P A R T I

ORGANOPHOSPHORUS PESTICIDES: GENERAL INTRODUCTION

1. INTRODUCTION:

Intensive research in the field of plant protection and pest control has been going on throughout the world. As a result various types of pesticidal compounds are being prepared, and their pesticidal, toxicological and other properties are being studied everyday. Of these, organophosphorus compounds constitute a class in which quite a large number of compounds have been synthesized and examined as effective pesticides, owing to their-high activity and biodegradability, their application in Agriculture, Public health, and related fields has been going rapidly. Several new compounds of this group are used for insecticidal, acaricidal, nematocidal, anthelmitic insect sterilizing, fungicidal, herbicidal, rotenticidal and other purposes.

2. ORGANOPHOSPHORUS FUNGICIDES:

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 later[1-3]. It is only recently they have been gaining importance in the control of pathogenic fungi[4-5]. In comparison to the heavy metal fungicides, the organophosphorus compounds are particularly favourable as regards to residue problem.

The simplest organophosphate is trimethyl phosphorothioate.

$(CH_3O)_3$. PS.

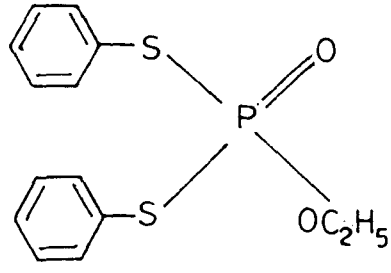
It is an effective, selective soil fungicide to control Pythium Sp.[6].

Trimethyl phosphorotetrathioate appear to be useful for the control of Pythium Sp.[6b, c].

$(CH_3S)_3$ PS.

The fungicidal activity of trialkyl phosphorotetrathioates decreases with increasing chain length of the alkyl group. These compounds are highly species selective in fungicidal activity. Thus, trimethyl phosphorothionate is almost ineffective against Rhizoctonia Fusarium and Verticillium[6c]. On the other hand an analogous compounds, O,O-diethyl S-methyl phosphorothioate, is a good fugicide against Rhizoctonia solani [6b]. The fungicidal activity of different other compounds are given below:

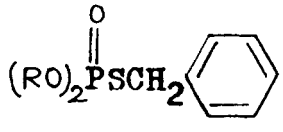
Edifenphos (Hinosan):



This is a fungicide with specific action against Pyricularia oryzae on rice at 30-50 g.a. i./L water using 800-1200 L/ha; 1 or 2 applications to wet paddy in the nursery, 2 or 3 applications after transplanting or in fields of broadcast rice before tillering has ceased. It is also effective against Pellicularia sasakii and ear blight and is well tolerated by rice varieties at effective fungicidal rates. It should not be used within 10 days before or after an application of propanil.

The n-propyl and isopropyl homologs have almost the same fungicidal activity as the ethyl edifenphos, but the methyl and butyl homologs are much less active than the later. The introduction of a chlorine atom into the benzene ring causes a remarkable decrease in fungicidal activity.

Kitazin:



R = $(CH_3)_2CH$ Kitazin P

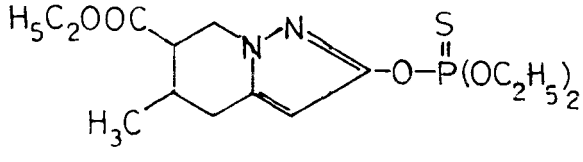
R = C_2H_5 Kitazin.

S-Benzyl diethyl phosphorothiolate was first introduced in 1965 as a fungicide under the trade name kitazin, but was replaced in 1967 by the isopropyl homolog kitazin P for commercialization.

It is a systematic fungicide used to control Pyricularia oryzae in rice. It is applied at 400-600g.a.i. (as e.c.) in 1000 L/ha as soon as the blast lesions appear. One or two sprays may be needed during the head-sprouting season. Kitazin and Kitazin P inhibit more strongly the mycelial growth and the spore formation of Pyricularia oryzae than the spore germination. Thus, they are effective curatively rather than prophytactically.

In the homologous series of dialkyl S-benzyl phosphorathiolates, the maximum fungicidal activity is obtained when the number of atoms in the alkyl group is three or four. The dimethyl homolog has poor activity. In the analogous series, the phosphorothionate, phosphorothiolothionate, and phosphate esters. Introduction of substituents such as chlorine atom or nitro group on the benzene ring has little effect on the increase of the fungicidal activity.

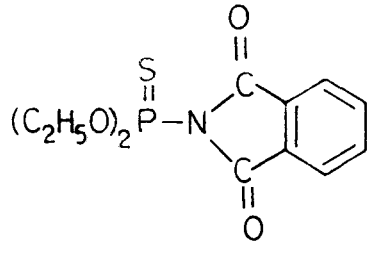
Pyrazophos (Afugan):



Pyrazophos is a systemic fungicide controlling powdery

mildews on a wide range of crops at 10-30 g. a.i./100L, and on cereals at 500-700 g/ha. It has both preventive and curative activity against powdery mildews. It is absorbed by foliage and green stems and translocated within the plant when applied to the soil or a seed dressing uptake by roots is insufficient for effective fungicidal action within the plant.

Ditalimfos (Dowco 199):



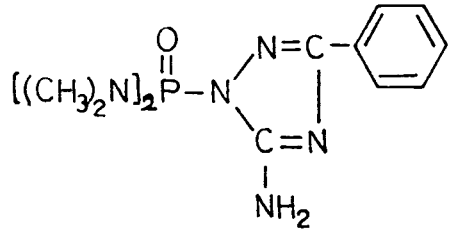
Ditalimfos is a non-systemic foliar fungicides with protectant and curative activity. It is used to control powdery mildew on ornamentals (primarily roses) and vegetables (cucurbits) under glass, as well as under field conditions, at 30-50 g.a.i./100L; on apples (25-50 g/100) and cereals (500-550 g/ha.). It is also used against Venturia inaequalis on apples at 37.5 - 100 g/100 L. It is liable to 'russet" certain apple cultivars, particularly Golden Delicious.

The isopropyl homolog of Ditalimfos has similar fungicidal activity but is about three times more toxic to mammals. The methyl homolog and the methylamide analog

are much less active in fungicidal action. The aromatic ring is necessary for the fungicidal activity, but any substitution on the ring causes a remarkable decrease in fungicidal activity. It is interesting to note that the fungicidal activity of Ditalimfos is lost by replacing the thiophosphoryl sulfur atom with an oxygen atom.

Furthermore, if the phthalimide-N is not directly attached to the phosphorus but through an $S-CH_2$ bridge or an oxygen atom, the phosphorus compounds are not fungicidal but insecticidal.

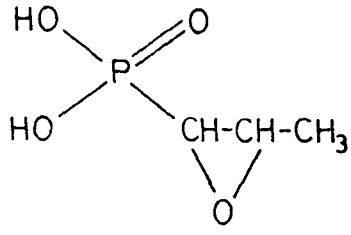
Triamiphos(wepsyn, wepsin):



Triamiphos is a fungicide for powdery mildew control and shows some systemic activity; it also has systemic insecticidal and acaricidal properties. Rates for powdery mildew control include; for apples 25 g. a.i.(as w.p) / 100 L every 10 d; for roses 25 g. a.i.(as water-miscible)/ 100 L. At these concentrations, it is non-phytotoxic and presents no hazard to wild life.

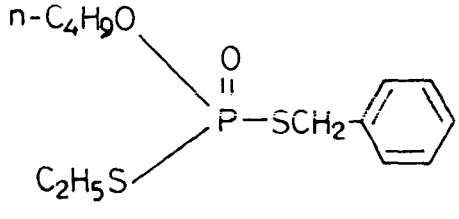
The 5-anilino-3-alkyl analogs of Triamiphos are also active as fungicides.

Phosphonomycin:



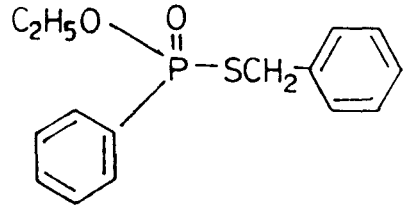
Phosphonomycin is a naturally occuring phosphonate antibiotic recently discovered by Merk & Co. Inc. It was isolated from fermentation broths on which Streptomyces fradiae was grown. Its structure was demonstrated by synthesis(7). This new antibiotic has a broad spectrum of activity and inhibits irreversibly pyruvateuridine diphospho-N-acetylglucosamine transferase in extracts of gram-positive and gram-Negative micro-organisms. It compares favourably with tetracycline and chloramphenicol.

Conen :



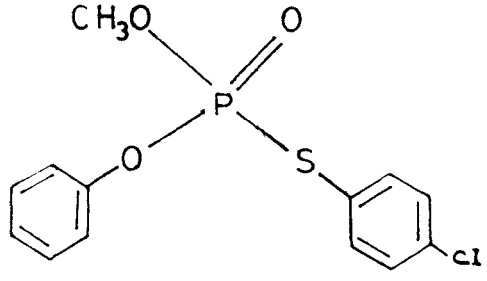
It is a fungicide to control rice blast disease.

Inezin:



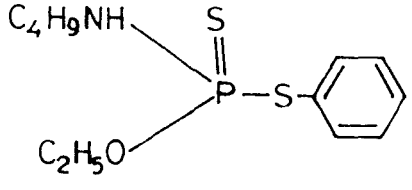
It is a fungicide to control rice blast and rice sheath blight.

Cerezin:



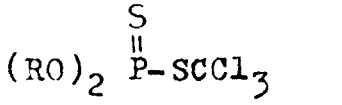
Cerezin has a curative effect on rice blast disease. It has also insecticidal activity against two hopper species, Nephotettix cincticeps and Delphacodes Striatella, which transmit virus disease to rice plants.

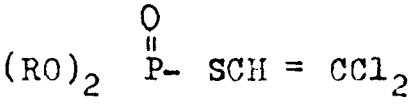
Phosbutyl:

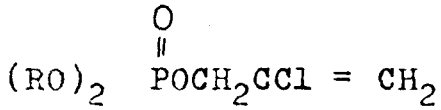


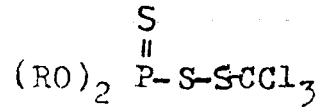
It is highly active against mycelial cells, but not active against spore germination. Being absorbed rapidly by the plant, it thus shows a good curative activity for many plants infected with pathogenic fungi.

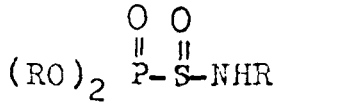
Certain organophosphorus compounds are known to have fungicidal activity. Structures of some selected compounds are given below:

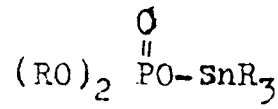


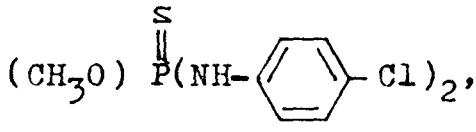


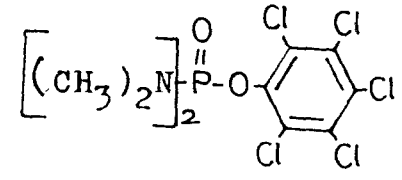


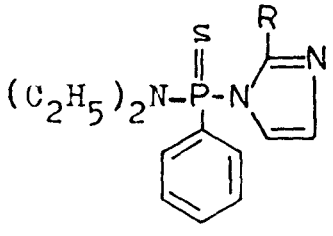


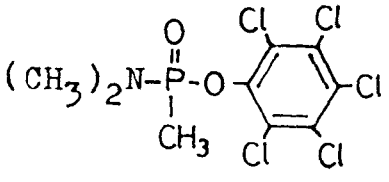


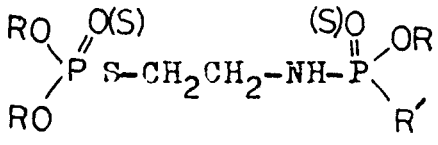


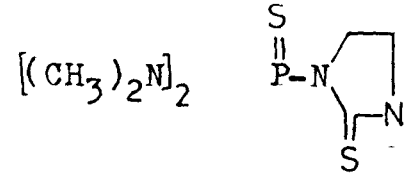








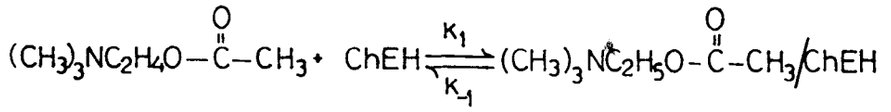




There are certain reviews on organophosphorus fungicides[6b,8] There is a interesting correlation among the alkylating activity, the inhibitory activity against SN ensymes', and the antifungal activity of some cyclic organophosphorus esters[10] many fungicides are known as the inhibitors of 'SH ensymes'[1]

3. REACTION WITH CHOLINESTERASE:

It is generally accepted that the organophosphorus compounds are toxic because they phosphorylate vital esterases, thus forming complexes that are either irreversible or do not readily release the enzymes[2]. The enzyme mainly affected is accepted to be cholinesterase, an enzyme that plays a vital role in hydrolysing acetylcholine. The reaction between choline (Ach) and cholimesterase (ChEH) takes place in three stages:



Acetyl choline Enzyme complex

At this stage there is an equilibrium between the enzyme and its substrate on the one hand and a complex of the two on the other.

$$(CH_3)_3\overset{+}{N}C_2H_4O\text{-}\overset{O}{\overset{\|}{C}}\text{-}CH_3/ChEH \xrightarrow{K_2} (CH_3)_3\overset{+}{N}C_2H_4OH + CH_3CoECh$$

Enzyme complex choline + Acetylated enzyme

The complex yields choline and acetylated enzyme in the second stage.

$$CH_3CO.ECh \xrightarrow[K_3]{H_2O} CH_3COOH + ChEH$$

Acetylated enzyme Acetic acid + Enzyme

The final stage is the deacetylation in which the acetylated enzyme is hydrolysed to give the free enzyme and acetic acid.

The active centre of acetylcholinesterase (AChE) is structurally complementary to its substrate acetylcholine which contains a trimethyl ammonium group with a positive charge on N and an ester linkage. The enzyme's active centre contains a negatively charged anionic site, which binds the trimethylammonium group, and a relatively "non-specific esteratic site, which catalyzes the hydrolysis of the ester linkage. In the esteratic site there are basic (histidine, imidazole, serine hydroxyl), and acidic (tyrosine hydroxyl) groups (Scheme - I); the reaction between an organophosphorus compound and AchE is

represented in scheme II, when the two chemicals interact there is a nucleophilic attack of the serine hydroxyl on the phosphorus atom that is aided by the acidic and basic groups present in the esteratic site of the enzyme. This results in the formation of a "reversible" complex that finally yields phosphorylated enzyme and nitro-saligenin. Aldridge[14] investigated the inhibition of cholines - terase by parathion and related compounds and found that the complex did not show significant reversibility. In other words, the inhibition of cholinesterase in this case followed first order kinetics and was bimolecular, i.e.

$$K = \frac{I}{t_I} \cdot \ln\frac{100}{b}$$

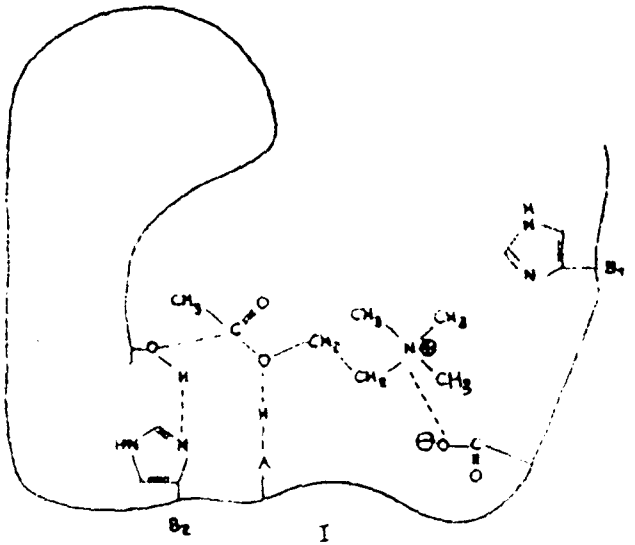
where, K = bimolecular rate constant,

t = time in minutes,

I = molar inhibitor concentration,

and b = percentage residual activity.

Correlation between the reactivity of an organo-phosphorus compound and its cholinesterase inhibition, however has not been ideal, and Main[15] introduced a kinetic treatment for the reaction that takes into account the reversibility of the complex. This reversi-bility is dependent on the affinity of the inhibiting compound for the active site of cholinesterase as well as on the rate of phosphorylation (scheme II).



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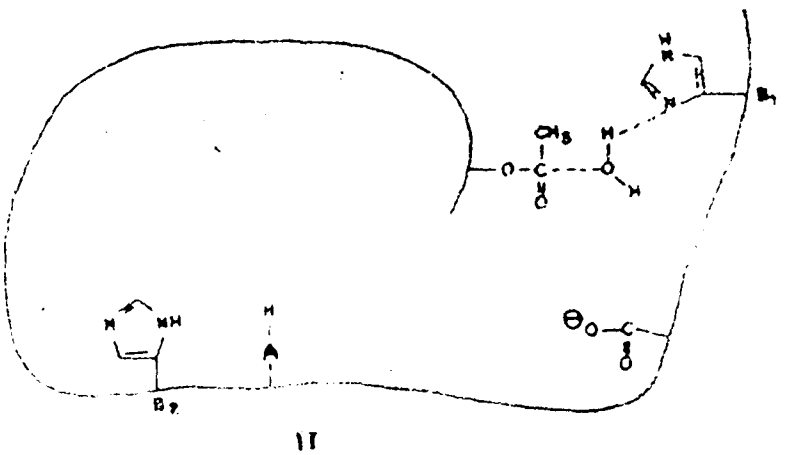
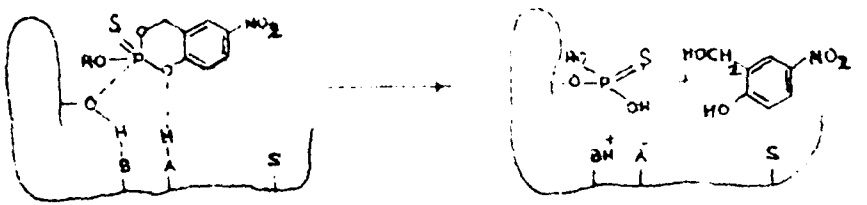


II

Schematic Mechanism of action of AChE. after Krupka
(I) Enzyme substrate complex in AChE
(II) Deacetylation of acetyl- AChE (Scheme I)



Schematic mechanism of reaction of organophosphate with AChE (Scheme II)

By utilising different kinetic methods the values for k_1 (affinity complex), K_p (phosphorylation constant), and K_e (bimolecular inhibition constant) may be determined[11,16].

If the acetylcholinesterase is destroyed or irreversibly bound, or forms a complex from which it is released more slowly than under normal condition, its substrate, acetylcholine, is not easily removed from the receptor surface of the muscle. This causes the muscle to be depolarised longer than usual and gives rise to several action potentials passing through the muscle. The result is a twitching of the muscle leading to tetanus and eventual paralysis of the muscles. Death in mammals occurs as a result of asphyxia caused by the paralysis of the respiratory muscles.

4. CHEMICAL HYDROLYSIS:

Since most organophosphorus pesticides hydrolyse their persistant and/or appearance of hydrolysis preducts may be obtained from kinetic studies. Hydrolysis rates of these compounds and their metabolites are of interest since chemical hydrolysis determines wheather or not toxic residues will persist. The first-order half-life of some common organophosphorus pesticides including some metabolites are listed in Table I[12].

Table I

Half-life of some organophosphorus pesticides in ethanol (temp. 70°c, pH 6.0, buffer solution 1:4)

Compound	Half-life (Hours)	Compound	Half-life (Hours)
Thimetoxon	0.50	Hemeton-S	18.00
Dichlorvos	1.35	Morphothion	18.40
Thimet	1.75	Baylex	22.40
Trichlorphon	3.20	Vamidothion	25.40
Mecarbam	5.90	Menazon	27.60
Malaoxon	7.00	Paraoxon	28.00
Demeton-S-methyl	7.60	Thionazin	29.20
Malathion	7.80		
Parathionmethyl	8.40	Disulfoton	32.00
Fenchlorphos	10.40	Diazinon	37.00
Sumithion	11.20	Parathion	43.00
Dimethoate	12.00	Chlorfenvinphos	93.00
Thiometon	17.00	Carbophenothion	110.00
Methyloxy-demeton	17.10	Dimefox	212.00

The hydrolysis rate is dependent upon the chemical structure and reaction condition such as pH, temperature, the kind of solvent used, and the existence of catalytic reagents[5]. In aqueous solution, between the pH range 1 to 5 many organophosphorus pesticides are most stable[13],

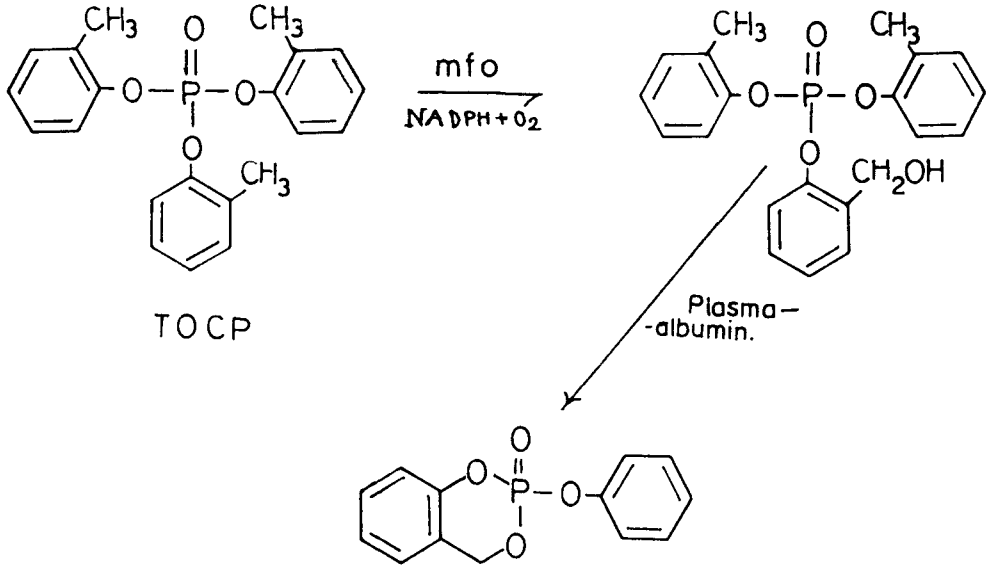
and in this range (pH 1 to 5), the variation in pH of the solution has practically on effect on the hydrolysis rate. But the hydrolysis rate increases steeply at pH higher than 7, and all organophosphorus pesticides are much more unstable under alkaline conditions. Very good discussions on chemical structure and hydrolyzability of various organophosphorus pesticides are given by E to[5] and Faust[12].

5. DISCOVERY OF SALITHON:

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)[17]. This was due to the phosphate triester of O-cresol, so called TOCP, which containated 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-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[18].

Because of very sensitive to the delayed neurotoxic action of organophosphorus compounds, hens have been used for the assay of the neurotoxicity of triaryl phosphate.

Aldridge and Barnes[19,5] observed that all neurotoxic triaryl phosphates except tri-p-ethyl phenyl phosphate have at least one alkyl group carrying α-hydrogen atom on the ortho position. This structure-neurotoxicity relationship of triaryl phosphates became clearly understandable by the isolation and characterisalition of the active metabolites of TOCP in 1951[20,21]. The principal metabolite (I) was O-tolyl saligenin cyclic phosphate (2-O-tolyloxy-4H-1,3,2-benzodioxaphosphorin 2-oxide). It is extraordinarily active in all the biological properties shown by TOCP: (I) was about 100 times more potent to cause ataxia in hens that TOCP; (I) was ten million times more active than TOCP in the <u>in vitro</u> inhibition of plasma cholinesterase[22].



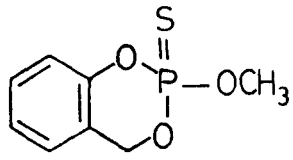
(I)

Fig. 1: Metabolic activation of TOCP.

The conversion of TOCP into the cyclic phosphate[21] via two steps is shown in Fig.1. The hydroxylation of the methyl group of TOCP is affected by the microsomal monoxygenase and then cyclization is followed by intermolecular transphosphorylation of the intermediate, di-O-tolyl O-(α-hydroxy) tolyl phosphate, eliminating one molecule of cresol. Ordinarily the later reaction is a slow one but greatly accelerated by the presence of plasma albumin[23].

Thus it is rational to presume that the triaryl phosphates having an O-alkyl group with the α-hydrogen atom may be similarly metabolised to give the corresponding active cyclic esters. In the cyclization reaction, no alkyl ester group participates as the leaving group[24]. Actually no aryl but alkyl saligenin cyclic phosphate is forned in vitro from alkyl di-O-tolyl phosphates. Such metabolic activation of TOCP or its analogs was observed in rate[21] hens[21], cats[25] and insects[26].

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 pesticides research-group of Kyushu University[27] 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.

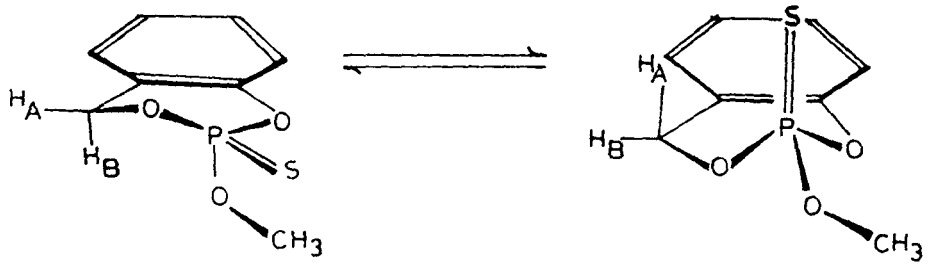
6. PROPERTIES OF SALITHION:

Referring back to Salithion, we pinpoint our discussion to its important properties[28] 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 (CS_2)ppm: 376(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 becames 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. 2). X-ray crystallographic analysis shows that the hetero ring of salithion is



I II

Fig. 2: Conformational change of salithion heter-ring.

a half-chair form in which the sulphide group in equatorial position (II). 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 α-cleavage occuring at the exocyclic ester group. Another characteristic fragmentation process is the direct loss of SH followed by the elimination of formaldehyde (Fig. 3).

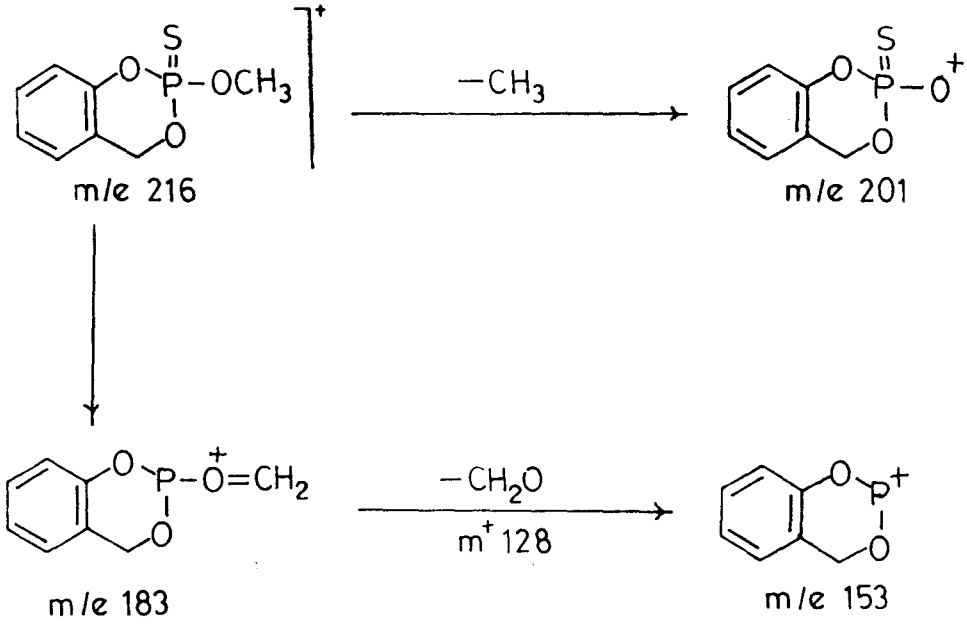


Fig. 3: Fragmentation of Salithion in Mass Spectrometry.

Salithion is relatively unstable in storage. Some secondary amines, such as carbazole and N-phenyl-α-naphthyl amine, stabilize the formulation[29]. In a phosphate buffer (pH 7.7), salithion is hydrolysed slowly through opening of the heteroring by the p-O-(aryl) bond cleavage: the hydrolysis rate constant (25^{o}) $K = 2.4 \times 10^{-4}$ min^{-1}. The hydrolysis rates of the corresponding cyclic methyl phosphate, S- methyl phosphorathiolate (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[30,31].

On exidation 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 reaction analysis of salithion[30].

Salithion is isomerized into S-alkyl saligenin cyclic phosphorothiolates by heating with iodides (the pistchimuka reaction)[32]. The reaction is greatly accelerated in such a polar solvent as dimethyl formamide. 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)[32,33]. Salithion is demethylated to form the salt of saligenin cyclic phosphorothionic acid by the action of certain nucleophils such as cyclohexylamine[28] and potassium dimethyl-dithiocarbamate[28,34]. The later 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. 4):

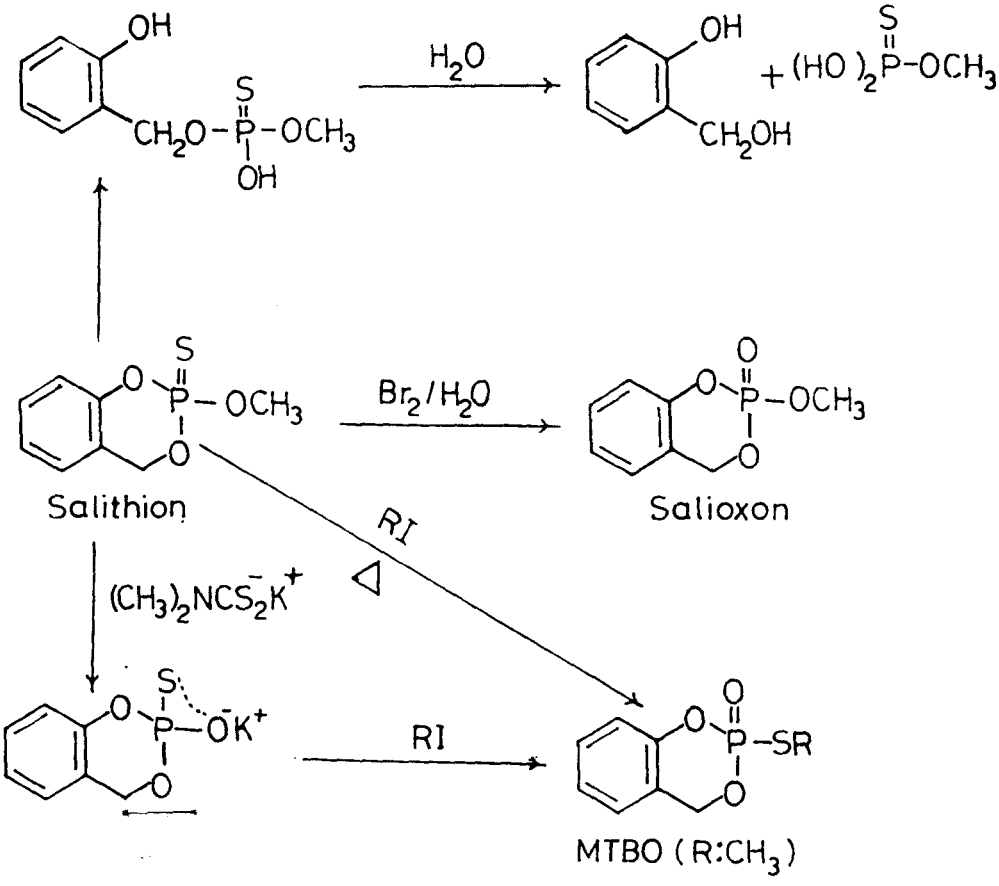


Fig. 4: Reaction of salithion.

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 stomach poisons but also as a fumigant[13]. Acute toxicity to mammals is mode-

rate. 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[35].

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[5]. It was shown that the biodegradation proceeds through demethylation and ring-opening by p-O-aryl-bond cleavage.

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 absorved in fertility of rats for three generations fed with 10 ppm salithion.

7. BIOLOGICAL ACTIVITIES AND STRUCTURAL RELATIONSHIP:

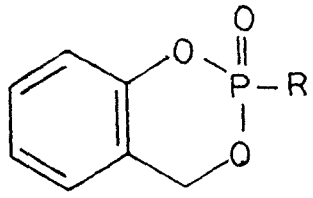
The Saligenin cyclic phosphate esters have interesting biological activities. Some of them are neurotoxic, causing ataxia in higher animals. Others do not show such harmful activity but do have high insecticidal activity, systemic activity and fungicidal activity. Their biological activities include also synergism with organophosphorus insecticides, nematocidal and antifilarial activity. The specificity in biological activities may be attributed to the steric effect of an exocyclic substituent group on the phosphorus atom as shown in Table II. All aryl saligenin cyclic phosphates manifest a high delayed neurotoxicity to cause ataxia in hens and high synergistic activity with malathion[20,41]. The aryl phosphate 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 weekly potentiated the toxicity of malathion[20]. The interesting feature is that, the alkyl derivatives reveal high insecticidal activity, whereas, the aryl cyclic esters donot[28].

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 Salicyloxy phosphinylenzymes (V)[21,22], Fig. 5. This involves by opening of the cyclic ester structure at the p-O aryl bond when the size of the exocyclic substituent (R in IV) increases, the ester becames a more selective inhibitor of aliesterase[43]. whereas, it becomes a more selective inhibitor of cholinesterase when the substituent is small. Thus the O-tolyl derivatives (M), for example, is 130 times more selective to inhibit aliesterase than cholinesterase.

Table II

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



(IV)

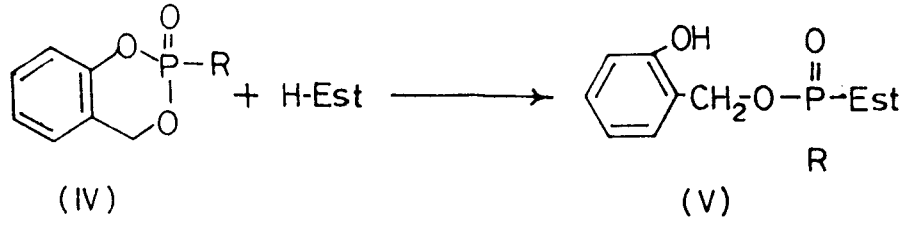
R	Delayed neurotoxicity MAD[a]	Synergism with malathion cotoxicity Coefficient.		Insecticidal activity
		Mice	Houseflies[b]	LD^{c}_{50}
$OCH_3-C_6H_4O$	2 - 5	16.7	7.8	(0)[d]
C_6H_5O	1.5-2	8.8	9.2	(3)[d]
C_6H_5	200	18.8	8.0	(0)[d]
C_2H_5	N.A.[e]	3.0	-	0.17
C_2H_5O	-	-	3.1	0.33
CH_3O	N.A.[e]	3.7	4.7	0.04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

a. Minimum atoxia dose for hens in mg/kg.

b. A resistant strain. c. 50% lethal dose by topical appplication to houseflies in μg/fly.

d. Percentage mortality at 10 μg/fly.

e. No ataxia signs evident with any sublethal dosages.



(IV) (V)

Fig. 5: Reaction of Saligenin cyclic phosphates with esterases (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 neucleophiles including the active site of esterase and is never requirement for the delayed neurotoxicity. As for example although Tri-o-ethyl phenyl phosphate (TEPP) has the neurotoxicity[19].

Johnson found "neurotoxic esterase" in nervous tissues which is specifically sensitive <u>invivo</u> to neurotoxic organophosphorus esters[45]. The esterase is unlike acetycholinesterase but similar to chymotrypsin and trypsin in the structure activity relationship of inhibitors[46].

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.

with this brief background of the relation of chemical structure to the biological activity of saligenin cyclic phosphorus esters, we will now discuss the specific activities such as insecticidal, synergistic, antiesterase, nematocidal, fungicidal etc.

7. (a) INSECTICIDAL ACTIVITY

Saligenin cyclic methyl phosphate (Table III), methyl phosphorothionate (Table IV), N-methyl phosphoramidothionate (Table V) are potent insecticides.

It is interesting to note that the exocyclic substituent of the most active cyclic phosphorus ester in each series (OCH_3, SCH_3, $NHCH_3$, 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 subsitituent at any position of the benzene ring and on the carbon atom of the hetero ring decreases the activity[50] (Table VI). The P-O-C aryl bond of the hetero ring of saligenin cyclic phosphorus esters appear to be active enough to phosphorylate cholinesterase to kill insects without any electron - withdrawing group.

7. (b) FUNGICIDAL ACTIVITY (Table VII and VIII, Page 41-42)

Salithion has no fungicidal activity, but some saligenin cyclic phosphorathiolates have fungicidal activity (Table VIII). These phosphorothiolate esters, particularly having an S-benzyl ester linkage, have activity to protect the rice plant from rice blast disease caused by the infection of Pyricularia oryzae[44]. The protective values against pyricularia oryzae of the cyclic phosphorothiolates and related compounds are shown in Table (VIII and IX). The data of some commercial fungicides including an organophosphorus compound, Hinosan (O-ethyl S, S diphenyl phosphorodithioate) are shown in Table (VIII) for comparison. 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 as insecticide but are almost in active as fungicide. In the series of dialkyl benzyl esters of phosphorus acids, only S-benzyl phosphorothiolates are highly active as fungicide but the others e.g. phosphates, phosphorothionates and phosphorodithionates are inactive[44].

It is important to note that some cyclic phosphorothiolates have both the high insecticidal as well as fungicidal activity, with only exception in the case of S-benzyl-O-O-diethyl phosphorothiolate (kitazin) which has weak insecticidal property but is a good fungicide

and now used in practice for the control of rice blast disease.

7. (c) ANTI-SH ENZYME ACTIVITY (Table VIII, page 42)

The saligenin cyclic phosphorothiolates have high activity to alkylate (Salicylate) mercaptans and to inhibit "SH-enzymes" such as yeast alcohol dehydrogenase[45]. 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 esters are shown in (Table VIII). 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 esterases. Salithion i.e., methyl phosphorothionate which have insecticidal property is almost inactive towards the enzyme.

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

The reaction mechanism is shown in Fig. 6.

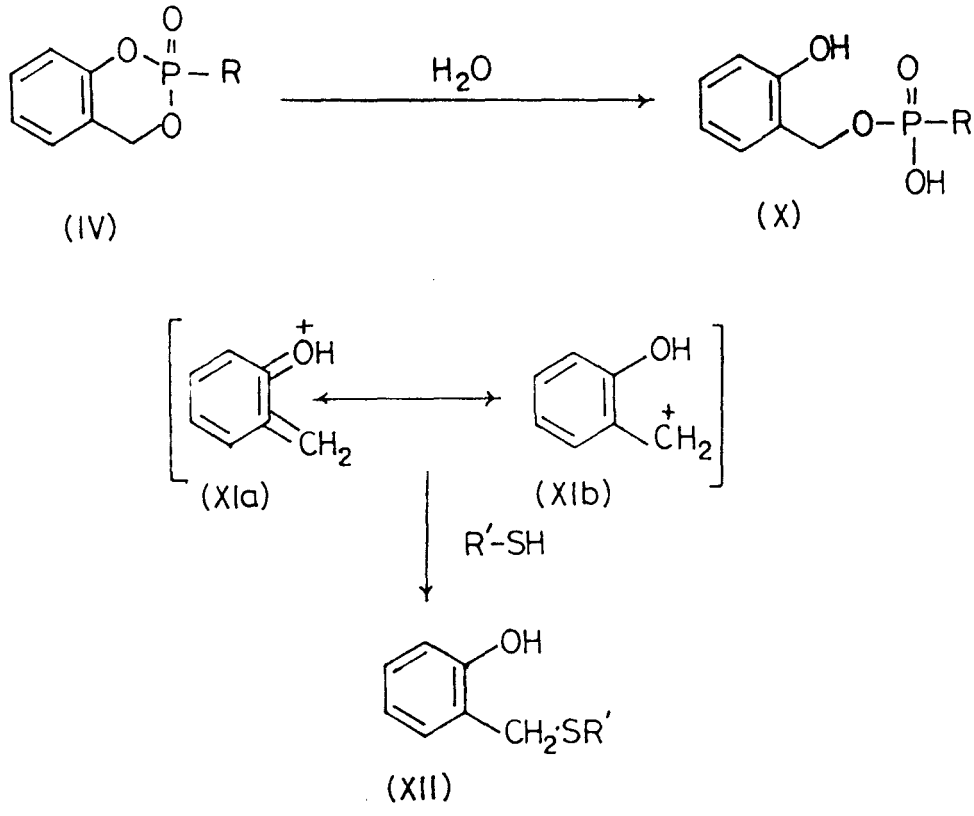


Fig. 6.

Saligenin cyclic phosphorothiolates are partially hydrolysed by opening of the heterocyclic P-O-C-aryl bond, more easily than phosphate esters. In Fig.6, the cyclic ester (IV) is hydrolysed by the attack of hydroxide ion to yield salicyl phosphote (X). The electron releasing-OH group of (X) may convert to a carbonium ion (XIb) which may actually react with a SH group to give a thioester (XII).

Cyclic methyl and ethyl phosphorothiolates are much more active in hydrolysis, alkylation and the inhibition of "SH-enzyme activities than the corresponding cyclic phosphates[45].

It seems reasonable to conclude that the decrease of electron density on phosphorus atom causes the high reactivity of the phosphorothiolates. This is supporting 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 VIII) 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.

7. (d) ANTIESTERASE ACTIVITY (Table IX, page 43)

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[5,43]. The

less neurotoxic aryl phosphonate 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 (IX). Aryl phosphonates are more specific inhibitors of pseudo-cholinesterase; alkyl phosphates are less specific and aryl phosphate are intermediate.

7. (e) SYNERGISTIC ACTIVITY

Saligenin cyclic aryl phosphates and phosphonates have synergistic activity with malathion against insects and mites, particularly their resistant strain[23].

The joint action of the activity of some saligenin cyclic phosphorus esters with malathion has been examined by Eto, Oshima, Kitakato, Tanaka and Kojima[41], 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.

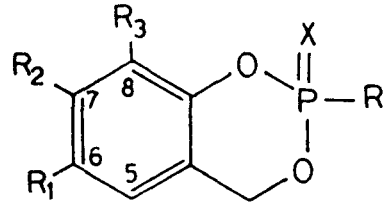
It has been observed that for a large number of organophosphorus compounds the synergism of malathion in mice and the degree of inhibition of ali-esterase invivo are generally related[20]. For insects, high

esterase activity hydrolysing malathion is supposed to be partly responsible for malathion-resistance in some strains of mosquito, housefly and green rice leaf hopper[41].

Eto et al[43] have shown that aryl derivatives of saligenin cyclic phosphorus esters are the selective inhibitors of alies terase, whereas small alkyl derivatives are not so selective to aliesterase inhibition. This appears to be responsible for their difference in synergistic properties.

Table III

Insecticidal activity of Ring-substituted Saligenin Cyclic Phosphorus Esters (Oxon-compounds):



X	R_1	R_2	R_3	R	LD_{50} (μg/housefly)
O	CH_3	H	H	OCH_3	0.1
O	CH_3	H	H	OC_2H_5	0.4
O	H	CH_3	H	OCH_3	0.43
O	H	CH_3	H	OC_2H_5	0.70

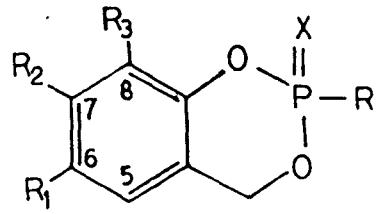
Contd.....

(Table III....Contd....)

X	R_1	R_2	R_3	R	LD_{50} (μg/house-fly)
O	H	CH_3	H	O-n-C_3H_7	7.2
O	H	CH_3	H	C_6H_5	10
O	H	CH_3	H	$NHCH_3$	0.14
O	H	H	CH_3	OCH_3	2.0
O	H	H	CH_3	OC_2H_5	2.1
O	H	H	CH_3	OC_6H_5	>10
O	Cl	H	H	OCH_3	0.09
O	Cl	H	H	OC_2H_5	0.13
O	Cl	H	H	O-n-C_3H_7	0.70
O	Cl	H	H	O-n-C_4H_9	2.5
O	Cl	H	H	OC_6H_5	> 10
O	Cl	H	H	$NHCH_3$	0.09
O	H	H	Cl	OCH_3	0.23
O	H	H	Cl	OC_2H_5	0.15
O	H	H	Cl	O-n-C_3H_7	0.30
O	H	H	Cl	O-i-C_3H_7	-
O	H	H	Cl	OC_6H_5	>10
O	H	H	Cl	$NHCH_3$	0.30

Table IV

Insecticidal activity of Ring-substituted saligenin cyclic phosphorus esters (thiono-compounds):



S	R_1 (6)	R_2 (7)	R_3 (8)	R	LD_{50} (μg/housefly)
S	CH_3	H	H	OCH_3	2.0
S	CH_3	H	H	OC_2H_5	>10
S	H	CH_3	H	OCH_3	0.23
S	H	CH_3	H	OC_2H_5	3.0
S	H	CH_3	H	O-n-C_3H_7	7.5
S	H	H	CH_3	OCH_3	1.3
S	H	H	CH_3	OC_2H_5	3.0
S	H	H	CH_3	O-n-C_3H_7	7.5
S	H	H	CH_3	$NHCH_3$	3.6
S	C_6H_5	H	H	OCH_3	0.4
S	C_6H_5	H	H	OC_2H_5	0.5
S	C_6H_5	H	H	O-n-C_3H_7	1.0
S	OCH_3	H	H	OCH_3	0.55
S	$COCH_3$	H	H	OCH_3	2.5
S	Cl	H	H	OCH_3	1.75
S	Cl	H	H	$NHCH_3$	0.06

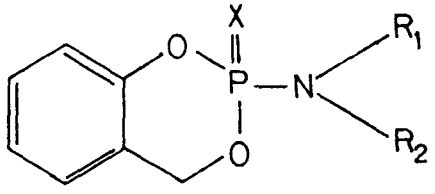
Contd.....

(Table IV contd....)

X	R_1	R_2	R_3	R	LD_{50} (µg/house-fly)
S	Cl	H	H	SCH_3	-
S	H	H	Cl	OCH_3	0.13
S	H	H	Cl	$NHCH_3$	0.09
S	H	H	Cl	SCH_3	-
S	Cl	H	C_6H_5	OCH_3	1.2
S	Cl	H	C_6H_5	OC_2H_5	3.0
S	Cl	H	C_6H_5	O-n-C_3H_7	>10
S	NO_2	H	H	OCH_3	3.0
S	C_6H_5	H	Cl	OCH_3	-
S	C_6H_5	H	Cl	OC_2H_5	-
S	C_6H_5	H	Cl	O-n-C_3H_7	-
S	Cl	H	Cl	OCH_3	0.3
S	Cl	H	Cl	OC_2H_5	4.0
S	Cl	H	Cl	$NHCH_3$	3.0

Table V

Saligenin cyclic phosphoramidates and phosphoramido thionates: Insecticidal activity, toxicity:



X	-N(R1)(R2)	LD_{50} (μg/mg Rice stem-borer)	LD_{50} μg/gm (Green rice leaf hopper)	LD_{50} (μg/female housefly)	LD_{50} mg/kg (mouse)
0	NHMe	2.84	0.04	0.05	5 - 7.5
0	NHEt	22.29	3.50	0.60	30 - 50
0	NH-N-Pr	33.60	33.0	1.50	>50
0	NH-i-Pr	103.34	>350	3.44	>50
0	NH-n-Bu	>214	>400	<10(54%)	>50
0	NH-ph	-	-	>10(5%)	-
0	$N(Me)_2$	13.80	4-0	0.3	-
0	$N(Et)_2$	167.80	34.10	>10(0%)	>50

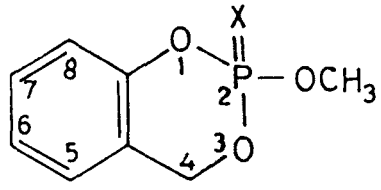
contd.....

(Table V contd.....)

X	$N\langle^{R_1}_{R_2}$	LD_{50} (μg/mg Rice stem-barer)	LD_{50} μg/gm (Green rice leaf hopper)	LD_{50} (μg/female house-fly)	LD_{50} mg/kg (Mouse)
S	NHMe	4.84	4.1	0.044	20 - 30
S	NH.Et	36.25	-	0.48	-
S	$N(Me)_2$	-	-	0.38	-
S	$N(Et)_2$	-	-	0.63	-
S	OMe (Salithion)	1.13	30.6	0.05	88
O	OMe (Salioxon)	2.16	1.8	0.035	52
Parathion		3.43	3.6	0.040	5 -7
Malathion		-	0.8	0.060	347
D-D mixture (mixture of 1.3 - diehloro-propane and 1,2-dichloropropane)		-	-	-	-

Table VI

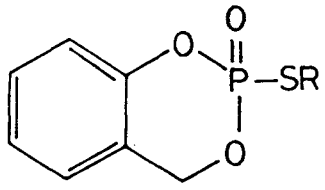
Effects of Substituent (R) on insecticidal activity(LD_{50} mg/H.Fly)



R	X	
	S	O
H	0.05 (Salithion)	0.035 (Salioxon)
4 - CH_3	-	3.35
6 - CH_3	2.00	0.1
7 - CH_3	0.23	0.43
8 - CH_3	1.30	2.0
6 - Cl	1.75	0.09
8 - Cl	0.13	0.23
β - CH_3	0.30	0.33
β - CH_3OH_2	3.55	0.99
β - Cl	-	2.07

Table VII

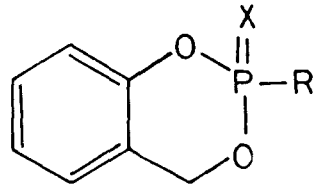
Insecticidal and Fungicidal activity of Saligenin cyclic Phosphorothiolates:



R	LD_{50} μg/ml (oriental housefly)	Protective value % against *Pyricularia oryzae*				Therapeutic value (%) to *P. oryzae* at 200 PPM
		200 ppm	100ppm	50ppm	25 ppm	
CH_3(MRBO)	3.00	100	100	100	84.8	7.1
C_2H_5	11.21	100	93.7	92.5	81.5	100
n-C_3H_7	94.50	100	57.1	34	-	-
i-C_3H_7	17.23	-	68.7	34.4	-	-
n-C_4H_9	211.8	100	91.7	93.3	75.6	97.6
C_6H_5	73.61	50.2	-	-	-	-
Salithion	1.60	52(at 500 ppm)	-	-	-	-
Hinosan	—	100	-	86.2	-	95.2 (at 250ppm)
Blasticidins	—	-	-	86.3	-	97.6
Pentachloro-benzyl alcohol	—	98.8	98.8	93.5	-	0.

Table VIII

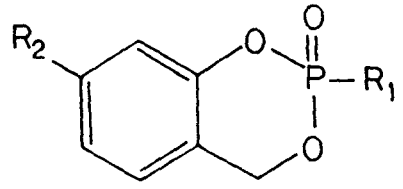
Chemical, Biological and Anti-Fungal Activities of Some Saligenin Cyclic Phosphotes and Their Thio Analogs:



X	R	Hydrolysis %	Cysteine reacted %	I_{50} yeast alcohol dehydronase ($Mx10^{-5}$)	Protective value against Pyricularia 50 ppm	Protective value against oryzae % 500ppm
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 IX

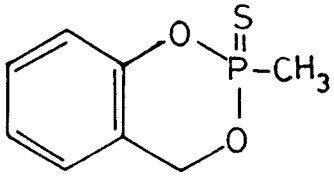
The inhibition of Housefly, Human Blood and Horse Serum-Esterases by some Saligenin cyclic Phosphorus compounds:



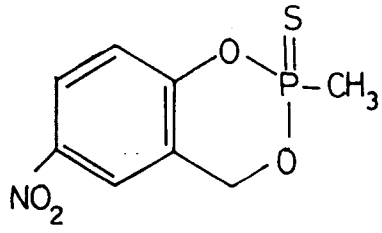
R_1	R_2	Housefly $I_{50} \times 10^8$(M)		Human Blood $I_{50} \times 10^8$(M)		Horse Serum $I_{50} \times 10^8$(M)	
		ChE	AliE	P-ChE	t-ChE	AliE	Malathionase
OCH_3	H (Salioxon)	7.6	8.4	1.8	17.0	230	620
OC_2H_5	H	13.2	2.1	1.6	25.0	240	-
O-n-C_3H_7	H	50.7	3.0	-	-	-	-
O-n-C_4H_9	H	37.5	2.3	-	-	-	-
OC_6H_5	H	-	-	0.5	12.0	120	120
C_6H_5	H	-	-	0.65	72.0	180	470
C_6H_5	CH_3	-	-	1.6	68.0	230	-
OPh-2-CH_3	H	-	-	1.3	39.0	200	-

8. AIMS AND OBJECTIVES OF THE PRESENT INVESTIGATION:

As stated previously (in part I) the cyclic organophosphorus esters of saligenin were discovered as the biologically active metabelites of tri-ortho-tolyl phosphates; many related compounds have been synthesized to study their chemical, biochemical and biological properties. Salithion (2-methoxy-4H-1,3,2-benzodioxaphosphorin 2-sulphide) is now commerciali ed as an insecticide. The introduction of any type of substituent at any position of the benzene ring decreases the insecticidal activity[50] It has been reported[50] that 2-methoxy-6-nitro-4H-1,3, 2-benzodioxaphosphorin 2-sulphide (BD-8) is obtained as a paste in the reaction of 2-hydroxy-6-nitro benzyl chloride with methylphosphorodichloridothionate. This methoxy compound has about sixty times less insecticidal activity compared to salithion[50]. However, it has been observed by Das[47, 48] that the methoxy compound (BD-8) is a solid (m.p. 84°C), and has about 1.5 to 2 times greater insecticidal activity against cockroach, Periplaneta americana (Linn) compared to salithion; it also decomposes more easily than salithion keeping less residues in the environment[47,48].

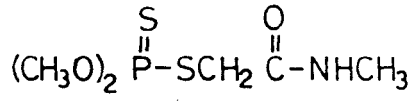


Salithion

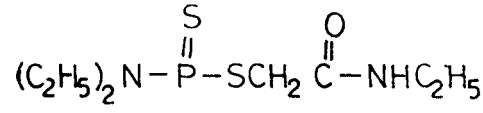


BD-8(m.p. 84°C)

Moreover, introduction of an amido group in place of an alkyl ester group often affords organophosphorus esters with fungicidal activity[9]. For example, although the insecticide dimethoate dimethyl S-(N-methyl-carbamoyl-methyl) phosphorothiolothionate has no fungicidal activity, its dialkylphosphoramidothiolothionate analogs, such as the compound V show some fungicidal as well as acaricidal activity[49]



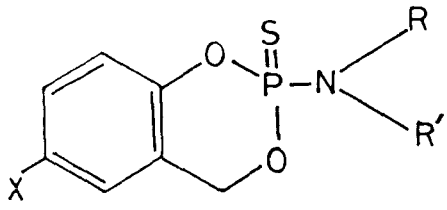
(Dimethoate)



(V)

There are several other examples in literature which clearly show that some phosphoramidothionates, phosphoramidothiolothionates, phosphoramides or phosphonamides in which the phosphorus atom is attached directly to the nitrogen atom of an amine or a hetarocyclic compound such as phthalimido, imidazole or triazole, have very good fungicidal activity[4,5,9].

These observations prompted me to undertake a systematic work on some 2-alkylamido-6-chloro/bromo/nitro-4H-1,3,2- benzodioxaphosphorin 2-sulphides having general structure (VI),



(VI)

where X = Cl/Br/No_2 and $-N\begin{matrix}R\\R'\end{matrix}$ is,

N-N-diisobutylamido,2,6-dimethylmorpholino or hexamethylenimido when X = Cl/Br/No_2. The work embodied in this dissertation is related to the investigation of the above mentioned compounds with reference to their chemical, pesticidal and toxicological properties besides structure elucidation by spectroscopic methods.

8. ACTUAL WORK:

i) Some new organophosphorus compounds (chloro/bromo/nitro saligenin cyclic phosphoramidothionates) mentioned above have been synthesised.

ii) The structures of all compounds have been established by chemical analysis, UV, Mass, IR and ^1H NMR spectral data.

iii) Insecticidal activities:

Insecticidal activities of these compounds against cockroach, periplaneta americana have been studied.

iv) Toxicological properties:

Acute oral toxicity on white albino rats and phytotoxic properties on the germination of rice seed (oryza sativa) have been studied.

v) Anticholinesterase activity:

Inhibition of the acetylcholinesterase activity in goat plasma have been studied.

vi) Hydrolytic properties:

Chemical hydrolysis of these compounds in alkaline PH have been studied.

vii) Antifungal activity:

Antifungal activities of these compounds against P. Oryzae, H. Oryzae, A.Solani and A.Candida by (growth inhibition method) and A.niger, P.oryzae and H.Oryzae,(by spore germination method) have been studied. An attempt has been made to study the quantitative structure activity relationship (QSAR).

viii) Toxic effect of some pesticides on algae:

Toxic effect of some commercial pesticides on Spirogyra sp., their degradation period and residue in L. river water by chlorophyllassaymethod have been studied.

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