ min^{-1}. The hydrolysis rates of the corresponding cyclic methyl phosphonate, S-methylphosphorothiolate (the thiolate isomer of salithion, MTBO), methyl phosphate (Salioxon), and N-methyl phosphoramidate are, respectively, 90, 60, 6 and 0.6 times more than that of salithion. Salithion is completely hydrolysed by heating at 100°C for 5 min. with N/6 sodium hydroxide to yield saligenin. This is applied for the colorimetric determination of salithion in formulations by allowing the formed saligenin to react, after adjusting pH 8, with 4-aminoantipyrine and then with potassium ferricyanide (19, 20).

On oxidation by bromine water salithion is converted to its oxon (salioxon). Since salioxon (2-methoxy-4H-1,3,2-benzodioxaphosphorin 2-oxide) is some thousand times more active in cholinesterase inhibition than salithion, an enzymatic method after the oxidation can be used for the residue analysis of salithion (19).

Salithion is isomerized into S-alkyl saligenin cyclic phosphorothiolates by heating with alkyl iodides (the Pistchimuka reaction) (21). The reaction is greatly accelerated in such a polar solvent as dimethylformamide. Potassium carbonate also assists the reaction. When methyl iodide is used, isomerization occurs to give 2-methylthio-4H-1,3,2-benzodioxaphosphorin-2-oxide (MTBO) (21,22). Salithion is demethylated to form the salt of saligenin cyclic phosphorothionic acid by the action of certain nucleophils such as cyclohexylamine (17) and potassium dimethyl-dithiocarbamate (17,23). The latter agent is particularly suitable for the preparation of MTBO by methylating the obtained salt with methyl iodide.

MTBO is a unique phosphorylating agent. The reactions of salithion are summarised in the following scheme (Fig. 5):

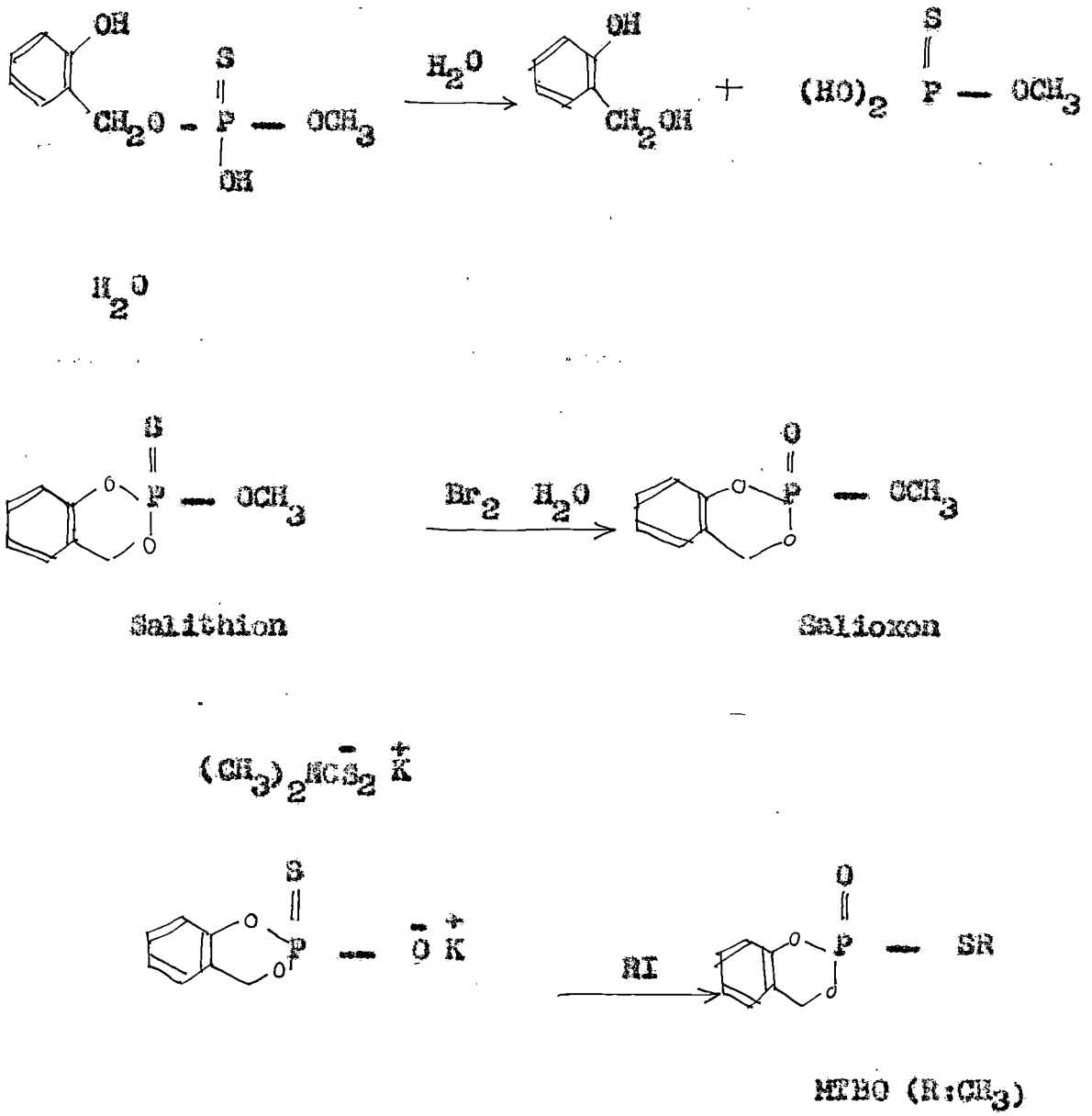


Fig. 5 : Reaction of Salithion.

Salithion is a wide-spectrum insecticide for use in orchards and vegetable gardens. It is particularly effective to control lepidopteran larvae, mealybugs, aphids and mites. It exhibits the insecticidal action not only as contact and stomach poisons but also as a fumigent (17). The residual toxicity of Salithion is so small that a natural enemy,

Pseudaphycus malinus, could be used co-operatively for the control of comstock mealybug (8).

Acute toxicity to mammals is moderate. LD_{50} in mice by oral administration is 91.3 mg/kg; for male rats 82-125 mg/kg, for female rats 102-180 mg/kg; for hens 110 mg/kg. Salithion ^{32}P applied topically to houseflies was rapidly absorbed in the body (42% after 1 hr.). The major part was degraded in the body and about 4% of applied or 10% of absorbed Salithion remained as Salithion and Salioxon for 24 hrs. On the other hand, Salithion ^{32}P administered orally to mice was rapidly degraded and excreted.

After 1 hr., 78% of the administered Salithion was hydrolysed in the body. After 3 hrs., 56.7% was excreted and only 2.4% remained in the body in chloroform soluble form (38).

About 10% of Salithion absorbed was found in the bean plant whose roots had been soacked in the nutrient solution containing the insecticide for 10 days. When Salithion was applied on the leaves about 10% was absorbed into the tissues and slightly translocated into other leaves. Most of Salithion applied on leaves or applied in solution form with nutrient vaporises. This causes a fumigant action to kill insects on the plant.

The metabolic pathways of Salithion in rats and plants have been studied (4). It was shown that the biodegradation proceeds through demethylation and ring-opening by P-O-aryl-bond cleavage.

Chronic toxicity tests (17) revealed that the rats fed for 24 months with 10 ppm Salithion showed slight decrease in cholinesterase

activities. No effect was however, observed in rats fed with 3 ppm Salithion. No histological lesion was found in any organs of rats fed with 100 ppm.

In men and women administered orally 0.02 mg/kg/day of Salithion for 21 days followed by 0.05 mg/kg/day for 14 days, no effect was found in the activity of erythrocyte acetyl cholinesterase. Carcinogenicity was not observed. No effect was observed in fertility of rats for three generations fed with 10 ppm Salithion.

4. Other Saligenin cyclic phosphorus esters:

A survey of literatures furnishes (14,15,16,24,25,26,27) a variety of Saligenin cyclic phosphorus esters in good number, which have been prepared and examined for insecticidal activity. They involve phosphates, phosphorothiolates, phosphoramidates, phosphonates and their thiono-analogs. A comprehensive but not a complete list of Saligenin and ring - substituted Saligenin cyclic phosphorus esters is given in Table - I.

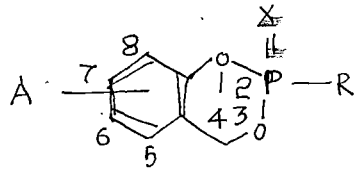


Table - I

Code No.	R	A	X	Procedure*	b.p.°C/mm Hg(mp °C)
K - 7	OCH_3	H	O	(P)	110 - 2°/0.05
K -13	O-n-C_3H_7	H	O	(P)	129 - 32°/0.05
K -18	O-n-C_4H_9	H	O	(P)	150 - 4°/0.05
K - 8	OC_2H_5	H	S	(P)	Liquid (not distilled)
K -16	OC_6H_5	H	S	(P)	(36°)
K -15	C_6H_5	H	S	(P)	(37°)
	CH_3	H	O	(P)	140°/0.5(35°)
	C_2H_5	H	O	(P)	143 - 9°/0.3(25°)
	i-C_3H_7	H	O	(P)	(30°)
	Sec-C_4H_9	H	O	(P)	110°/0.5
	t-C_4H_9	H	O	(P)	(74°)
	$CH = CH_2$	H	O	(P)	155°/2.5
	CH_2Cl	H	O	(P)	160/0.8 (51°)
	CH_2CH_2Cl	H	O	(P)	139 141/0.1
	CH_3	H	S	(P)	130/0.6
	C_2H_5	H	S	(P)	120/0.6
	i-C_3H_7	H	S	(P)	108/0.6
	CH_2Cl	H	S	(P)	146 - 155/0.4
	OCH_3	6-CH_3	O	(P)	139 - 140/0.3
	OC_2H_5	6-CH_3	O	(P)	152- 156/0.3
	OCH_3	7-CH_3	O	(P)	109/0.05
	OC_2H_5	7-CH_3	O	(P)	112 118/0.05

*Pyridine (P) or aqueous Sodium hydroxide solution (S) was used as dehydrogenchloride agent.

Table - I (Contd.....)

Code No.	R	A	X	Procedure*	b.p.°C/mm Hg(m.p.°C)
	O-n-C_3H_7	7-CH_3	O	(P)	141 - 147/0.1
	C_6H_5	7-CH_3	O	(P)	(93 - 95)
	$NHCH_3$	7-CH_3	O	(P)	(145 - 146)
	O-CH_3	8-CH_3	O	(P)	(118 - 120/0.6)
	OC_2H_5	8-CH_3	O	(P)	165/0.6
	OC_6H_5	8-CH_3	O	(P)	135 - 140/0.6
	OCH_3	6-Cl	O	(P)	145 - 152/0.2
	OC_2H_5	6-Cl	O	(P)	160/0.2
	O-n-C_3H_7	6-Cl	O	(P)	167 - 169/0.15
	O-n-C_4H_9	6-Cl	O	(P)	127/0.18
	OC_6H_5	6-Cl	O	(P)	(89°)
	$NHCH_3$	6-Cl	O	(P)	(148°)
	OCH_3	8-Cl	O	(P)	170 - 171/0.15
	OC_2H_5	8-Cl	O	(P)	151/0.18
	O-n-C_3H_7	8-Cl	O	(P)	183/0.18
	O-i-C_3H_7	8-Cl	O	(P)	137/0.04
	OC_6H_5	8-Cl	O	(P)	203/0.52(54°)
	$NHCH_3$	8-Cl	O	(P)	(128 - 129°)
	OCH_3	6-CH_3	S	(S)	(34 - 35°)
	OC_2H_5	6-CH_3	S	(S)	(71 - 72°)
	O-n-C_3H_7	6-CH_3	S	(S)	158 - 160/0.2
	OCH_3	7-CH_3	S	(S)	110 - 115/0.65

*Pyridine (P) or aqueous Sodium hydroxide solution(S) was used as dehydrogenchloride agent.

**These compounds were purified through Silicic acid Column-Chromatography.

Table - I (Contd.....)

R	A	X	Procedure*	b.p.°C/mm Hg(m.p. °C)
OC_2H_5	7-CH_3	S	(S)	125 - 130/0.65
O-n-C_3H_7	7-CH_3	S	(S)	140 - 142/0.65
OCH_3	8-CH_3	S	(S)	68 - 70/0.15
OC_2H_5	8-CH_3	S	(S)	103 - 109/0.15
O-n-C_3H_7	8-CH_3	S	(S)	120 - 124/0.15
$NHCH_3$	8-CH_3	S	(S)	(30°)
OCH_3	6-C_6H_5	S	(S)	Oil **
OC_2H_5	6-C_6H_5	S	(S)	Oil **
O-n-C_3H_7	6-C_6H_5	S	(S)	Oil **
OCH_3	6-OCH_3	S	(S)	Paste **
OCH_3	6-$COCH_3$	S	(S)	Paste **
OCH_3	6-Cl	S	(P)	170 - 178/0.2
$NHCH_3$	6-Cl	S	(P)	175 - 180/0.25
SCH_3	6-Cl	S	(S)	160 - 170/0.2
OCH_3	6-Cl	S	(S,P)	(72 - 73°)
$NHCH_3$	8-Cl	S	(P)	(46 - 47°)
SCH_3	8-Cl	S	(S)	Oil **
OCH_3	6-NO_2	S	(S)	Paste **
OCH_3	6-Cl 8-C_6H_5	S	(S)	Paste **
OC_2H_5	" "	S	(S)	Paste **
O-n-C_3H_7	" "	S	(S)	Paste **

*Pyridine (P) or aqueous Sodium hydroxide Solution (S) was used as dehydrogenchloride agent.

**These compounds were purified through Silicic acid Column-Chromatography.

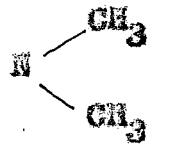
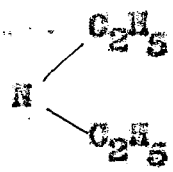
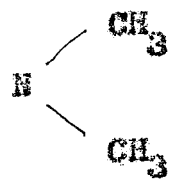
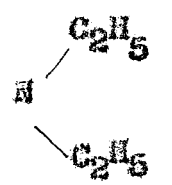
Table - I (Contd.....)

R	A	X	Procedure*	bp. °C/mm Hg. (m.p. °C)
OCH_3	{6-C_6H_5 8-Cl	S	(S)	Paste **
OC_2H_5	" "	S	(S)	Paste **
O-n-C_3H_7	" "	S	(S)	Paste **
OCH_3	6,8-Cl	S	(S)	(57 - 58°)
OC_2H_5	"	S	(S)	Oil**
$NHCH_3$	"	S	(S)	Oil **
SCH_3	H	S	(S)	(69 - 70)
SC_2H_5	H	S	(S)	145 - 147/0.2
S-n-C_3H_7	H	S	(S)	145 - 150/0.25
S-i-C_3H_7	H	S	(S)	140 - 143/0.1
S-C_3H_5	H	S	(S)	140 - 147/0.3
S-n-C_4H_9	H	S	(S)	160 - 167/0.25
S-C_6H_5	H	S	(S)	(79 - 80)
SCH_3	H	O	(P)	144 ~ 5/0.1
SC_2H_5	H	O	(P)	140 ~ 5/0.04
S-i-C_3H_7	H	O	(P)	155 ~ 8/0.1
S-n-C_4H_9	H	O	(P)	157 - 60/0.02
SC_6H_5	H	O	(P)	(88 ~ 9)

* Pyridine (P) or aqueous Sodium hydroxide Solution (S) was used as dehydrogenchloride agent.

** These compounds were purified through Silicic acid Column-Chromatography.

Table - I (Contd.....)

Code No.	R	A	X	Procedure*	b.p. ^{o}C/mm Hg. (m.p. ^{o}C)
K-19	$NHCH_3$	H	O	(triethylamine)	(87^{o})
K-22	NHC_2H_5	H	O	(P)	(68^{o})
K-20		N	O	(triethylamine	(121^{o})
K-23		H	O	(Potassium carbonate)	133 6/0.5
K-35	$NHCH_3$	H	S	(P)	120 - 3/0.2
K-37	NHC_2H_5	H	S	(Potassium carbonate)	undistilled liquid.
K-36		H	S	(Potassium carbonate)	113 22/0.2
K-38		H	S	(Potassium carbonate)	110/0.2

* Pyridine (P) or aqueous Sodium hydroxide Solution (S) was used as dehydrogenchloride agent.

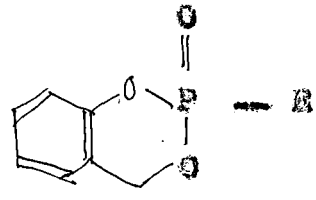
** These compounds were purified through Silicic acid Column-Chromatography.

5. Structure and Specificity in Biological Activities:

The results of the investigation on the activity - structure relationship suggest that biologically active substances may have two important sites in the molecule in order to manifest biological activities; One reacts actually with a target and another decides the specificity in biological activity. The biological activities of Saligenin cyclic phosphates are greatly influenced by the exocyclic substituent on Phosphorus atom as shown in Table 2. Aryl Saligenin cyclic Phosphates

Table - 2

Effect of the exocyclic substituent (R) on biological activities of Saligening cyclic phosphates (I)



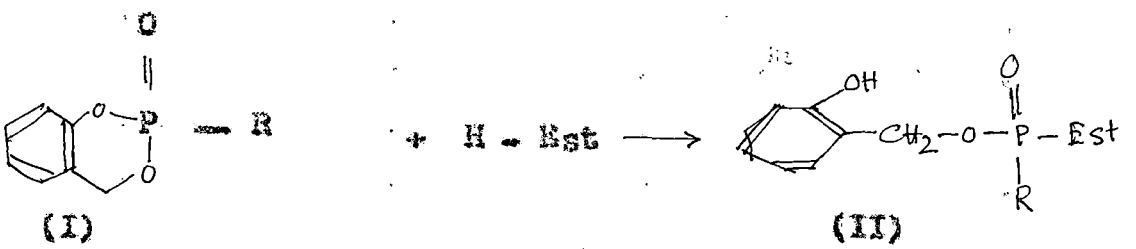
(I)

R	Delayed neurotoxicity MAD[a]	Synergism with Cotoxicity Co-efficient Mice	Houseflies[b]	Insecticidal activity LD^{c}_{50}
o-$CH_3C_6H_5O$	2 - 5	16.7	7.8	(0)[d]
C_6H_5O	1.5 - 2	8.8	9.2	(3)[d]
C_6H_5	200	18.8	8.0	(0)[d]
C_2H_5	n.a.[e]	3.0	-	0.17
C_2H_5O	-	-	3.1	0.33
CH_3O	n.a.[e]	3.7	4.7	0.04
$(CH_3)_2N$	n.a.[e]	1.1	-	0.3

a. Minimum ataxic dose for hens in mg/kg.
b. A resistant strain.
c. 50% Lethal dose by topical application to houseflies in μg/fly.
d. Percentage mortality at 10 μg/fly.
e. No ataxia signs evident with any sublethal dosages.

manifested a highly delayed neurotoxicity to cause ataxia in hens and high synergistic activity with malathion (5,28). The aryl phosphonate analogs showed similar biological activities but less in the neurotoxicity. A sharp contrast was, however, observed in the corresponding cyclic esters having a small alkyl group on phosphorus, i.e. 2-alkyl-, 2-alkoxy-, and 2-alkylamido-4H- 1,3,2-benzodioxaphosphorin-2-oxides, did not cause ataxia in hens with any sublethal doses and weakly potentiate the toxicity of malathion (5). Surprisingly the alkyl derivatives showed high insecticidal activity, whereas the aryl esters did not (29). This finding has impelled the inventors of Salithion to examine the saligenin cyclic phosphate esters carrying a small exocyclic alkyl substituent on the Phosphorus atom as potential insecticide candidates.

The specificity of Saligening cyclic phosphates in the biological activity relates to their selectivity in enzyme inhibition. These phosphates inhibit various serine enzymes, probably, due to the formation of Salicyloxy-phosphinylenzymes (II) (6,7) Fig. 6 by phosphorylating the enzyme after opening of the cyclic ester structure at the P— O— aryl bond



(I) (II)

Fig. 6: Reaction of Saligenin cyclic Phosphates with esterases (H-Est.).

The ester becomes a more selective inhibitor of aliesterase when the size of exocyclic substituent (R in I) increases, whereas it becomes a more selective inhibitor of cholinesterase when the substituent is small. Thus, the O-totyl derivative (A), for example, inhibits aliesterase 130 times more than cholinesterase. Therefore, the exocyclic substituent of Saligenin cyclic Phosphate esters is regarded as the selectophore in biological actions. (30)

The heterocyclic structure of Saligenin cyclic phosphorus esters is none to contribute towards the delayed neurotoxicity, but it merely induces the chemical reactivity of the Phosphorus atom for nucleophiles including the active site of esterases.

In nervous tissues, Johnson found "neurotoxic esterase" which is specifically sensitive <u>in vivo</u> to neurotoxic organophosphorus esters[31]. The esterase is unlike acetylcholinesterase but similar to chymotrypsin and trypsin in structure activity relationship of inhibitors[32]. Although the structure-neurotoxicity relationship is too complicated to be generalized, the neurotoxicity appears to relate more with the structure of nonleaving group than that of the leaving one. Neurotoxic esterase is remarkably resistant to most of the methyl esters[33].

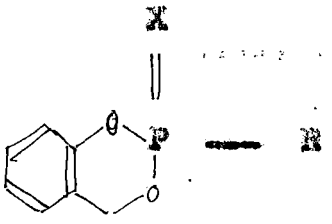
With this brief background of structure-biological activity relationship of Saligenin cyclic phosphorus esters, an emphasis on their specific activities such as insecticidal, synergistic, antiesterase, nematocidal, fungicidal, etc., will be laid in subsequent paragraphs.

6. Insecticidal activity:

It has already been stated that the cyclic esters in any particular series having a small alkyl group have high insecticidal activity[8] (Table-3). Methyl derivatives are much more active than higher alkyl and aryl derivatives, except for phosphate series in which ethyl derivative is more active[24] than the methyl one. N, N-Dialkyl Phosphoramidates are much less active than mono-alkyl derivatives. Thus, Saligenin cyclic methyl phosphate, methyl phosphorothionate, N-methyl phosphoramidate, N-methyl Phosphoramidothionate, methyl phosphorothiolate and ethyl phosphonothionate are potent insecticides. It is interesting to note that the exocyclic substituents of the most active cyclic phosphorus esters (OCH_3, $NHCH_3$, CH_2CH_3, SCH_3) differ from each other in electronic characteristics, but resembles in steric property such as the distance (about 2.9A°) between phosphorus and carbon atoms in the P-X-C function, if supposing the bond angle of divalent sulphur is near 90° rather than 109.5°[17].

Table - 3

Effect of exocyclic substituent (R) on insecticidal activity of (III) (LD_{50} μg/housefly).



(III)

R	X		R	X	
	O	S		O	S
CH_3	0.13	0.31	CH_3S	0.09	0.18
C_2H_5	0.17	0.08	C_2H_5S	0.23	0.9
CH_3O	0.04	0.05	CH_3NH	0.05	0.04
C_2H_5O	0.33	0.30	C_2H_5NH	0.66	0.48

The phosphorothiolothionates have not enough insecticidal activity[16]. The phosphates, phosphorothiolates and phosphonates appear too unstabbe to be used practically as insecticides. The phosphoramidates are several times as toxic to mammals as the phosphorothionates.

Furthermore, the introduction of any substituents on the benzene ring, the hetero ring, or the exocyclic ester group brings down the insecticidal activity[25,34] (Table-4). Thus Salithion, the simplest

phosphorothionate, was the most promising compound as insecticide amongst all the series of Saligenin cyclic phosphorus esters.

Table - 4

Effect of substituent (R) on insecticidal activity (LD_{50} µg/housefly).

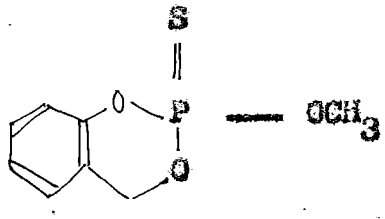
R	X		R	X	
	S	O		S	O
H	0.05 (Salithion)	0.035 (Salioxon)	6-Cl	1.75	0.09
4-CH_3	-	3.35	8-Cl	0.13	0.23
6-CH_3	2.00	0.1	β-CH_3	0.30	0.33
7-CH_3	0.23	0.43	β-CH_3OCH_2	3.55	0.99
8-CH_3	1.30	2.0	β-Cl	-	2.07

An outstanding contrast in the effect of para-substitution between Salithion series and parathion is noteworthy. The insecticidal activity of diethyl phenyl phosphorothionate (V) is progressively increased by p-substitution of phenyl ring in the increasing order of the electron-withdrawing activity of the substituent, whereas neither electron-withdrawing nor electron-releasing group enhances the activity of Salithion (IV) (Table-5)[17]. It seems evident, therefore, that the P-O-C (aryl) bond of the hetero ring of Saligenin cyclic phosphorus esters without any substituent anywhere, neither in benzene ring nor

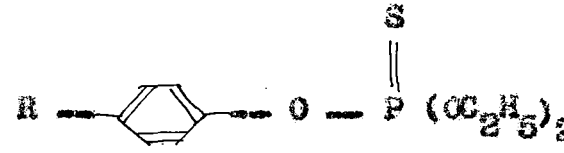
in hetero cyclic ring, appears to be optimum for the reactivity to phosphorylate cholinesterase to kill the insects.

Table - 5

Effect of p-substitution on insecticidal activity of Salithion (IV) and parathion (V) series.



(IV)



(V)

R	σ [a]	Relative insecticidal activity[b] IV	V
OCH_3	-0.263	9.2	0.1
CH_3	-0.170	2.6	0.1
H	0.000	100.0	0.1
C_6H_5	+0.009	12.8	-
Cl	+0.226	3.0	0.33
$COCH_3$	+0.87	2.0	2.5
NO_2	+1.27	1.7	100.0

a. Hammett's substituent constant.

b. Percentage of the most active compound in each series.

The reactivity of the cyclic phosphate ester of Saligenin is surprisingly greater than what is expected from the acidity consideration of Saligenin though the hetero-ring is not much strained and the endocyclic O-P-O angle of Salithion (104°) is in the range of angle of acylic phosphate esters (102 - 108°)[4]. Many five and six-membered cyclic phosphorus esters have been prepared from 1,2-and 1,3-alkanediols and examined for antiesterase and insecticidal activities by Fukuto[35] and Edmundson[36]. These cyclic esters showed high reactivity but exhibited only poor anticholinesterase and insecticidal activities. Cyclic phosphorothionates of Catechol inhibit plasma cholinesterase but show almost no insecticidal activity[37]. Therefore, the high activity of Saligenin cyclic phosphorus esters may be attributed to the special hetero-ring involving an enol and a benzyl ester linkage[17].

7. Activity as systemic insecticides:

Some known systemic insecticides, such as Schradan (Octamethyl Pyrophosphoramide) and Mipafox (N,N '-diisopropyl-phosphorodiamidic fluoride), have phosphoramide linkage. On analogy, it deemed probable that Saligenin cyclic phosphorus esters can also be endowed with systemic insecticidal activity by the introduction of an alkyl amino group on the phosphorus atom. Actually, Saligenin cyclic N-methyl phosphoramidate and phosphoramidothionate (2-methylamido-4H-1,3,2 benzodioxaphosphorin-2-oxide and sulphide) revealed a considerable systemic insecticidal activity against rice stem-borers and green rice leafhoppers on rice plants[26].

Referring to the metabolism of tri-o-toyl phosphate[6,11] *in vivo* it is reasonable to suppose that Saligenin cyclic phosphoramidates might be produced *in vivo* from di-o-tolyl phosphoramidates. On examination, di-o-tolyl N-methyl phosphoramidate was found to exhibit systemic insecticidal activity against rice stem-borer[8]. It was metabolically transformed into the active cyclic ester by the homogenate of rice stem-borers but not that of rice plants[9]. Salithion showed more or less systemic activity against armyworm and mite.

8. Synergistic activity:

Saligenin cyclic aryl phosphates and phosphonates have synergistic activity with Malathion against insects[28,29] and mites[39] particularly against their resistant strains (Table-2).

At least two esterases having the ability to hydrolyse Malathion or its phenoxy carbonyl homologue are detected in the homogenate of the resistant strain G. One of these is more potent and specific esterase for hydrolysis of Malathion. Both the esterases are completely inhibited by Saligenin cyclic phenyl phosphate[40]. For the resistant strains of red citrus mites, Panonychys citri McGregor, Saligenin cyclic phenylphosphonate displayed a high synergistic action with Malathion, by inhibiting the degradation of carboxylic ester linkage in Malathion molecule[41]. 2-Methyl-2-phenyl-4H-1,3,2-benzodioxaphosphorin-2-oxide is the most active synergist against resistant houseflies and green rice leaf-hoppers.

9. Antiesterase Activity:

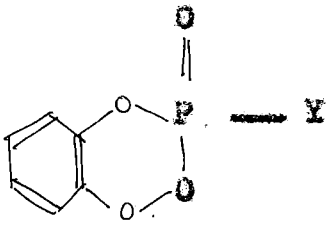
Reference has already been made that the specificity in

biological activities of Saligenin cyclic phosphorus esters are remarkably influenced by the steric characteristics of the exocyclic substituent on the phosphorus atom. This is evident when one compares their specificities in enzyme inhibition [30]. Saligenin cyclic phosphorus esters reacts with nucleophilic agents, including esterases, to phosphorylate by opening of the cyclic ester structure at P-O-C (aryl) bond (Fig. 6) [6,29,42].

The chemical and biological activities of three representative Saligenin cyclic esters, methyl phosphate, phenyl phosphate and phenyl phosphonate are compared in Table-6. The insecticidal methyl

Table - 6

Effect on substituents on chemical and biological activities of (VI)



(VI)

Y	K_{hyd} x 10^3 min^{-1}	I_{50} ChE x 10^8 M (C)	I_{50} AliE x 10^8 M (A)	C/A	LD_{50} *	Synergism **	Ataxia ***
OCH_3	1.4	7.6	8.4	0.9	0.04	0.6	n.a
OC_6H_5	6.3	155	1.4	119	10(3)	2.3	2
C_6H_5	12.8	89	3.2	27.8	10(0)	2.5	200

* µg/housefly; ** Cotoxicity Co-efficient;

*** Minimum ataxic dose in hens (mg/kg); n.a. no ataxia.

phosphate is very active as an inhibitor of cholinesterase. However, the highly neurotoxic aryl phosphate is a poor inhibitor of cholinesterase but is a very specific inhibitor of aliesterase. The less neurotixic aryl phosphonate occupies an intermediate position.

10. Nematocidal Activity:

A number of Saligenin cyclic phosphorus esters are effective to kill nematodes (29). N-Methylphosphoramidate is most active but N,N-dimethylphosphoramidate is inactive against the non-parasitic soil nematode Rhabditis suspended in water (26). Owing to instability in water, the cyclic phosphates and phosphonates are practically inactive but their thiono analogues are considerably active against Rhabditis (24). Some Saligenin cyclic aryl phosphonothionates are more effective against the rice white tip nematode (Aphelenchoides besseyi Christie) than the cyclic N-methyl phosphoramidate, though the formers are poor in insecticidal activity (43). These arylphosphonothionates also show a high activity against filaria in cotton rats (Litomosoides Carinii). It is interesting to note that these arylphosphonothionates are poor insecticides, whereas they are more potent to kill nematodes than Salithion, suggesting the cholinesterase or other critical target of nematodes may differ in nature from the insect cholinesterase (17).

11. Fungicidal Activity:

Recently some phosphorothiolate esters, particularly having S-benzyl ester-linkage, have been developed as fungicides. S-Benzyl-O, O-diethyl phosphorothiolate (Kitazin) is a typical fungicide now used

in practice for control of rice blast disease. S-Alkyl Saligenin cyclic phosphorothiolate esters, which have no S-benzyl ester - linkage but O-benzyl and S-alkyl ester linkages, were examined for fungicidal activity [44,27]. They were found active not only as insecticides but also as fungicides. Some of them are effective to protect rice plants from rice-blast disease caused by the infection of Pyricularia oryzae. Ethyl and n-butyl esters are most promising as fungicides.

The S-alkyl cyclic phosphorothiolates inhibit not only serine enzymes including cholinesterase but also SH-enzymes such as Papain and alcohol dehydrogenase [45,46]. The activity to inhibit SH-enzymes appears to relate to fungicidal activity. The cyclic phosphorothiolates (a) are highly reactive and rapidly S-alkyl O-Salicyl hydrogen phosphorothiolates (b) which may react readily with nucleophiles like mercaptan to alkylate them.

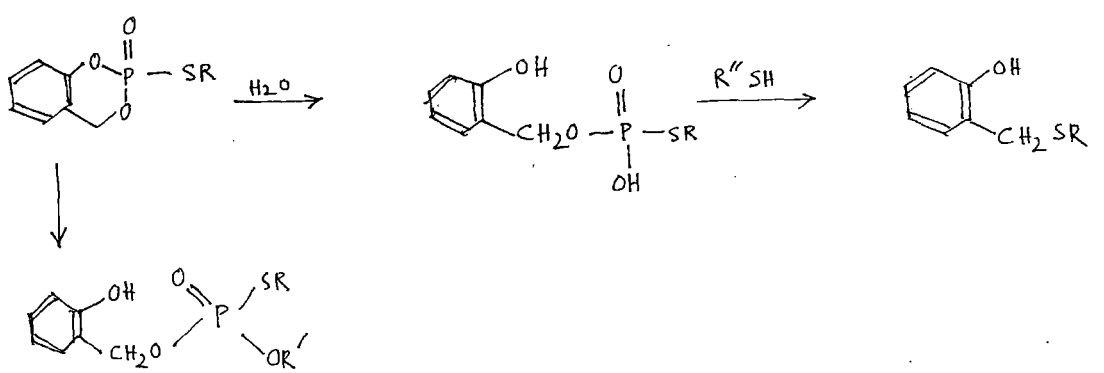
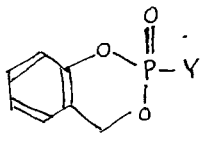
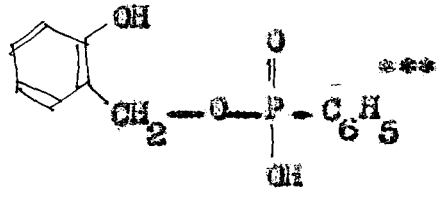


Fig.7: Phosphorylation and alkylation reactions by Saligenin cyclic phosphorothiolates. (R'OH = alcohol & serine enzyme; R"SH = mercaptans and SH-enzymes).

It is the open-ringed O-hydroxy benzyl ester which actually inhibits the alcohol dehydrogenase instead of Saligenin cyclic hydrogen phosphate (Table-7)[8]. The hydroxy group in the ortho position may promote the alkylating property of benzyl ester, giving a benzyl carbonium ion[45].

Table - 7

Reactivity of Saligenin cyclic phosphorus esters and related compounds with SH-compound and enzyme.

Y	Reaction with Cysteine*.	I_{50} alcohol dehydrogenase x 10^{5}M
 SCH_3	+	4.5
C_6H_5	+	5.0
OH	-	-**
 ***	+	10

*S-substituted cysteine was produced (+) or not (-) at pH 7.6, 25°C for 30 min.

** No inhibition occured at 5 x 10^{-4}M.

*** Cyclohexylammonium salt was used.

It is interesting to notice the similarity in structure between fungicidal S-benzyl phosphorothiolates and intermediate partial hydrolyzate of saligenin cyclic phosphorothiolates. Metabolic hydroxylation at the para or ortho position of S-benzyl phosphorothiolate fungicides may be assumed to occur in vivo.

12. Conclusion:

The high biological activities of saligenin cyclic phosphorus compounds may be attributed to the hetero-ring involving enol and benzyl ester linkages. The alkylation reaction may be responsible for SH enzyme and fungicidal activity. The phosphorylation reaction is responsible for esterase inhibition, and animal toxicity and insecticidal activity. An exocyclic substituent group affects physical and biological properties by virtue of its electronic and steric characteristics. Thus, methylphosphorothionate is useful as an insecticide, alkylamidates have systemic activity, alkylamidophosphorothiolates have fungicidal activity, phenyl phosphonates have entifilarial activity, and aryl phosphates are neurotoxic and have synergestic activity.

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