CHAPTER 1

GENERAL INTRODUCTION AND REVIEW ON CALCIUM AND RELATED COMPOUNDS

INTRODUCTION

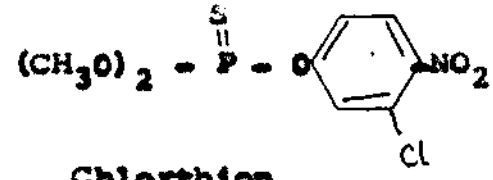
Chemicals play a very important role in the agricultural industry. One major group included in the category in fertilizers, over 250 billion pounds of which were used in 1983 by developing and developed countries of the world. Also included in this category are pesticides. According to one report, the total loss to worldwide food production from pests amounts to an estimated 45% of total annual food production. We can imagine the increase in global food availability that would result of such pests could be controlled. Chemicals are often the weapons of choice in this battle.

Pesticides (herbicides, fungicides, insecticides and etc.) are important in controlling not only many types of agricultural pests, but also insects that are vectors of disease such as the mosquitoes that carry encephalities, yellow fever and malaria; bodylice that carry typhus; and fleas that can spread plague. Pesticides also play a very important part in the leaves of consumers.

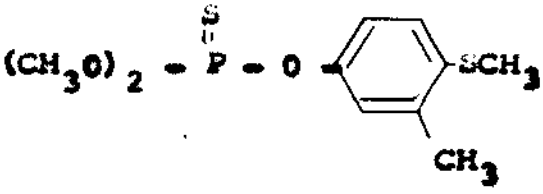
As a result various types of pesticidal compounds are being prepared and their pesticidal, toxicological and other properties are being studied everyday. Of them, organophosphorus compounds constitute a class in which quite a large number of compounds have been synthesized and examined as the effective pesticides, owing to their high activities and bio-degradabilities.

their applications in agriculture, public health and related fields have been going rapidly. The development of new organophorus compounds was dominated for a long time almost exclusively by one single guiding principle namely the "Acyl Rule" of Schrader(1.2.3). The great advancement in agricultural practice, scientific knowledge of the structure activity relationship and mode of action of organophosphorus pesticides were achieved by the discovery of parathion by Schrader in 1944. Parathion is extremely toxic to mammals as well as insects. Many less toxic pesticides have been synthesized by slight structural modification of parathion, for example, Chlorthion (in 1952), fenthion (in 1958) and fenitrothion (in 1959) were discovered.

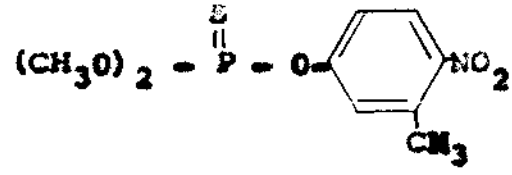
Parathion



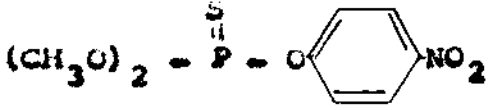
Chlorthion



Fenthion

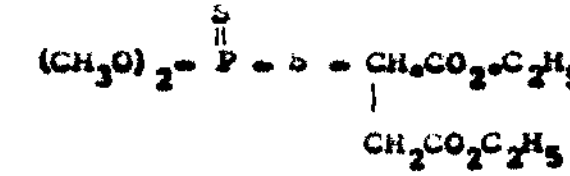


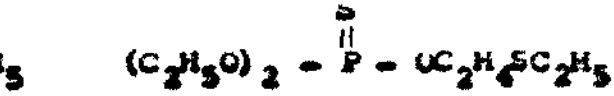
Fenitrothion



Parathion-methyl

Malathion was discovered in 1950 and demeton in 1951.





Malathion

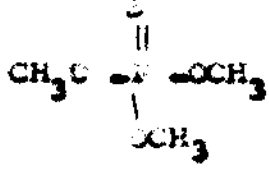
Demeton - S

In 1952, the perkow reaction was discovered, and many important vinyl phosphate esters have been introduced as practical pesticides. Since then several new compounds have been developed and are in commercial use(3).

Fungicides help to control the diseases that wither the leaves of our fruit trees, cucumber vines or rose bushes.

The first studies in which the microbiological action of organophosphorus compounds was noted, were made at the beginning of the 1940's, but systematic investigations of their fungicidal and bactericidal properties were begun much latter(4,5). Recently it is only they have been gaining importance in the control of pathogenic fungi(2,3). In comparison to the heavy metal fungicides, the organophosphorus compounds are particularly favourable as regards to residue problem.

The simplest organophosphate is trimethyl phosphorothioate.



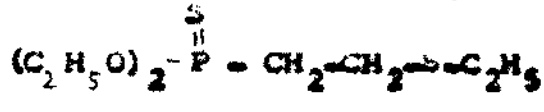
It is effective, selective soil fungicide to control Pythium sp.(6) .

The other fungicides are Edifenophos, Kitasin, Pyrazophos (Afugan), Tolclofos (Rhizolex 199), Triamiphos (Wepsin), phosphonomycin, Conen, Inezin, Cerezin phosbutyl etc.

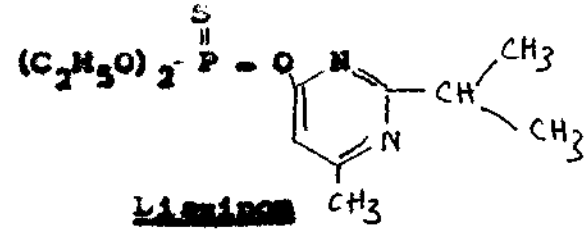
There are certain review on organophosphorus fungicides (7,8,9) . There is an interesting correlation among the alkylating activity, the inhibitory activity against "SH enzymes" and the antifungal activity of some cyclic organophosphorus esters (10) , many fungicides are known as the inhibitors of "SH enzymes"(11).

First among the insecticide came the organochlorine compounds, of which DDT is the grandfather and most famous. It and many of its relatives are now banned from further use in the various countries. Exceptions include chlordane which is used to treat termites and Kelthane, Lindane and Methoxychlor that are reserved primarily for outdoor applications.

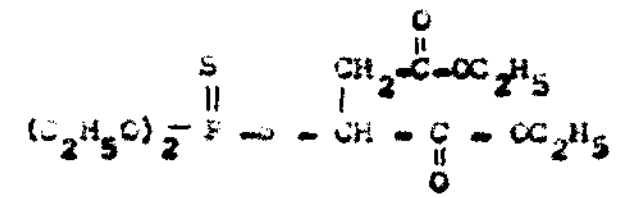
Organochlorine pesticides have been replaced largely by organophosphates. These compounds are generally not very persistent but are, as a group, the most toxic of all pesticides to vertebrates. The most popular organophosphate insecticide and of the safest is Diazinon.

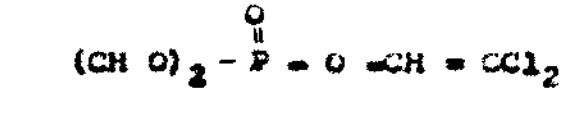


Malathion



Diazinon





Malathion **DDVP**

It is used in concentrations of less than 1% in sprays for house plants and in treating home pests such as roaches and ants. In higher concentrations, it is used to control outdoor insects or vegetables, flowering plants and lawns. Other important organophosphates are Malathion which has been used to treat fruit fly infestations, Dy-syston and DDVP. Dy-syston is a systemic that is used on indoor plant bug-darts, while DDVP is used no-pest strips because of high vapour pressure, which gives it slow-release quality that is important. For the same reason, other closely related organophosphates are the active ingredients in many brands of flea and tick collars.

Some of the most powerful-anticholinesterases known today are found among organic derivatives of phosphorus. Indeed, certain of these substances are so potent as to be extremely poisonous, and much of the development work in this field was done in the interest of preparing chemical warfare agents. It is a paradox that a number of compounds which were developed for this purpose are now employed for the benefit of mankind as drugs, insecticides and pesticides.

Schrader in Germany, originated the development of the organophosphorus insecticides in 1934, but his work was not

published until after world war (II)(12). Later a great many such compounds were prepared as potential insecticides phosphorus containing anticholinesterases are also of great theoretical interest because of their mode of biochemical action.

Since most organophosphorus pesticides hydrolyse their persistance and/or appearance of hydrolysis products may be obtained from kinetic studies. Hydrolysis rates of these compounds and their metabolites are of interest since chemical hydrolysis determines whether or not toxic residues will persist. The first order half-lives of some common organophosphorus pesticides including some metabolites are listed in table 1.1 (13).

Table 1.1

Half-lives of some organophosphorus pesticide in ethanol (temp. 70 °C, pH 6.0, buffer solution 1:4)

Compound	Half-life (hrs)	Compound	Half-life (hrs)
Dichlorvos	1.35	Demeton-S	18.0
Thimet	1.75	Morphothion	18.4
Trichorphon	3.2	Vamidothion	25.4
Mecarbam	5.9		
Malaoxon	7.0	Paraoxon	28.0
Malathion	7.8	Thionazin	29.2
Parathion methyl	8.4	Disulfoton	32.0
Fenchlor phos	10.4	Diazinon	37.0
Azinphos methyl	10.4	Ethion	37.5
Sumithion	11.2	Parathion	43.0
Dimethoate	12.0	Phenkapton	92.0
		Chlorfenvinphos	93.0
		Carbophenothion	110.0
		Dimefox	212.0

The hydrolysis rate is dependent upon chemical structure and reaction conditions such as pH, temperature the kind of solvent used, and the existence of catalytic reagents(3). In aqueous solution, between pH range 1 to 5 many organophosphorus pesticides are most stable (14) and in this range (pH 1 to 5), the variation in pH of the solution has practically no effect on the hydrolysis rate. But the hydrolysis rate increases steeply at pH higher than 7, and all organophosphorus pesticides are much more unstable under alkaline conditions. Very good discussions on chemical structure and hydrolyzability of various organophosphorus pesticides are given by Eto(3) and Faust(13).

REVIEW ON SALITHION AND RELATED COMPOUNDS

Discovery of saligenic cyclic phosphate as a biologically active metabolite of tri-O-cresyl phosphate (TOCP)(3,15,16) has led to extensive studies on synthesis chemical and biological properties of many related compounds (17,18). Analogous cyclic phosphorus esters have been synthetically prepared for examination of their chemical properties (19,20) and biological activities[21,22] are not always coincident with the chemical reactivities and appear to be influenced by the size of an exocyclic substituent on the phosphorus atom. The TOCP metabolite causes ataxia in hens but has no insecticidal activity, while its analogous cyclic phosphate carrying a small alkyl group have insecticidal activity (23). Among the saligenin cyclic phosphorus compounds, salithion (2-methoxy-4H-1, 3, 2-benzodioxaphosphorin 2-sulphide) has been prepared in a large quantity and practically used as an insecticide in Japan (3,18).

Organophosphorus compounds are delayed neurotoxic. A large outbreak of flaccid paralysis took place in Morocco(1959)[25] and USA (1930)[24] due to adulteration of fluid extract of ginger and cooking oil with TOCP. Aldridge and Barnes (26) observed that all neurotoxic triaryl phosphate except tri-p-ethyl phenyl phosphate have at least one alkyl group carrying the α-hydrogen atom on the O-position. This structure-neurotoxicity relationship of triaryl phosphate is characterized by the isolation of active metabolite TOCP (15,16). The main active metabolite (M) is o-tolyl

saligenin cyclic phosphate (2-O-tolyl-4H-1, 3, 2-benzodioxaphosphorin 2-oxide). It extraordinarily active in all the biological properties shown by TOCP. This compound (M) is about 100 times more potent to cause ataxia in hens than TOCP and also ten million times more active than TOCP in the **in vitro** inhibition of plasma cholinesterase (M).

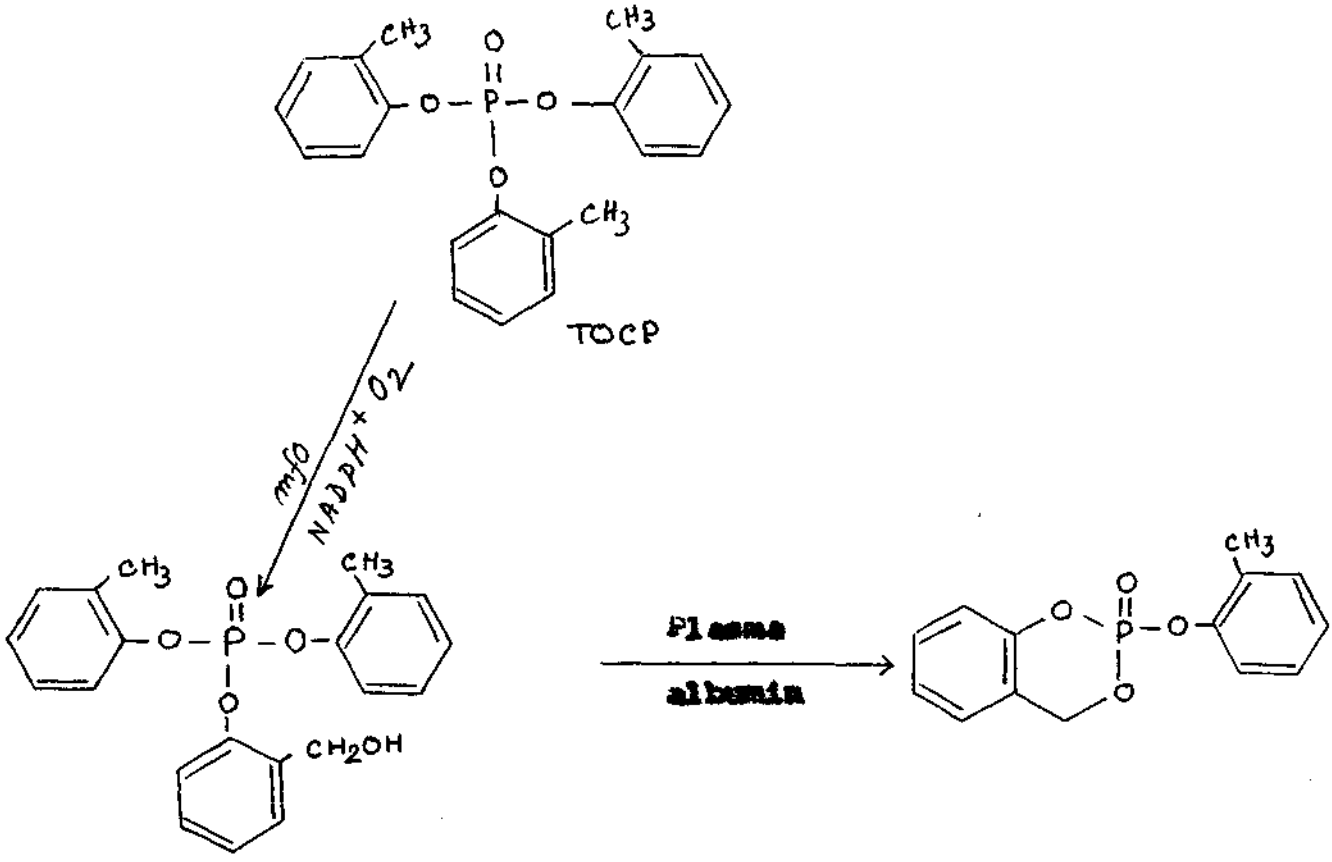
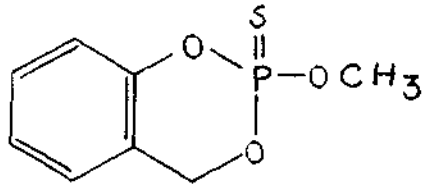


Fig.1.1 Metabolic activation of TOCP

All aryl saligenin cyclic phosphates have showed no insecticidal activity but manifested a high delayed neurotoxicity to cause ataxia in hens; surprisingly the corresponding cyclic

esters (both P=O and P=S compounds) having a small alkyl group on phosphorus revealed high insecticidal activity (18,21).

The pesticide research group of Kyushu University [23] in 1963 discovered "Salithion" (2-methoxy-4H-1, 3, 2-benzodioxaphosphorin 2-sulphide) an organophosphorus insecticide having a unique cyclic ester structure and developed into a commercial insecticide in 1968 by Sumitomo Chemical Co. with the cooperation of Toa-Noyaku Co. (now Kumiai Chemical Co.) and Mikasa Chemical Co. of Japan.



Salithion

The cyclic phosphate and phosphonate esters of saligenin are synthesised by the reaction of saligenin and substituted phosphorylidichlorides in the presence of a dehydrochlorinating agent such as tertiary amine in a dry solvent e.g. chloroform, toluene at low temperature (19). In some cases instead of tertiary amine potassium carbonate and finely divided copper powder at high temperature (31), are used. Thus salithion was first prepared in very low yield. This difficulty has been overcome later by applying the Schotten-Baumann acylation procedure using an aqueous solution of sodium hydroxide (Fig. 1.2).

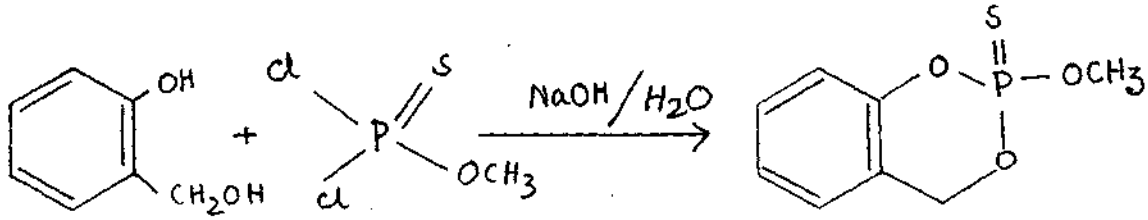
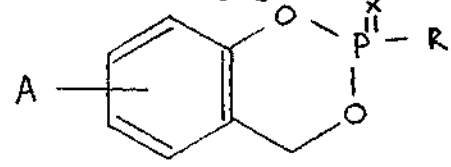


Fig. 1.2. Synthesis of Salithion

Careful observation of the literature reveals (31,32,33,30, 34,35,36,37) that a variety of saligenin cyclic phosphorus esters have been prepared and examined for insecticidal activity as well as other biological properties. A list containing these type of compounds is given in Table 1.2.

Table 1.2

Substituted Saligenin cyclic phosphorus Esters with physical Properties.



R	A	X	*Procedure	b.p. °C/mm Hg(m.p. °C)
OCH_3	H	S	(S)	55-56°
OCH_3	H	O	(P)	110-2°/0.05
o-nC_3H_7	H	O	(P)	129-32°/0.05
OC_2H_5	H	S	(P)	Liquid (not distilled)
OC_6H_5	H	S	(P)	30°
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°)
iC_3H_7	H	S	(P)	108°/0.6

Table contd...

R	A	X	*Procedure	b.p. °C/mm Hg(m.p.°C)
OCH_3	6-CH_3	O	(F)	139-140/0.3
OC_2H_5	6-CH_3	O	(F)	152-156/0.3
OCH_3	7-CH_3	O	(F)	109/0.05
$NHCH_3$	7-CH_3	O	(F)	145-146
OCH_3	8-CH_3	O	(F)	118-120/0.5
OC_6H_5	8-CH_3	O	(F)	135-140/0.6
OCH_3	6-Cl	O	(F)	145-152/0.2
OC_2H_5	6-Cl	O	(F)	160/0.2
O-n-C_3H_7	6-Cl	O	(F)	167-169/0.15
O-n-C_4H_9	6-Cl	O	(F)	187/0.18
OC_6H_5	6-Cl	O	(F)	89°
$NHCH_3$	6-Cl	O	(F)	148°
OCH_3	8-Cl	O	(F)	170-171/0.15
OC_2H_5	8-Cl	O	(F)	181/0.18
O-n-C_3H_7	8-Cl	O	(F)	183/0.18
O-i-C_3H_7	8-Cl	O	(F)	137/0.04
OC_6H_5	8-Cl	O	(F)	203/0.52 (54°)
$NHCH_3$	8-Cl	O	(F)	128-129°
OCH_3	6-Cl	S	(F)	170-178/0.2
$NHCH_3$	6-Cl	S	(F)	175-180/0.25
SCH_3	6-Cl	S	(F)	160-170/0.2
OCH_3	8-Cl	S	(G)	72-73°

Table contd....

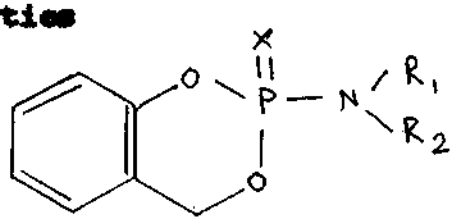
R	A	X	*Procedure	b.p.°C/mm Hg (m.p.°C)
$NHCH_3$	8-Cl	S	(P)	46-47°
CH_3	8-Cl	S	(S)	Oil**
OCH_3	6-NO_2	S	(S)	Paste**
OCH_3	6-Cl 8-C_6H_5	S	(S)	Paste*
OC_2H_5	6-Cl 8-C_6H_5	S	(S)	Paste*
OCH_3	6-C_6H_5 8-Cl	S	(S)	Paste*
OCH_3	6,8-Cl	S	(S)	57.58°
OC_2H_5	6,8-Cl	S	(S)	Oil**
$NHCH_3$	6,8-Cl	S	(S)	Oil**
SCH_3	H	S	(S)	69-70°

* Pyridine (P) or aqueous sodium hydroxide solution (S) was used as dehydrogen chloride agent.

** These compounds were purified through silicic acid column chromatography.

Table 1.3

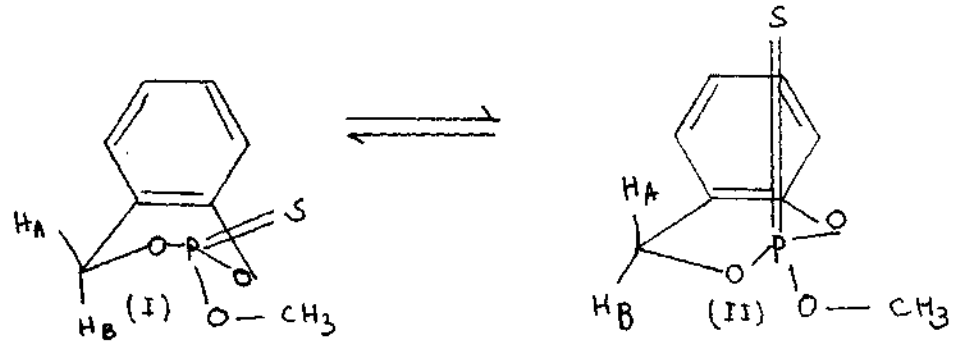
Saligenin cyclic phosphoramidates and Phosphoramidothionates with physical properties



Compound Code No.	R_1	R_2	X	*Procedure	b.p. °C/mm Hg (m.p. °C)
K-19	CH_3	H	O	A	87°
K-22	C_2H_5	H	O	A	68°
K-41	n-C_3H_7	H	O	A	135-140/0.5
K-40	i-C_3H_7	H	O	A	84
K-42	n-C_4H_9	H	O	A	46-47
K-10	C_6H_5	H	O	A	46-47
K-20	CH_3	CH_3	S	A	121
K-23	C_2H_5	C_2H_5	S	B	133-6/0.5(44)
K-35	CH_3	H	S	B	120-123/0.2
K-37	C_2H_5	CH_3	S	B	Undistilled liquid
K-36	CH_3	CH_3	S	B	118-122/0.2
K-38	C_2H_5	C_2H_5	S	B	110/0.2

* Tertiary amine (A) or Potassium Carbonate (B) was used as dehydrogen chloride agent.

Salithion is a colourless crystal m.p. 55-56°C, practically insoluble in water but easily soluble in acetone and benzene, moderately soluble in cyclohexane, toluene and xylene; vapour pressure 1.5×10^{-6} mm Hg at 250°C, UV λ_{max}^{nm} (ε) 274(860), 267(860). It has characteristic IR band at 1020cm^{-1} P-O-CH_2 in the hetero ring. NMR (CS_2) ppm; 3.76(3H, doublet, J_{P-H} = 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 upper field of the doublet at 5.21 ppm slightly splits further (1.5 Hz). This becomes much significant at -30°C, suggesting the methylene protons (H_A, H_B) are not equivalent with each other, but the dioxaphosphorin ring is conformationally mobile in solution (Fig.1.3).



(Fig.1.3. Conformational change of Salithion heteroring

...-ray crystallographic analysis shows that the hetero ring of Salithion is a half chair form in which the sulphide group is in equatorial position (I). The strain in the ring appears little; the endocyclic O-P-O angle is 104°.

Salithion gives a characteristic modes of fragmentation in mass spectrometry(38). It gives an intense peak of $(M-CH_3)^+$ (M/e 201) by β -cleavage occuring at the exocyclic ester group.

Another fragmentation process is the direct loss of SH followed by the elimination of formaldehyde (Fig.1.4).

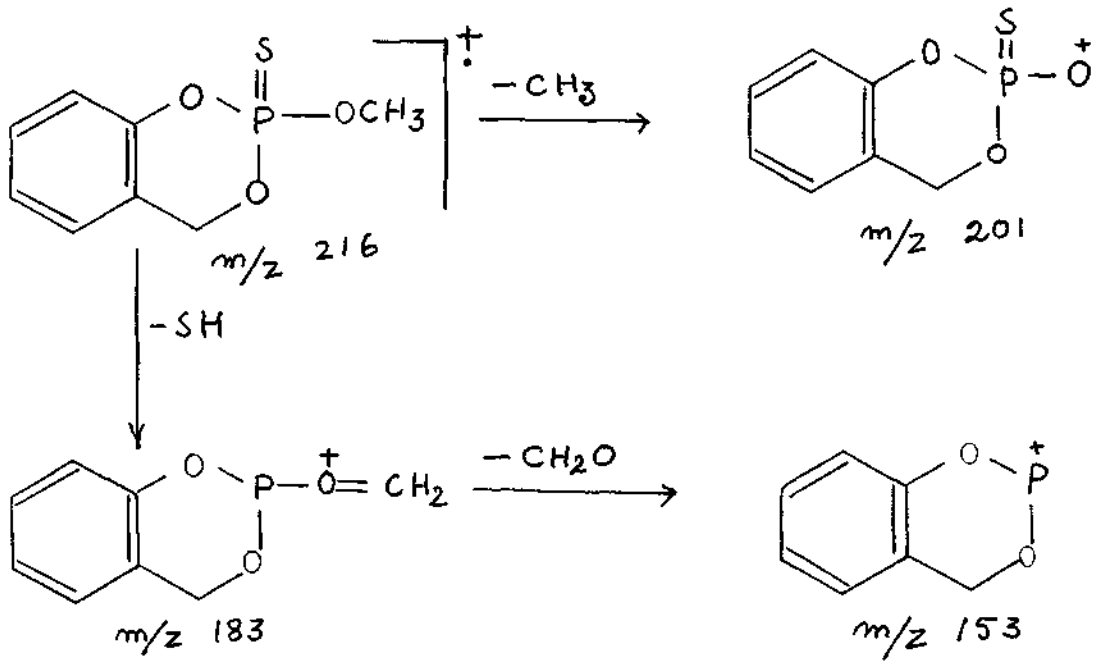


Fig.1.4. Modes of Fragmentation of Salithion in mass spectrometry.

Salithion reacts with bromine water to give salioxon(39). Since salioxon (2-methoxy-4H-1, 3, 2-benzodioxaphosphorin 2-oxide) is several thousand times more active in cholinesterase inhibition than salithion, an enzymatic method after the oxidation can be used for the residue analysis of salithion(39). It is converted into S-alkyl saligenin cyclic phosphorothionate by heating with alkyliodide (the Fistchimuka reaction)(40) in dimethyl formamide. Saligenin is demethylated to form the salt of saligenin cyclic phosphorothionic acid by the action of certain nucleophiles such as cyclohexylamine and potassium dimethyldithiocarbamate(18,42)(41)

Salithion is relatively unstable in storage. Some

secondary amines, such as carbazole and N-phenyl ∝-naphthylamine, stabilised the formulation(43). In a phosphate buffer pH 7.7 salithion is slowly hydrolysed by the P-O (aryl) bond cleavage(18). The hydrolysis rate constant (25°C) is $k = 2.4 \times 10^{-4}$ min^{-1}. Under these conditions give saligenin as the sole product. The rates of hydrolysis of the corresponding cyclic methylphosphonate, O-methylphosphorothiolate (the thiolate isomer of salithion, MTPO), methyl phosphate (salioxon), and N-methyl phosphoramidate are, respectively 90, 60, 6 and 0.6 times greater than that of salithion. The hydrolysis of salithion is shown in Fig. 1.5

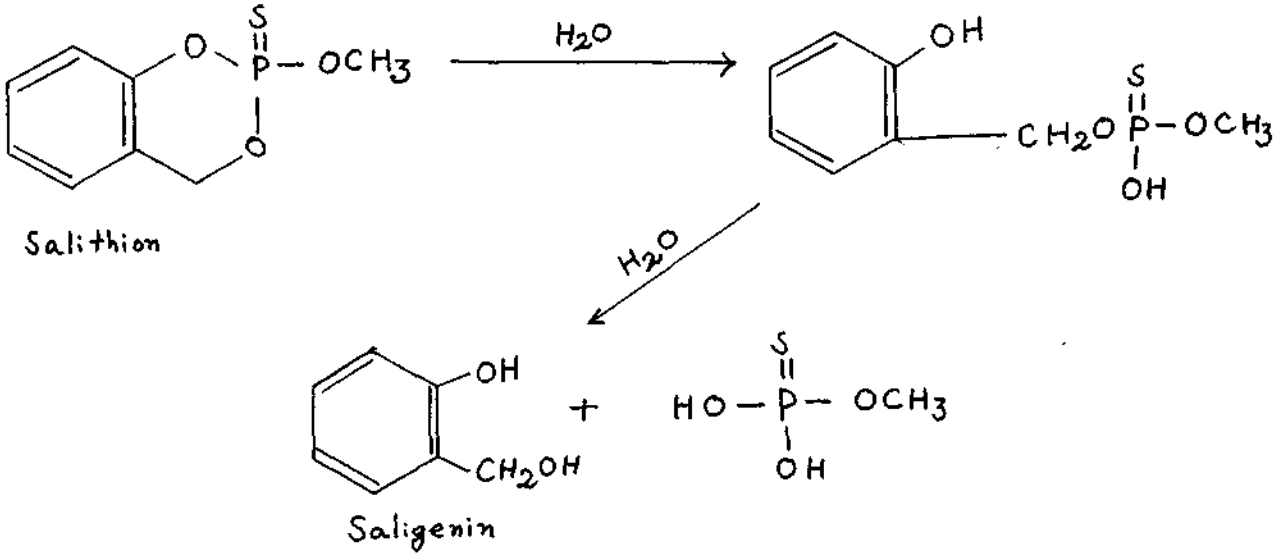


Fig.1.5 Hydrolysis of Salithion.

The metabolic pathway of salithion in rats and plants(46) have stucied(3). These are summarised(18) in Fig. 1.6.

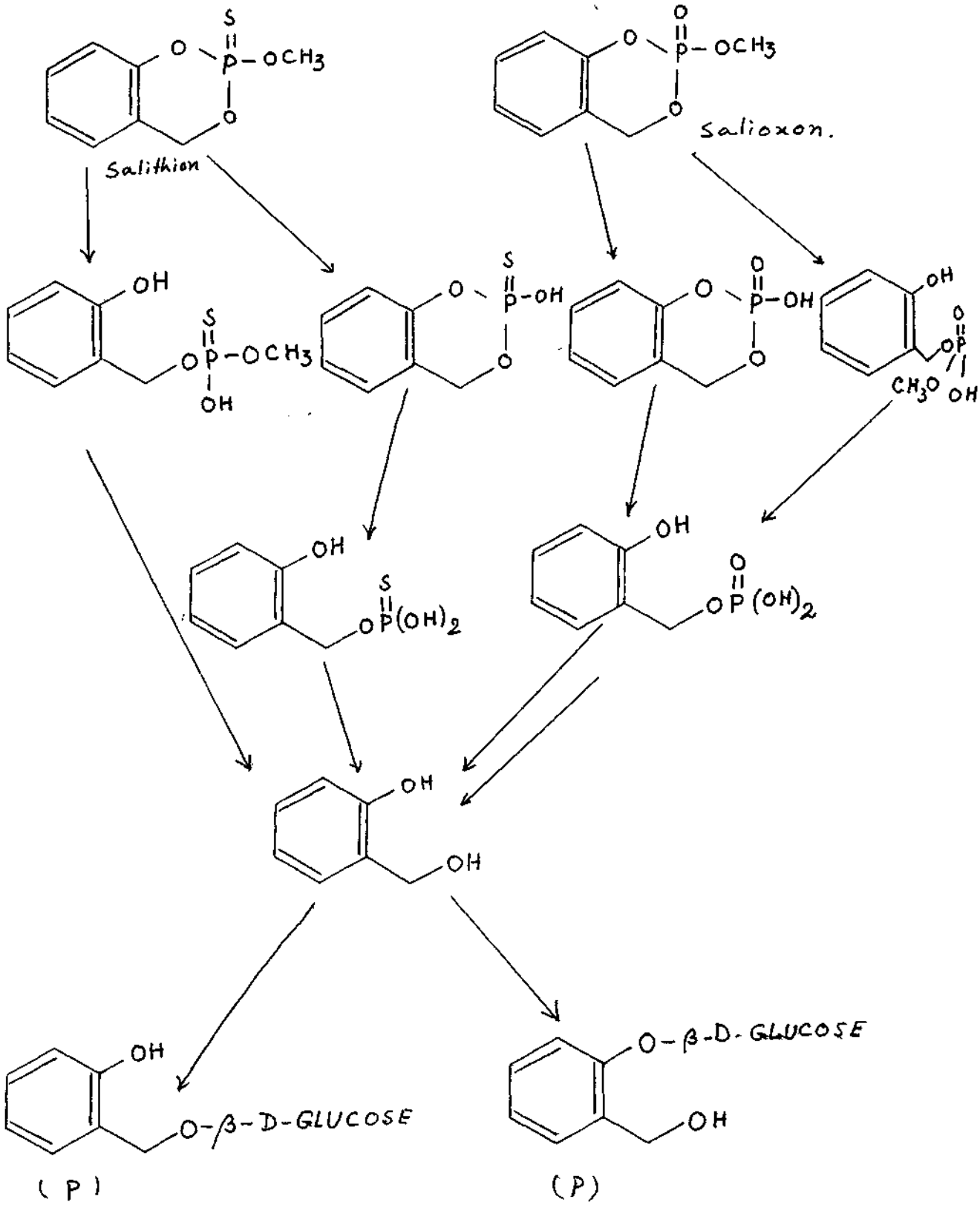
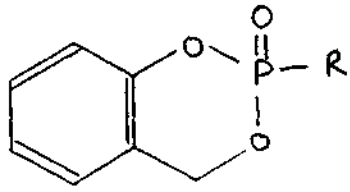


Fig.1.6. Metabolic pathways of salithion in rats and plants (P-denotes the metabolites found in plant only).

In the series of alkyl derivatives there is a relationship between hydrolysis rate constants and insecticidal activity (LD_{50}) (Table1.4). The higher the reactivity is, the stronger the insecticidal activity. However, the aryl derivatives do not follow this relationship. they are more reactive than alkyl derivatives, but are almost non-insecticidal. The size of the substituent appears to be more important for biological activity than the electronic property of the substituent.

Table 1.4

The relationship between rate-constant and insecticidal activity of some saligenin cyclic phosphorus esters.



R	k_{hyd} min^{-1}	LD_{50} (μg/female house fly)
CH_3	2.23×10^{-2}	0.19
C_2H_5	4.25×10^{-3}	0.17
i-C_3H_7	-	0.33
Sec-C_4H_9	-	7.0
t-C_4H_9	-	10 (0%)
$CH = CH_2$	1.39×10^{-2}	0.68
CH_2Cl	2×10^{-1}	10 (60%)
Cl_2CH_2Cl	1.41×10^{-2}	0.99

contd....

R	K_{hyd}^{alk}	LD_{50}(μg/female house fly)
C_6H_5	1.28×10^{2}	10(10%)
OCH_3	1.42×10^{-3}	0.035
OC_2H_5	5.04×10^{-3}	0.33
O-n-C_3H_7	3.79×10^{-4}	7.1
O-n-C_4H_9	3.29×10^{-4}	10(40%)
$O-CH_2CH_2Cl$	2.58×10^{-3}	0.49
$O-C_6H_5$	6.30×10^{-3}	10(3%)
$NHCH_3$	1.54×10^{-4}	0.05
$N(CH_3)_2$	Negligibly small	0.40
NHC_6H_5	2.40×10^{-4}	10

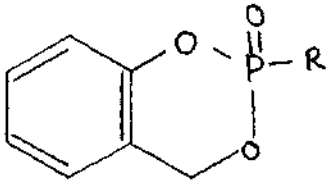
Biological Activities and structural Relationship :

The saligenin cyclic phosphate esters have interesting biological activities. Some of them are neurotoxic, causing ataxia in higher animals. Others did not show such harmful activity but do have high insecticidal activity, systemic activity and fumgicidal activity. Their biological activities include also synergism with organophosphorus insecticides, nematocidal and antifilarial activity. The specificity in biological activities may be attributed to the steric effect of an exocyclic substitu nt group on the phosphorus atom as shown in Table 5. All aryl saligenin cyclic phosphates manifest a high delayed neurotoxicity to cause ataxia in hens and high synergistic activity with malathion(22, 48). The arylphosphate

analogs showed similar biological activities but less in the neurotoxicity. On the other hand 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 only weakly potentiated the toxicity of malathion(22). The interesting feature is that, the alkyl derivatives reveal

Table 1.5

Effects of the exocyclic substituent (R) on biological activities of saligenin cyclic phosphate

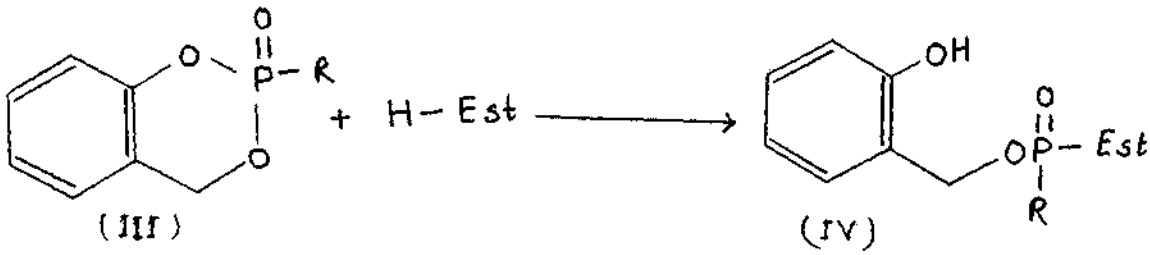


R	Delayed neurotoxicity MAD[a]	Synergism with malathion cotoxicity co-efficient		Insecticidal activity
		Mice	House flies[b]	LD_{50}[c]
$OCH_3-C_6H_4O$	2-5	16.7	7.8	(0)[d]
C_6H_5O	1.5-2	8.8	9.2	(3)[d]
C_6H_5	200	18.8	8.0	(0)[d]
C_2H_5	N.A[e]	3.0	-	0.17
C_2H_5O	-	-	3.1	0.33
CH_3O	N.A[e]	3.7	4.7	0.04
$(CH_3)_2N$	N.A[e]	1.1	-	0.30

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

high insecticidal activity, whereas, the aryl cyclic esters do not (21) .

The specificity of saligenin cyclic phosphates in the biological activity relates to their selectivity in enzyme inhibition. These phosphates inhibit various serine enzyme by phosphorylation, producing probably salicyloxyphosphinyl-enzymes (17,27). This involves by opening of the cyclic ester structure at the P-O aryl bond. When the size of the exocyclic substituent R in (III) increases, to give (IV) the ester becomes a more selective inhibitor of aliesterase (19). Whereas, it becomes a more selective inhibitor of cholinesterase when the substituent is small. Thus the O-tolyl derivative, for example, is 130 times more selective to inhibit aliesterase than cholinesterase.



(III) (IV)

Fig.1.7. Reaction of Saligenin cyclic phosphates with esterase (H-Est).

Therefore, the exocyclic substituent of saligenin cyclic phosphate esters is regarded as the selectophore in the biological actions.

The heterocyclic structure of saligenin cyclic phosphorus esters is merely for the chemical reactivity of the phosphorus atom towards nucleophiles including the active site of esterase and is never requirement for the delayed neurotoxicity. As for example, although Tri-p-ethyl phenyl phosphate (TEPP) has the neurotoxicity(26), is unable to be transformed into a cyclic ester structure.

Johnson found "neurotoxic esterase" in nervous tissues which is specifically sensitive in vivo to neurotoxic organophosphorus esters(50). The esterase is unlike acetylcholinesterase but similar to chymotrypsin and trypsin in the structure activity relationship of inhibitors (51).

Although the structure-neurotoxicity relationship is too complicated to generalize, the neurotoxicity appears to be rather closely related to the structure of the non-leaving group than that of the leaving group.

(a) INSECTICIDAL ACTIVITY (Table 1.6)

Saligenin cyclic methyl phosphate, methylphosphorothionate, N-methyl phosphoramidothionate are potent insecticides. It is important to note that the exocyclic substituent of the most active cyclic phosphorus ester in each series (OCH_3, SCH_3, $NHCH_3$ and CH_3CH_2) differs from each other in electronic characteristics, but resembles in steric property such as the distance (about 2.9°A) between phosphorus and carbon atom in the

P-X-C function, if the bond angle of divalent sulfur is near 90° rather than 109.5°. The introduction of any type of substituent at any position of the benzene ring and on the carbon atom of the hetero ring decreases the activity. Thus salithion is the most effective insecticide in the whole series of saligenin cyclic phosphorus esters. The P-O-C aryl bond of the hetero ring of saligenin cyclic phosphorus esters appear to be active enough to phosphorylate chlinesterase to kill insects without any electron withdrawing group.

(b) FUNGICIDAL ACTIVITY

Salithion has no fungicidal activity, but some saligenin cyclic phosphorothiolates having S-benzyl ester linkage, to protect the rice plant from rice blast disease (Pyricularia Oryzae)(52). The methyl, ethyl and n-butyl-phosphorothiolates have high fungitoxicity comparable to other commercial fungicides. The normal and isopropyl derivatives are less effective. Saligenin cyclic methyl phosphate and phosphorothionate (salithion) are highly active and as insecticide but are almost inactive as fungicide.

(c) ANTI-SH ENZYME ACTIVITY

The saligenin cyclic phosphorothiol have high activity to alkylate (salicylate) mercaptans and to inhibit "SH-enzymes" such as yeast alcohol dehydrogenase(38). The activity seems to be related to fungicidal property but not with the insecticidal

activity.

I_{50} values for alcohol dehydrogenase of some saligenin cyclic phosphorus ester are shown in Table1.7. Cyclic methyl and ethyl phosphorothiolates are most active in this series. On the other hand, cyclic phosphates have only weak activities, though they are potent inhibitors of esterase. Salithion i.e., methyl phosphorothionate which have high insecticidal property is almost inactive toward the enzyme.

The rate of alkylation reaction by the cyclic esters looks parallel with the hydrolysis of the ester and the alkylation proceeds with a considerable time lag. These facts suggest that the alkylation occurs hydrolysis. Actually, the partial hydrolysate of saligenin cyclic esters react immediately with mercaptans. The reaction mechanism is shown in Fig.1.8.

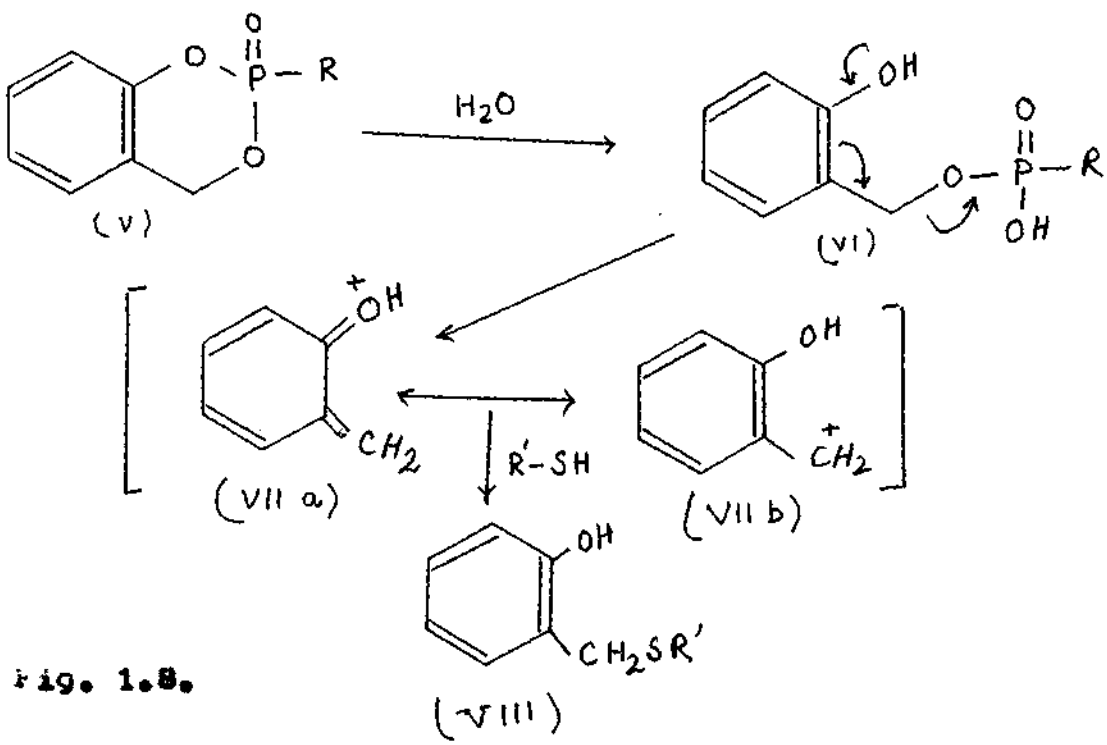


Fig. 1.8.

Saligenin cyclic phosphorothiolates are partially hydrolysed by opening of the heterocyclic R-O-C aryl bond, more easily than phosphate esters. In Fig.1.8, the cyclic ester (V) is hydrolysed by the attack of hydroxide ion to yield salicyl phosphate (VI). The electron releasing -OH group of (VI) may convert to a carbonium ion (VIIb) which may actually react with a SH group to give a thioester.

Cyclic methyl and ethyl phosphorothiolates are much more active in hydrolysis, alkylation and the inhibition of "SH-enzyme" activities than the corresponding cyclic phosphates(38). It seems reasonable to conclude that the decrease of electron density on phosphorus atom causes the high reactivity of the phosphorothiolates. This supported by the lower P=O frequency (1280 cm^{-1}) of the phosphorothiolates in comparison with that of the phosphates (1310 cm^{-1}).

Further investigation shows Table that there is an interesting correlation among the alkylating activity, the inhibitory activity against "SH-enzymes" and the antifungal activity of the cyclic esters. Cyclic methyl and ethyl phosphorothiolates are highly active in all three functions. Cyclic phosphates have very weak activities but they are potent inhibitors of esterases. These facts suggest that high inhibitory activity against "SH-enzymes" may be an important factor for the fungicidal activity of the cyclic phosphorothiolates.

(d) ANTIESTERASE ACTIVITY

The most insecticidal saligenin cyclic methyl phosphate (salioxon) is the strongest inhibitor of insect cholinesterase. However, the highly neurotoxic aryl phosphate is a poor inhibitor of cholinesterase, but is a very specific inhibitor of aliesterase(3,49). The less neurotoxic arylphosphonate occupies an intermediate position. In any series, when the size of the exocyclic substituent increases, the compound becomes a more selective inhibitor of aliesterase; in contrast, the compound carrying a small substituent is a more selective inhibitor of cholinesterase(Table 8). Aryl phosphonates are more specific inhibitors of Pseudo-cholinesterase; alkyl phosphates are less specific and aryl phosphate are intermediate.

(e) SYNERGISTIC ACTIVITY

Saligenin cyclic aryl phosphates and phosphonates have synergistic activity with malathion against insects and mites, particularly their resistant strain(53).

The joint action of the activity of some saligenin cyclic phosphorus esters with malathion has been examined by Eto, Oshima, Kitakato, Tanaka and Kojima(46) and compared with some phosphorus esters which are known as the synergists of malathion. They increase the toxicity of malathion 2.3 to 3.4 times at a 1:1 mixing ratio. The activities of them are more

than propyl paraoxon but less than Dibrom and isopropyl paraoxon.

It has been observed that for a large number of organophosphorus compounds the synergism of malathion in mice and the degree of inhibition of ali-esterase *in vivo* are generally related (22). For insects, high esterase activity hydrolysing malathion is supposed to be partly responsible for malathion-resistance in some strains of mosquito, housefly and green rice Leaf hopper (48).

Eto *et al* (49) have shown that aryl derivatives of saligenin cyclic phosphorus esters are the selective inhibitors of aliesterase, whereas small alkyl derivatives are not so selective to aliesterase inhibition. This appears to be responsible for their difference in synergistic properties.

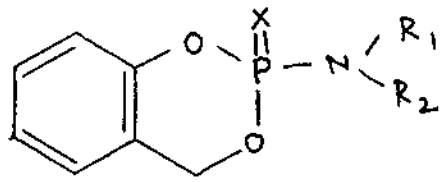
Conclusion

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 "all-enzyme" inhibition 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, methyl phosphorothionate is useful as

an insecticide, alkyl amidates have systemic activity, alkyl phosphorothiolates have fungicidal activity, phenyl-phosphonates have antifilarial activity, and aryl phosphates are neurotoxic and have synergistic activity.

Table 1.6

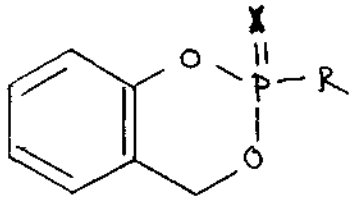
Saligenin cyclic phosphoramidates and phosphoramidothionates : Insecticidal activity and toxicity



X	$-N\begin{matrix}R_1\\R_2\end{matrix}$	LD_{50} (μg/female House fly)	LD_{50} (mg/kg(Mouse)
O	NHMe	0.05	5-7.5
O	NHEt	0.60	30-50
O	NH-n-Pr	1.50	>50
O	NH-n-Bu		> 50
O	NH-Ph	> 10 (5%)	-
O	$N(Me)_2$	0.3	-
O	$N(Me)_2$	0.3	-
O	$N(Et)_2$	> 10	> 50
S	NHMe	0.044	20-30
S	NHet	0.48	-
S	$N(Me)_2$	0.38	-
S	$N(Et)_2$	0.63	-
S	OMe	0.05	88
O	OMe	0.045	52
Parathion		0.040	347
Malathion		0.060	-

Table 1.7

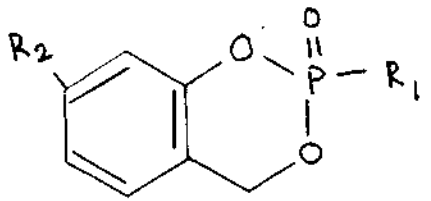
Chemical, Biological and Anti-Fungal activities of some Saligenin cyclic phosphates and their thio analogous



X	R	Hydrolysis (%)	Cystein reacted (%)	I_{50} Yeast Alcohol dehydrogenase ($M \times 10^{-5}$)	Protective value against Piricularia Oryzae (%)	
					50 ppm	500 ppm
O	SCH_3	86	55	4.5	100	-
O	SC_2H_5	81	50	4.4	93	-
O	OC_6H_5	55	45	6.8	-	-
O	OCH_3	17	10	62	-	65
S	OCH_3	5	5	100	-	52

Table 1.8

The Inhibition of Housefly, Human blood and Horse Serum-Esterase by some Saligenin cyclic phosphorus Compounds.



R_1	R_1	Housefly $I_{50} \times 10^{8}$ (M)		Human blood $I_{50} \times 10^{8}$ (M)		Horse plasma $I_{50} \times 10^{8}$ (M)	
		ChE	AliE	P-ChE	t-ChE	AliE	Malathionase
OCH_3	H (Salioxon)	7.6	8.4	1.8	17.0	280	620
OC_2H_5	H	13.2	2.1	1.6	25.0	240	-
O-n-C_3H_7	H	50.7	3.0	-	-	-	-
O-n-C_4H_9	H	37.5	2.3	-	-	-	-
OC_6H_5	H	-	-	-	-	-	-
C_6H_5	CH_3	-	-	1.6	68.0	230	-
OPh-2-CH_3	H	-	-	1.3	39.0	200	-

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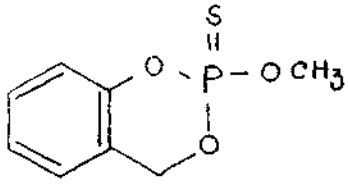
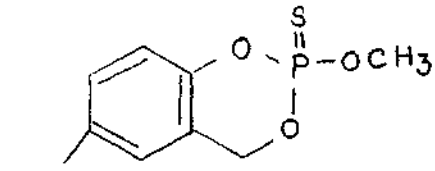
AIMS AND OBJECTIVES OF THE PRESENT INVESTIGATION

Since the cyclic organophosphorus esters of saligenin were discovered as the biologically active metabolites of triorthotolyl phosphate, many related compounds have been synthesized to study their chemical, biochemical and biological properties including pesticidal activities (1). The 2-methoxy-4H-1, 3, 2-benzodioxaphosphorin 2-sulphide is now commercialized as an insecticide named salithion (2). It has been reported that the 2-methoxy-6-nitro 4H-1, 3, 2-benzodioxaphosphorin 2-sulphide (MO-8) is obtained as a paste in the reaction of 5-nitro saligenin with methyldichloridophosphorothionate after purification through silicic acid column chromatography; and this methoxy compound has about sixty times less insecticidal activity as compared with salithion (3).



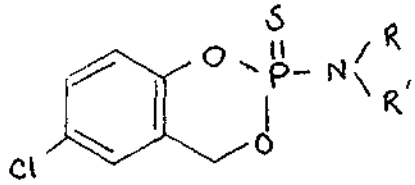
Salithion



MO-8

However, it has been observed by Das (4) that the said methoxy compound (MO-8) is a solid (m.p. 84°C) and has about 1.5-2 times greater oral insecticidal activity to cockroaches, Periplaneta

americana (Linn) compared with salithion. These observations prompted us to undertake a systematic investigation on some substituted saligenin cyclic phosphorus compounds. This work is mainly concerned with the chemical, spectral, biochemical, chemical hydrolysis, insecticidal, phytotoxic and fungicidal properties of some 2-alkylamido-6-chloro-4H-1, 3, 2-benzodioxaphosphorin 2-sulphides of the general structure given below.



where $-N\begin{matrix}R\\R'\end{matrix}$ = Diisobutylamido

= Dipropylamido

= Dibutylamido

= 2, 6-Dimethylmorpholino

= 2-Ethylpiperidino

= 4-Benzylpiperidino

= Hexamethyleniamido.

ACTUAL WORKS

1. Synthesis :

Syntheses of some 2-alkylamido-6-chloro-4H-1, 3, 2-benzodioxaphosphorin 2-sulphides have been presented.

2. Spectral studies :

UV, IR, Mass and ^{1}H NMR spectral data of these compounds have been presented. ^{13}C and ^{31}P NMR spectral data of some 2-alkylamido-6-chloro-4H-1, 3, 2-benzodioxaphosphorin 2-sulphides have been given.

3. Studies of Biological Properties :

(i) Insecticidal activity test of these compounds on cockroach and blow-fly has been performed.

(ii) Acute oral toxicity study of these compounds on rats has been performed.

(iii) Anticholinesterase activity of these compounds has been determined on goat whole blood and blow-fly head homogenate.

(iv) Phytotoxic properties of these compounds on the germination of rice seed have been studied.

(v) Antifungal activity of these compounds against Pyricularia oryzae has also been determined.

4. Studies of Chemical Hydrolysis :

Chemical hydrolysis of these compounds at alkaline, pH 11.85 has been given.

5. Quantitative Structural Activity Relationship :

Relationship between chemical structure and antifungal activities has been studied using different parameters, hydrophobic constant, Es and structural Information content(-IC) of the various molecules.

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