CHAPTER 3

BIOLOGICAL ACTIVITIES AND CHEMICAL HYDROLYSIS OF SOME 2-ALKYLCARBAMOYL-6-CHLORO GALLOCYANIN CYCLIC PHOSPHOROTHIOLATES

MATERIALS AND METHODS

3.1.1. ORAL INSECTICIDAL ACTIVITY ON COCKROACHES

Insecticidal tests were performed on the American cockroach, Periplaneta americana (Linn) according to Busvine (16) with minor modifications. Adults of P. americana weighing about 1 g to 1.2 g were collected in the month of May-June, 1987 from a particular location of North Bengal University campus. In the field they were never exposed to any organophosphorus insecticides. For preliminary experiment ten cockroaches in each pot were exposed to hours the mortality was determined and the approximate minimum concentration required for 100% mortality (LC_{100}, μg/g basis) were found out.

To determine more precise LC_{100} (the minimum concentration required for 100 per cent mortality) value of each compound, one cockroach of known weight, in each pot was exposed to known quantity of the phosphoramidothionates progressively increasing its concentration by 2μ g/g ; for salithion and the methoxy compound (MD-8) the concentration was increased by 1μ g/g. Each experiment was duplicated and the average LC_{100} value was found out by using the simple arithmatic procedure(17). Before concluding the experiment on them, the cockroaches were kept starved for 24 hrs. However, the varying susceptibility of male and female cockroaches to different compounds were ignored during the experiment.

3.1.2. ORAL INSECTICIDAL ACTIVITY ON BLOWFLY

Blow-fly (Chrysomya megacephala) was selected for oral toxicity tests. The average body weight of each blow-fly was 18 mg. Two sets of blow-fly were treated with two different dosages of the different compounds. Required quantity of ethanol solution of the compound was smeared uniformly with the mango-juice and was exposed to blow-fly. For each set twenty blowflies were separately treated in this manner. After 24 h the mortality was determined and LD_{50}(μg/blow-fly) was found out.

3.2. ACUTE ORAL TOXICITY ON RATS

Oral toxicity testing was conducted on 6-12 months old male white albino rats, weighing 140-200 g, each housed in separate compartments of a cage. All animals had free access to food and water. Different dosages of a compound were mixed with boiled fish and given to the animals at their habitual[19] feeding time. The mortality within 48 h were recorded along with the toxic symptoms. Acute oral toxic dosage was found out by varying the amount of compound proportionately. The negligible amount of compound wasted by the animals during dieting was roughly accounted for determining the dosage.

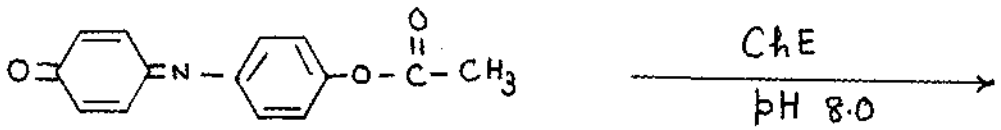
3.3. ANTICHOLINESTERASE ACTIVITY

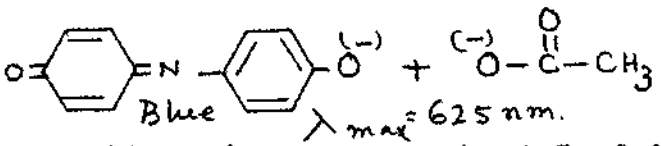
The organophosphorus compounds have a common pharmacological property which is the ability to inhibit the activity of a

group of enzymes, especially the anticholinesterase (AChE), involved in the hydrolysis of esters of choline. Since these enzymes present widely in insects and mammals, many of the organophorus compounds, used as insecticides also exhibit high mammalian toxicity. There are four major methods for assaying cholinesterase activity and effect of the inhibitor. The methods are potentiometric, manometric, and chlorimetric.

3.3.1. ANTICHOLINESTERASE ACTIVITY IN BLOW-FLY HEAD HOMOGENATE

The acetylcholinesterase inhibition of blow-fly head brie (BFAChE) had been measured by colorimetric method of Kramer and Gamson (1949) using indophenyl acetate as an internal substrate-indicator in 0.05 M phosphate buffer of pH 8.0. The enzymatic reaction for indophenyl acetate is as follows :





The reaction mixture contained 5 ml buffer containing blow fly head brie (5 ml of the solution contained 1 fly head and 0.15 ml indophenylacetate (total volume = 5.15 ml, concentration of indophenyl acetate $\sim 10^{-5}$). The absorbance of control and sample were taken at wavelength 625 nm after exactly 30 mins. incubation.

Reagents :

(i) Buffer solution : (0.05M Potassium dihydrogen phosphate) Clark & Lubs buffer of PH 8.0; 6.8 g KH_2PO_4 dissolved in 500 ml of water was mixed with 475 ml of 0.1(N)NaOH solution and diluted to 1 litre after pH was adjusted to 8.0.

(ii) Indophenyl acetate : working solution :

0.008 g of indophenylacetate when dissolved in 10 ml of absolute ethyl alcohol gave a 3.3×10^{-3} (M) solution so that the final concentration of indophenyl acetate in the reaction vessel was always 9.6×10^{-5}(M).

(iii) Glycerol solution : 10 ml of Glycerol was diluted to 100 ml with absolute alcohol.

(iv) Saline solution : 9 g of NaCl was dissolved in 1 litre distilled water.

(v) Salt Solution : 2.03 g of Manganous chloride and 2.15 g of NaCl were dissolved in in 250 ml of water.

(vi) Preparation of working solution of acetylcholinesterase from blow-fly heads.

About 500 blow-fly (Chrysomaya megacephala) closed in a glass vessel were stored in a deep freeze for 1-2 h. They were then transferred in a container dicapitated with a shaving blade and forceps. 250 heads were combined with 2 ml of salt solution and 2 g of washed sand in a prechilled size no.1 mortar.

The heads were slowly ground, then transferred to 50 ml plastic centrifuge tube with one 3 ml aliqot of cold saline solution and two 5 ml aliqots of buffer solution. The head fragments were removed by centrifugation for 10 mins. at 10,000 r.p.m. in superspeed centrifuge at 4°C. The supernatant liquid was decanted into a graduated cylinder and the fragmented heads were mixed with 10 ml buffer solution and centrifuged again at 10,000 r.p.m. This extraction procedure was repeated twice. The super-entanant solutions were combined and the volume was adjusted to 250 ml with buffer solution so that each ml was equivalent to 1 fly-head. This solution was stocked frozen in deep-freeze. One ml of this solution was diluted to 5 ml with buffer solution so that each 5 ml of the diluted solution contained single fly-head and used for each set of experiment.

Methods :

A series of 15 ml. pyrex beakers (numbered 1, 2, 3.... etc.) containing different amounts of inhibitors (different phosphoramidothioates) in acetone along with one marked 'control' without inhibitors were arranged. 0.5 ml of glycerol solution was poured in each of the remaining beakers including the 'control' the acetone (in the beaker 1, 2, 3.... etc.) was removed by blowing cold air. To the "control" 5 ml of working ensyme--buffer solution was added and simultaneously the stop-watch was started. At the interval of exactly 2 mins. 0.5 ml of the ensyme buffer solution was added to each of the remaining beaker.

After exactly 30 mins. 0.15 ml of the indophenylacetate solution (3.3×10^{-3}M) was added to the beaker marked "control" and subsequently to each beaker of the series at the interval of 2 mins. and then kept to be incubated at 30°C. After incubation for exactly 30 mins. the absorbance of control and remaining solution were successively noted in the spectrophotometer Shemadzu UV-240 at 625 nm with reference to enzyme buffer (reagent blank) solution.

Calculation

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

3.3.2. ANTICHOLINESTERASE ACTIVITY IN GOAT WHOLE BLOOD

The method employed to determine the inhibition of the activity of acetyl cholinesterase in goat whole blood by organophosphorus compounds was by colorimetric method of Kramer and Gamson (19), using indophenylacetate as an internal substrate indicator in 0.05M phosphate buffer of pH 8.0. The reaction mixture contained 5 ml of enzyme buffer solution (4.8 ml phosphate buffer solution along with 0.2 ml goat whole blood) and 0.15 ml indophenyl acetate (total volume = 5.15 ml, concentration of indophenylacetate in the reaction mixture $\sim 10^{-5}$M). The readings of "control" and "sample" were taken at 625 nm, after exactly 30 minutes incubation.

MATERIALS AND METHOD

150 ml fresh blood was collected from goat and mixed with 15 mg ammonium oxalate (anticoagulating agent) in a 250 ml standard joint bottle and was shaken well. The bottle containing blood was then kept in the freezer at 0°c.

The other reagents are phosphate buffer, indophenyl acetate and glycerol solution were prepared just as the same as mentioned in [3.3].

3.4. PHYTOTOXICITY TESTS

Phytotoxicity testing was conducted according to Eto et al(20). Acetone solution of the compounds mixed with fixed amount of water containing 0.1% Tween 80 was prepared 5 ml of this aqueous suspension containing 200, 100 and 50 ppm of the compounds was poured into a petridish bottom covered with absorbent cotton. Ten seeds of paddy (Pusa 2-21 variety supplied by Rice Research station, Chinsurah, West Bengal) were placed on the cotton and kept at room temperature (25-27°C) for six days.

Occasionally 2-4 ml water added in each petridishes so that the seeds remained in moist condition. Each test was triplicated. Number of germination was counted after 6 days.

3.5. CHEMICAL HYDROLYSIS

The chemical hydrolysis studies were performed in

0.0095(M) NaOH in 50 per cent ethanol of pH 11.85 at 30°C. 6-chlorosaligenin cyclic phosphoramidothionates on hydrolysis produced chlorosaligenin. The rate of hydrolysis was monitored by following the rate of formation of 5-Chloro-saligenin spectrophotometrically in a Shimadzu UV-240 instrument at the wave length λ = 292, 294, 296 and 298 nm. These wave lengths were chosen as the difference in optical densities of 5-chloro saligenin and any of the phosphoramidothionates at the same concentration was maximum at this region.

Determination of the hydrolysis constant 6-chloro saligenin cyclic phosphoramidothionates involve the following stages (i) Determination of the molar extinction coefficients (ϵ_2) of 5 chlorosaligenin in the alkali solution (0.0095 M) NaOH in 50% ethanol of pH 11.85).

Solution of 5 chloro-saligenin of known concentration in the alkali solution was made and the optical densities of the solution at λ = 292, 294, 296 and 298 nm were measured; from the values of the optical densities and concentration the molar extinction co-efficient (ϵ_2) of 5-chloro saligenin at λ = 292, 294, 296 and 298 nm were determined.

(ii) Determination of the molar extinction co-efficients (ϵ_1) of the 6-chloro-saligenin cyclic phosphoramidothionates in the alkali solution (0.0095 M NaOH in 50% ethanol of pH 11.85).

As the rate of hydrolysis of the 6-chloro-saligenin cyclic phosphoramidothionates were extremely slow in the alkali solution (0.0095 M NaOH in 50% ethanol of pH 11.85).

As the rate of hydrolysis of the 6-chloro-saligenin cyclic phosphoramidothionates were extremely slow in the alkali solution (pH 11.85) the optical densities (at λ = 292, 294 296, and 298 nm) of the alkali solution of any of the phosphoramidothionates was measured as soon as the alkali solution was added; the optical densities were assumed to be due to the phosphoramidothionates only. From these values of optical densities $(O.D.)_t$ and the concentration of the phosphoramidothionates, the molar extinction coefficients of the phophoramidothionates (ϵ_1) were determined.

(iii) Determination of the amount of the phosphoramidothionate (C_t) hydrolysed after a certain interval.

After 240 hours of the addition of the alkali solution the optical densities $(O.D.)_t$ of the mixture of unhydrolysed phosphorasaidothionates and the hydrolysed product were measured at λ= 292, 294, 296 and 298 nm.

The concentrations of the phosphoramidothionates hydrolysed (C_t) were calculated from the equation :

$$C_t = \frac{(O.D)_t - (O.D)_1}{\epsilon_2 - \epsilon_1}$$

(iv) Determination of the hydrolysis rate constant (K_{hyd}) of the

6-chloro-saligenin cyclic phosphoramidothionates.

Hydrolysis rate constants (K_{hyd}) were calculated from the 1st order rate equation

$$K_{hyd} = \frac{1}{t} \ln \frac{C_o}{C_o - C_t}$$

where

C_o = initial concentration of the 6-chloro saligenin cyclic phosphoramidothionates

C_t = Concentration of the compound hydrolyzed after time t.

3.6. FUNGICIDAL ACTIVITY

(a) Test Organism :

Pyricularia oryzae cav. - causal fungus of blast disease of rice

(b) Culture Medium :

Solid medium : Malt extract agar.

20 g malt (Difco) extract was boiled in water till dissolved. 20g agar (Kobe Japan) was added and boiled until agar was well dissolved. 0.05g chloramphenicol was suspended in 10 ml of 95% alcohol and added to the medium as antibacterial agent. The volume of the medium was then made upto 1 litre by

adding water. pH of the medium was adjusted to 6.5 by adding NaOH solution. Medium was sterilized at 15 p.s.i. for 20 mins.

(c) Growth Inhibition Method :

Growth inhibition studies were made by using "Poison food technique"(21) . Acetone solution of suitable quantity of the compounds in sterile water containing 0.1% Tween 80 was incorporated into melted malt agar so as to get the desired concentration of the compounds in the media. The test medium was poured into the sterile petridishes and after solidification the 7-8 days old culture disc was placed asceptically at the centre of the petridish. Three replications on each test with appropriate control under same conditions were maintained. These petridishes were incubated at $30^{\circ} \pm 1^{\circ}C$ in dark. Linear growth of the fungal disc was measured after 24, 48, 72 and 96 h interval. Per cent inhibition over control was calculated following the equation given by Vincent (1927) (22).

3.7 QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP :

The optimization of bioactivity in a class of pesticides by variation of the substitution pattern is one of the aims in pesticide design. A major contribution to a rational approach in this area was initiated by Hansch (23). An essential prerequisite of this quantitative approach to structure-activity relationships by regression analysis is the development of reliable substituent constants. In this dissertation I have undertaken a SAR study of a series of compounds using some molecular parameters such as partition co-efficient log P, Taft's steric constant Es, hydrophobic constant π and structural information content SIC of various molecules (24).

(i) Determination of first-order topological information content(-IC)

Studies on Drug/Pesticide design gained momentum in the recent years, and it has been observed that the utility of topological indices in Quantitative Structure Activity Relationship is indispensable. A topological index is ideally, a graph-theoretical invariant defined on the structure (Chemical-graph) of the molecule and is regarded as a 'quantitative descriptor of the general topological characteristics of the molecular structure. The sensitivity of the topological indices to structural alterations signifies the efficacy of such parameters in the quantitative correlation and prediction of the properties

of pesticidal agents arising out of molecular manipulations. Information theory has been applied to the total molecular graph to define two topological indices, information contents (IC) and structural information content (SIC) - which have been found to be well correlated with the biological activities of drugs/pesticides[25]. The detailed mathematical theory of the partitioning of the vertices (atoms) of a molecular graph to determine its embedded geometrical complexity is given in the following pages along with an illustrative example of the partitioning process.

Following the method of Sankar et al[26] an equivalence relation is defined on the set of vertices X(G) of a chemical graph G, representing a molecule. Let $A_1, A_2, \ldots\ldots A_k$ be a partition of X(G) and $P_1, P_2, \ldots\ldots P_k$ be their corresponding probabilities, then the probability scheme is given by

$$A_1, A_2, \ldots\ldots A_k$$

$$P_1, P_2, \ldots\ldots P_k$$

where

pi = ai/n and ai and n are the cardinalities of A_i and X(G) respectively. The information content IC [26] considering the class partition of vertices with respect to first order neighbour is given by Shannon's formula[27] :

$$IC\left[X(G)\right] = -\sum_{K} pi \log_2 pi \text{ bits}$$

for i=1 to i=K. The logarithm is taken at a base 2 to measure the information in bits.

The calculation of SIC of CL-12 (Fig) is given as an illustrative example of the partitioning process.

There are 37 vertices in the graph representing a molecule of the compound CL-12.

Out of the 37 vertices 17 represent hydrogen atoms, 1 represents nitrogen atom, 1 represents sulphur atom, 1 represents phosphorus atom, 3 represents oxygen atoms, 1 represents Chlorine atom and 13 represents carbon atoms.

In our formalism it was very difficult to distinguish hydrogen and halogen atoms with the help of a co-ordinate system which reflects the first order topological neighbourhood. So we have not distinguished between the two atoms.

Since the graph is connected, each vertex of this graph is incident with at least one vertex of it. Actually each vertex representing a hydrogen atom is incident with just one other vertex of the graph. We see from the graph that all the 17 vertices representing hydrogen atoms are such that all of them are incident with vertex representing carbon atom.

So the co-ordinate of each hydrogen is : (1^4). One vertex represents nitrogen atom. Valency of nitrogen is three.

The vertices are incident with two carbon atoms and one with phosphorus atom.

Therefore, the co-ordinates of this vertex is : $(1^5, 1^4, 1^4)$.

The valency of sulphur is two, and that of phosphorus is five. The vertex representing sulphur atom is incident with that of phosphorus atom by a double bond. So the co-ordinate is given as : (2^5)

The vertex representing that of phosphorus is incident with that of two oxygen atoms by two single bonds, a nitrogen by a single bond and to sulphur by a double bond. The co-ordinate is given as $(1^2, 2^2, 1^3, 1^2)$.

There are three vertices representing that of oxygen. Two are similar, which are incident with the vertex representing carbon atom and phosphorus atom. Another oxygen atom is incident with two carbon atoms by single bonds. Therefore, the co-ordinates of these three vertices are given as :

$(1^5, 1^4) \times 2$

and $(1^4, 1^4)$

The remaining fourteen vertices representing carbon atoms have distinct co-ordinates which are :

$(2^4, 1^4, 1^1) \times 4$

$(1^4, 2^4, 1^4) \times 1$

$(1^4, 2^4, 1^2) \times 1$

$(1^1, 1^1, 1^2, 1^4) \times 1$

$(1^3, 1^1, 1^1, 1^4) \times 2$

$(1^4, 1^1, 1^1, 1^4) \times 2$

and $(1^4, 1^1, 1^1, 1^1) \times 2$

Moreover there is one vertex representing Chlorine atom. The Chlorine atom is incident with a carbon atom. So the co-ordinate is given as : (1^4).

The following table gives a simplified version of the partitioned class and their probability.

Table 3.1

Partition class Number with co-ordinates	Number of atoms in the Partitioned Class	Probability pi
I (1^4)	17	17/37
II $(1^5, 1^4, 1^4)$	1	1/37
III (1^4)	1	1/37
IV (2^5)	1	1/37
V $(1^2, 1^2, 1^3, 1^2)$	1	1/37
VI $(1^5, 1^4)$	2	2/37

Partition Class Number with co-ordinates	Number of atoms in the partitioned class	Probability pi
VII $(1^4,1^4)$	1	1/37
VIII $(2^4,1^4,1^1)$	4	4/37
IX $(1^4,2^4,1^4)$	1	1/37
X $(1^4,2^4,1^2)$	1	1/37
XI $(1^1,1^1,1^2,1^4)$	1	1/37
XII $(1^3,1^1,1^1,1^4)$	2	2/37
XIII $(1^4,1^1,1^1,1^4)$	2	2/37
XIV $(1^4,1^1,1^1,1^1)$	2	2/37

Therefore,

$I'(C)$ = First order topological information Content of CL-12

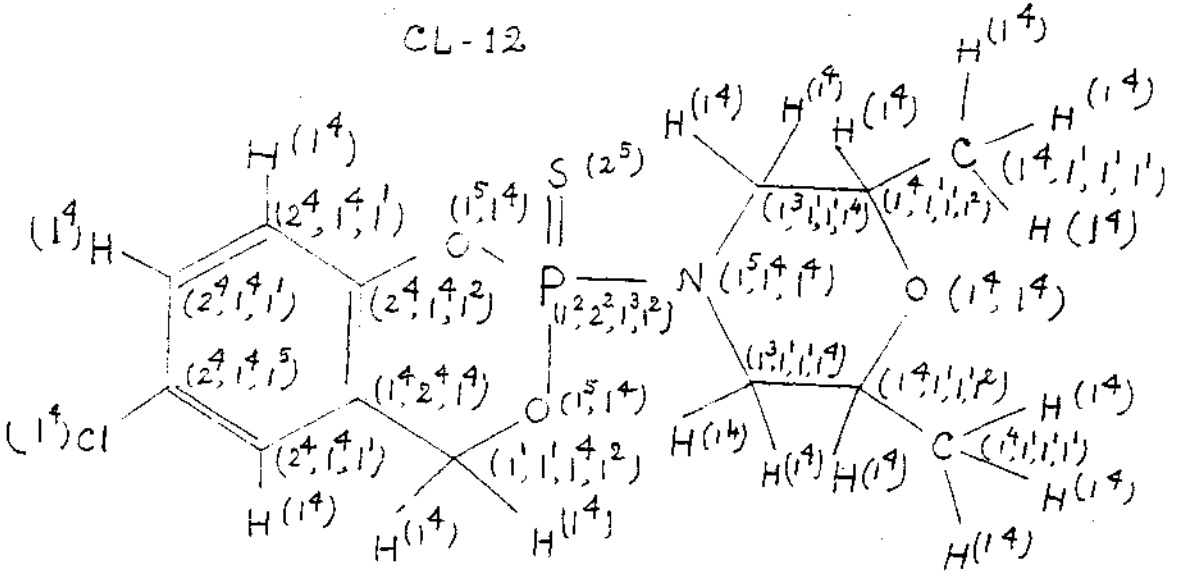
$= - 1X17/37 \log_2 17/37 + 4X2/37 \log_2 2/37 + 1X4/37 \log_2 4/37 + 8X1/37 \log_2 1/37$

$= - 17/37 \log_2 17 + 8/37 \log_2 2 + 4/37 \log_2 4 + \log_2 1/37$

$= 2.8994$ bits

If the maximum of all possible information contents of CL-12 corresponding to all possible modes partition of the set of vertices of the graph be represented by the symbol $I(C)$.

CL-12



H: $\{(1^4)\times 17\}$

N: $\{(1^{15}, 1^4, 1^4)\times 1\}$

Cl: $\{(1^4)\times 1\}$

S: $\{(2^5)\times 1\}$

P: $\{(1^2, 2^2, 1^3, 1^2)\times 1\}$

O: $\{(1^5, 1^4)\times 2\}$
$\{(1^4, 1^4)\times 1\}$

C: $\{(2^4, 1^4, 1^1)\times 4\}$; $\{(1^3, 1^1, 1^1, 1^4)\times 2\}$
$\{(1^4, 2^4, 1^4)\times 1\}$; $\{(1^4, 1^1, 1^1, 1^4)\times 2\}$
$\{(1^4, 2^4, 1^2)\times 1\}$; $\{(1^4, 1^1, 1^1, 1^1)\times 2\}$
$\{(1^1, 1^1, 1^2, 1^4)\times 1\}$

$$I'(A) = -\left\{1\times\frac{17}{37}\log_2\frac{17}{37} + 4\times\frac{2}{37}\log_2\frac{2}{37} + 1\times\frac{4}{37}\log_2\frac{4}{37} + 8\times\frac{1}{37}\log_2\frac{1}{37}\right\}$$

$$= -\left\{\frac{17}{37}\log_2 17 + \frac{8}{37}\log_2 2 + \frac{4}{37}\log_2 4 + \log_2\frac{1}{37}\right\}$$

= 2.8994 bits

$I(C) = -37 \times 1/37 \log_2 1/37$

$= \log_2 1/37$

$= 5.21$ bits

$\therefore \delta.I.C. = 2.8994/5.21$

$= 0.5565$

$R.N.\delta.I.C. = (1-\delta.I.C.)$

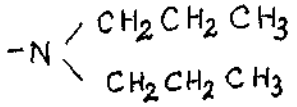
$= 0.4434$

The $\delta.I.C.$ of the other molecules have been calculated in the above method.

(ii) Determination of π and Es.

Hydrophobic constant (π) has been estimated from the fragment values developed by Leo and Hansch (23).

An illustrative example for the estimation of value is given below. For the compound CL-7 the substituent taken is dipropylamido



Thus, when the π value of

$-CH_3$, 0.89; $-CH_2$, 0.66; chain length, $-0.12 \times n-1$;

$= 2(-CH_3) + 4(CH_2) - 0.12 \ldots 4$

$= 2(0.89) + 4(0.66) - 0.48$

$= 3.94$

The (π) values for the various other molecules have been calculated in the above way.

Taft's steric constants (Es) have been obtained from the Pomona Medicinal Chemistry Data Base[23].

(iii) Determination of partition coefficient

Partition coefficients for all these compounds were determined experimentally. The compounds (2 mg) were partitioned between 1-Octanol (10 ml) and water (100 ml) on a mechanical shaker till the equilibrium was reached (12 h). The octanol layer was separated and centrifuged at 1000 rpm for 30 mins. and the concentration of the compounds in octanol layer determined before and after partition by measuring absorbance at 279 nm on a Shimadzu UV-240 spectrophotometer from the standard curve. The partition was calculated as P = (C)octanol/(C) water.

3.8 RESULTS AND DISCUSSION

3.8.1 INSECTICIDAL ACTIVITY AGAINST COCKROACHES

All the organophosphorus compounds have been evaluated for their oral insecticidal activity against cockroaches, P. americana (Linn) and mortality have been observed after 24h and are listed in table 3.2. The results have been compared with malathion and 2-methoxy-4-nitro-4h-1, 3, 2-benzodioxaphosphorin 2-sulphide (MD-8).

Table 3.2

Insecticidal activity against cockroaches (P. americana)

Compound Code No.	Conc. showing 100% mortality (LC_{100}) µg/g
CL-6	> 100
CL-7	> 50
CL-10	> 100
CL-12	10
CL-14	> 100
CL-15	> 75
CL-17	> 75
MD-8	6
Malathion	10

The data presented in table 3.2 reveal that all phosphoramidothionates have less oral insecticidal activity

except CL-12 than ED-8 and salithion. CL-12 compound has similar insecticidal activity to salithion.

3.8.2 INSECTICIDAL ACTIVITY AGAINST BLOW-FLY

All the organophosphorus compounds have been evaluated for their oral insecticidal activity against blow-fly (Chrysomaya megacephala) and mortality have been observed after 24 h and are listed in table 3.3 . The results have been compared with salithion and ED-8.

Table 3.3

Insecticidal activity against blow-fly (Chrysomaya megacephala)

Compound Code No.	LD_{50} (μg/blow-fly)
CL-6	> 10
CL-7	> 10
CL-10	> 10
CL-12	> 10
CL-14	> 10
CL-15	> 10
CL-17	> 10
ED-8	> 10
Salithion	0.05

The data presented in table 3.3 reveal that all phosphoramidothionates have less oral insecticidal activity than salithion.

3.8.3 ACUTE ORAL TOXICITY ON RATS

The acute oral toxicity data (LD_{50}) of the chloro-saligenin cyclic phosphoramidothionates on male white albino rats are presented in table 3.4 and the results have been compared with salithion and 2-methoxy-6-nitro-4H-1, 3, 2--benzodioxaphosphorin 2-sulphide (MO-8).

Table 3.4

Compound Code No.	LD_{50} (mg/kg) Male rat
CL-6	>250
CL-7	> 250
CL-10	> 250
CL-12	>250
CL-14	> 250
CL-15	> 250
CL-17	> 250
MO-8	120-135
Salithion	>120

The data presented in the table 3.4 show that all the compound is less toxic than salithion. Before death the rats were found to suffer from acute respiratory trouble. In some cases a fluid with blood-stain oozed out of nostrils and eyes of the animals. In all cases, the decrease of spontaneous motor activity occurred

after 2-4h salivation and irregular respiration were observed.

The LD_{50} values given here are only after preliminary experiment and this requires further work for accurate LD_{50} value determination. However, the LD_{50} values given are appreciably fair to enable one to judge the relative toxicity of the compounds to male white albino rats.

3.8.4 ANTICHOLINESTERASE ACTIVITY

The anticholinesterase activity data (molar I_{50}) of 6-chloro saligenin cyclic phosphoramidothionates for blow-fly head homogenate (BFAChE) and goat whole blood (AChE)* are summarized in table 3.5 .

Table 3.5

Compound Code No.	I_{50}(M) x 10^3 (BFAChE) blow-fly	I_{50}(M) x 10^3 (AChE) goat whole blood
CL-6	90.37	4.25
CL-7	0.99	2.19
CL-10	9.40	4.13
CL-12	0.20	2.07
CL-14	322.0	21.50
CL-15	18.95	0.36
CL-17	207.0	5.11

* Ref. Exp 1 - 14

Due to very low solubility of the 6-chloro-saligenin cyclic phosphoramidothionates the actual I_{50} values of the

compounds could not be determined accurately. As the reaction medium becomes turbid at a concentration above 10^{-5}M and the optical density values become inconsistent, the highest concentration used for the compounds is 10^{-5} M. Upto this concentration these compounds are very poor inhibitors of acetylcholinesterase (both in blow-fly and goat whole blood) and as a result the I_{50} values obtained from the regression analysis are of little importance.

3.8.5 PHYTOTOXIC PROPERTIES

The phytotoxicity data against rice (Pusa 2-21 variety) supplied from Rice Research Station, Chinsurah, West Bengal) for the phosphoramidothionates are listed in Table 3.6.

Table 3.6

The effect of phosphoramidothionates on germination of Rice seed (Pusa 2-21 Variety)

Compound Code No.	Per cent germination at different concentrations		
	500 ppm	250 ppm	100 ppm
CL-6	100	100	100
CL-7	100	100	100
CL-10	100	100	100
CL-12	100	100	100
CL-14	100	100	100
CL-15	100	100	100
CL-17	100	100	100

At 500, 250 and 100 ppm none of the compounds is phytotoxic to rice seeds (Pusa 2-21 variety).

3.8.6 CHEMICAL HYDROLYSIS

Rates of hydrolysis of 2-alkylamido-6-chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphides in ethanol-water(50%) in the presence of alkali (pH 11.85) have been determined by following the liberation of 2-hydroxy 5-chlorobenzylalcohol. The release is quantitative and shows strict first order kinetics dependence. The first order rate constants are proportional to base, the rate of neutral hydrolysis being quite negligible and the first order rate constants of different chloroaligenin cyclic phosphoramidothionates are summarised in table 3.7 .

Table 3.7

Alkaline hydrolysis, pH 11.85, 0.0095(M)NaOH (50% ethanol-water), Temperature 30°C *

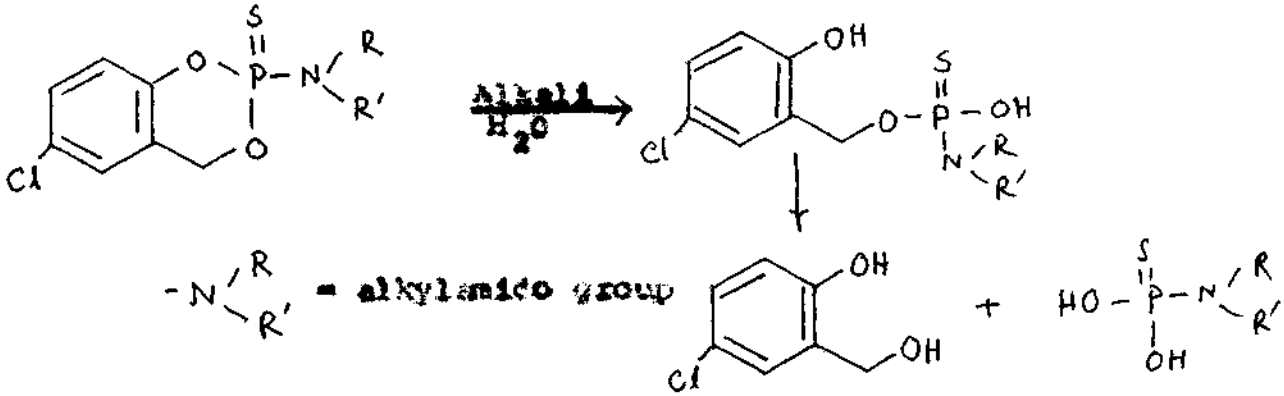
Compound Code No.	Average 10^{-5} K_{hyd} (min^{-1})	$T_{\frac{1}{2}}$ hrs.
CL-6	0.575	2008
CL-7	1.37	843
CL-10	0.885	1305
CL-12	4.06	284
CL-14	0.686	1552
CL-15	1.11	1034
CL-17	-	-

* Ref. Exp. 15-20.

The average hydrolysis constants recorded in table show that the nature of alkylamido group in the exocyclic side chain influences the stability of the compounds to alkaline hydrolysis.

The rate of hydrolysis increases in the following order CL-6 < CL-14 < CL-10 < CL-15 < CL-7 < CL-12. The 2,6 Dimethylmorpholine Compound (CL-12) is least stable and the Diisobutylamido compound (CL-6) is most stable towards hydrolysis (alkaline) and CL-17 compound does not undergo hydrolysis under these conditions.

With ethanol-NaOH solution (pH 11.85) under these conditions gave Chlorosaligenin and alkylamidothionates as the sole products, it is established that compounds are hydrolysed under the above condition exclusively by attack on phosphorus following the work of Eto (28). The proposed mechanism of the reaction is given below.



3.8.7 FUNGICIDAL ACTIVITY

Pyricularia oryzae

The data (% inhibition) of some 5-chloro saligenin cyclic phosphoramidothionates for *in vitro* growth inhibition

studies against *P. oryzae* (causal organism of blast disease of rice) are listed in Ex.R.21-27. The data for Hinosan have been presented in table 3.8&3.9, ED_{50} and ED_{95} (μg/ml) values calculated by least square regression analysis are given in table 3.8&3.9.

Table 3.8

Antifungal activity of the phosphoramidothionates against *P. oryzae* (Cav)

Compound Code No.	ED_{50} in μg/ml			
	48 hrs	72 hrs	96 hrs	120 hrs
CL-6	0.61	0.88	1.27	1.85
CL-7	0.52	0.91	1.78	2.95
CL-10	0.33	0.58	0.98	2.08
CL-12	0.32	0.38	0.48	0.85
CL-14	0.91	1.40	2.22	3.29
CL-15	1.12	2.00	2.59	3.66
CL-17	0.43	0.73	0.95	1.42
Hinosan (Standard)	<5.00	<5.00	<5.00	<5.00

Table 3.9

Antifungal activity of the phosphoramidothionates against *P. Oryzae* (Cav)

Compound Code No.	ED_{95} in µg/ml			
	48 hrs.	72 hrs.	96 hrs.	120 hrs.
CL-6	3.18	4.70	6.96	8.71
CL-7	2.95	6.20	13.51	16.65
CL-10	2.63	3.99	6.94	14.03
CL-12	1.56	2.64	3.78	5.57
CL-14	5.94	7.94	13.37	19.79
CL-15	7.91	12.03	14.43	25.03
CL-17	3.19	3.83	6.04	7.37
Hinosan	>5.00	>12.50	>25.00	>25.00
(Standard)	<12.50	<25.00	<50.00	<50.00

From the antifungal activity data it has been observed that 6-chlorosaligenin cyclic phosphoramidothionates show good inhibitory effect on the growth of *P.oryzae*.

For the 6-chloro saligenin cyclic phosphoramidothionates at 72 h the ED_{50} value increases in the following order :

CL-12 < CL-10 < CL-17 < CL-6 < CL-7 < CL-14 < CL-15 < Hinosan

i.e. CL-12 is most active and CL-15 is least active, their inhibitory effects are greater than that of Hinosan. At 72 h the ED_{95} value of the 6-chloro saligenin cyclic phosphoramidothionates increases in the following order :

CL-12 < CL-17 < CL-10 < CL-6 < CL-7 < CL-14 < CL-15 < Hinosan i.e. CL-12 is most active and the CL-15 is least active. The activity of CL-12 is about ten times greater than that of Hinosan.

To sum up 2-(2,6-Dimethyl morpholino-6-Chloro-4H-1,3,2 benzodioxaphosphorin 2-sulphide (CL-12) is most effective against P. oryzae.

In my present study I have tried to elucidate the structure - antifungal activity relationship of the twelve compounds against the fungus Pyricularia oryzae applying Hansch's method (23), using the different parameters such as hydrophobic (log P or π), Taft's steric constant (E_s) and structural information content (SIC).

For structure activity correlation, regression yields several equation presented in table 3.11 . The best fit Eq. is Eq.8.

$$p\,ED_{50} = -0.167\,\pi - 8.318\,SIC + 10.464$$
$$(\pm 0.070) \quad (\pm 2.260) \quad (\pm 1.431)$$
$$n = 12,\ r = 0.78\ s = 0.19\ F_{2,9}(calc) = 6.98$$
$$tab\ (0.05) = 4.3 \ldots\ldots \text{Eq.(8)}$$

In these equations, p ED_{50} represents - log ED_{50}(M), n is the number of compounds, s is the standard deviation, r is the correlation coefficient and F is the statistical measure of the significance of correlation. The figures in parenthesis are the 95% confidence intervals of the corresponding constants. The conclusion is as follows:

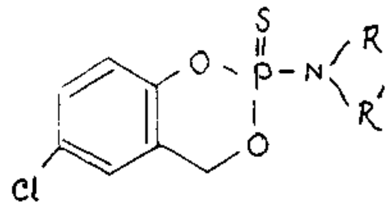
(i) None of the steric, ΔIC or hydrophobic parameters alone can account for the biological response (antifungal activity).

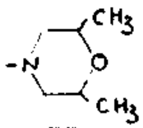
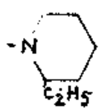
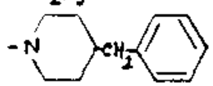
(ii) A combination of two or more parameters is always necessary indicating the involvement of more than one factor for the biological activity.

(iii) For *P. oryzae* the regression equation involving pID_{50} with π and ΔIC provides the best fit Equation 8 as judged by r, s and F values. Since π is a hydrophobic parameter and ΔIC is a topological steric parameter, it suggests that the stereo-hydrophobic make up and topology of the bioactive molecule are major determinants of the bioresponse.

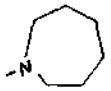
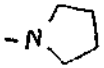
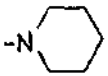
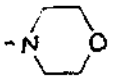
Table 3.10

Value of substituent constants and calculated and observed Fungicidal activity of the Compounds (*P. oryzae*)



Compound code No.	$-N\langle^{R}_{R'}$	π	E_s	log k	$(\log k)^2$	ΔIC	$(\Delta IC)^2$	π^2	$pED_{50}(M)$ (Cal)	$pED_{50}(M)$ (obs.)	Δ pED_{50}
CL-6	$-N(CH_2CH(CH_3)(CH_3))_2$	4.76	-4.34	1.43	2.04	0.47	0.22	22.65	5.75	5.61	-0.14
CL-7	$-N(CH_2CH_2CH_3)_2$	3.94	-3.20	0.98	0.96	0.51	0.26	15.52	5.56	5.54	-0.02
CL-10	$-N(CH_2(CH_2)_2CH_3)_2$	5.02	-3.26	1.24	1.54	0.47	0.22	25.20	5.72	5.70	-0.02
CL-12		2.51	-4.58	1.37	1.87	0.55	0.30	6.30	5.47	5.82	+0.35
CL-14		4.76	-3.41	1.27	1.61	0.52	0.27	22.65	5.34	5.37	+0.03
CL-15		6.45	-4.20	0.97	0.94	0.49	0.24	41.60	5.31	5.29	0.02

....contd...

Compound Code No.	$-N\begin{matrix}A\\A'\end{matrix}$	π	Es	log P	$(\log P)^2$	ΔIC	$(\Delta IC)^2$	π^2	pED_{50} (M) (Cal)	pED_{50} (M) (obs.)	ΔpED_{50}
CL-17		3.87	-2.30	1.12	1.25	0.52	0.27	14.97	5.49	5.63	-0.13
*CL-1		2.55	-2.36	1.40	2.19	0.58	0.32	6.50	5.21	4.99	-0.22
*CL-2		5.07	-2.10	1.41	1.99	0.55	0.30	25.70	5.04	4.96	-0.08
*CL-3		1.78	-2.10	1.31	1.87	0.59	0.35	3.16	5.25	4.94	-0.31
*CL-4	$-N\begin{matrix}CH_3\\CH_3\end{matrix}$	2.86	-1.68	1.52	2.37	0.59	0.35	8.16	5.07	5.33	+0.25
*CL-5	$-N\begin{matrix}C_2H_5\\C_2H_5\end{matrix}$	4.76	-2.62	1.60	2.56	0.54	0.29	22.66	5.17	5.22	+0.05

*CL-1 to *CL-5 compound prepared in our Laboratory by Das et al (29) have been taken for regression analysis.

Table 3.11

Regression Equations for *E. coli*

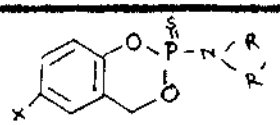
	n	r	s	F_{cal}	$F_{tab(0.05)}$	
$pED_{50} = 0.012\ \pi + 5.264$ (±0.056) (±0.217)	12	0.07	0.92	1,10=0.04	5.0	...(1)
$= -0.449 \log P + 5.95$ (±0.446) (±0.590)	12	0.30	0.30	1,10=1.01	5.0	...(2)
$= 0.219\ \Sigma IC + 5.237$ (± 0.450) (±0.281)	12	0.15	0.31	1,10=0.23	5.0	...(3)
$= -0.125\ Es + 4.933$ (±0.079) (±0.247)	12	0.45	0.82	1,10=2.52	5.0	...(4)
$= -\pi + 0.003\ \pi^2 + 5.307$ (±0.178) (±0.020) (±0.450)	12	0.14	0.32	2,9=0.10	4.3	...(5)
$= 6.626 \log P - 2.763 (\log P)^2 + 1.577$ (±3.904) (±1.517) (±2.460)	12	0.58	0.27	2,9=2,98	4.3	...(6)
$= -7.725\ \Sigma IC + 3.284\ (\Sigma IC)^2 + 8.53$ (±28.318) (±26.696) (±7.520)	12	0.60	0.25	2,9=2.57	4.3	...(7)
$= -0.167\ \pi - 8.318\ \Sigma IC + 10.464$ (±0.070) (±2.260) (±1.431)	12	0.78	0.19	2,9=6.98	4.3	...(8)
$= -0.013 \log P - 4.217\ \Sigma IC + 7.626$ (±0.452) (±2.155) (±1.00)	12	0.60	0.26	2,9=2.56	4.3	...(9)
$= -0.179\ Es - 0.169 \log P + 5.049$ (±0.084) (±0.407) (±0.666)	12	0.62	0.26	2,9=2.90	4.3	...(10)
$= -0.171\ \pi - 1.204\ \Sigma IC - 6.825(\Sigma IC)^2 + 8.633$ (±0.759) (±23.613) (±22.550) (±6.231)	12	0.78	0.20	3,8=4.21	4.1	...(11)
$= -4.219 \log P - 1.705(\log P)^2 - 2.916\ \Sigma IC + 4.4092$ (±4.373) (±1.752) (±2.541) (±3.454)	12	0.65	0.23	3,8=2.01	4.1	...(12)
$= -0.152\ Es - 0.691 \log P + 0.188(\log P)^2 + 5.504$ (±0.087) (±0.635) (±0.176) (±0.787)	12	0.68	0.25	3,8=2.34	4.1	...(13)

FURTHER STUDIES OF SOME 6-NITRO/BROMO SALICYLIDEN CYCLIC PHOSPHORAMIDOTHIOATES HAVING ANTIFUNGAL ACTIVITIES

FURTHER STUDIES ON SOME NITRO/BROMO SALIGENIN CYCLIC PHOSPHORAMIDOTHIONATES HAVING PESTICIDAL ACTIVITIES

In our laboratory some nitro/bromo saligenin cyclic phosphoramidothionates have been prepared and structure of these compounds have been elucidated by Das et al (unpublished). I have studied some biological properties and chemical hydrolysis of these compounds such as oral insecticidal activity on cockroaches (P. americana), acute oral toxicity on rats, anticholinesterase activity in goat whole blood and blow-fly head homogenate, fungicidal activity on Pyricularis oryzae and chemical hydrolysis in 0.0095(M) NaOH in 50% ethanol (pH 11.85) at 30°c.

The physical properties of some 6-nitro/bromo saligenin cyclic phosphoramidothionate are given below.

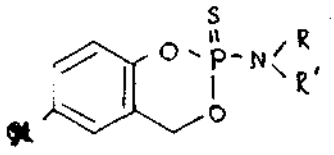


Compound Code No.	X	$-N\begin{matrix}R\\R'\end{matrix}$	Yield (%)	Mol.Wt.	M.P.(°C)
ED-25	NO_2	Diisobutylamido	80	258	136
ED-27	NO_2	Dipentylamido	60	386	Liquid
ED-29	NO_2	Dibutylamido	80	358	101
BR-6	Br	Diisobutylamido	90	392	90
BR-8	Br	Dipentylamido	75	420	Liquid
BR-10	Br	Dibutylamido	70	392	Liquid.

CHEMICAL HYDROLYSIS

The chemical hydrolysis in 0.0095(M) NaOH 50% ethanol-water (pH 11.85) of these compounds were studied according to the method described in section 3.5. The results of average hydrolysis constant of these compounds are given in table 3.12.

Table 3.12



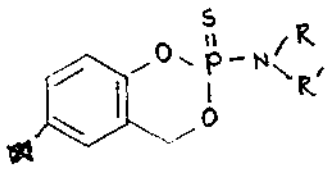
Compound Code No.	X	$-N\begin{matrix}R\\R'\end{matrix}$	Average $K_{hyd}(min^{-1})$
EL-25	NO_2	Diisobutylamido	3.54×10^{-5}
ED-27	NO_2	Dipentylamido	28.02×10^{-5}
EL-29	NO_2	Dibutylamido	3.88×10^{-5}
BR-6	Br	Diisobutylamido	1.48×10^{-4}
BR-8	Br	Dipentylamido	1.95×10^{-5}
BR-10	Br	Dibutylamido	1.72×10^{-5}

The average hydrolysis constants recorded in table 3.12 show that nitro/bromo saligenin cyclic phosphoramidothionate are stable towards alkaline hydrolysis (pH 11.85).

FUNGICIDAL ACTIVITY

Antifungal activities of these compounds against Pyricularia oryzae have been studied according to the method described in section 3.6. The ED_{50} (μg/ml) and ED_{95} (μg/ml) values of fungicidal activity at 72 h have been summarised in Table 3.13.

Table 3.13



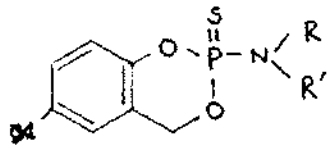
Compound Code No.	X	$-N\begin{matrix}R\\R'\end{matrix}$	ED_{50} (μg/ml) at 72 hrs.	ED_{95} (μg/ml) at 72 hrs.
BR-25	NO_2	Diisobutylamido	12.60	85.94
BR-29	NO_2	Dipentylamido	12.65	78.24
BR-6	Br	Diisobutylamido	0.41	2.31
BR-8	Br	Dipentylamido	0.61	3.85
BR-10	Br	Dibutylamido	1.21	7.70

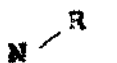
The data presented in table 3.13 show that the bromo saligenin cyclic phosphoramidothionates (BR-6, BR-8 and BR-10) have good antifungal activity at 72 hrs compared with that of nitro saligenin cyclic phosphoramidothionates.

ANTICHOLINESTERASE ACTIVITY

The anticholinesterase activity of some phosphoramidothioates against blow-fly head homogenate and goat whole blood has been measured by the method described in section 3.3 and the results of anticholinesterase activity have been summarized in table 3.14

Table 3.14



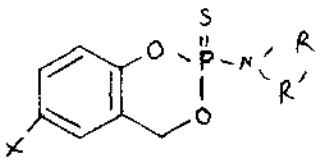
Compound Code No.	X		I_{50}(M) (BPAChE) blow-fly	I_{50}(M) (AChE) goat whole blood
ED-25	NO_2	Diisobutylamido	2.76×10^{-5}	7.20×10^{-5}
ED-27	NO_2	Dipentylamido	2.0×10^{-5}	11.34×10^{-5}
ED-29	NO_2	Dibutylamido	9.48×10^{-5}	4.16×10^{-5}
BR-6	Br	Diisobutylamido	9.34×10^{-3}	20.93×10^{-3}
BR-8	Br	Dipentylamido	2.34×10^{-3}	14.02×10^{-3}
BR-10	Br	Dibutylamido	1.14×10^{-3}	1.22×10^{-3}

The data presented in table 3.14 reveal that the ED-25, ED-27 and ED-29 compound are good inhibitor of acetylcholinesterase (both in blow-fly and goat whole blood) compared with that of BR-6, BR-8 and BR-10 compound.

ACUTE ORAL TOXICITY ON RATS

The acute oral toxicity on male white albino rats was performed according to the method described in section and the results of acute oral toxicity have been listed in table 3.15

Table 3.15



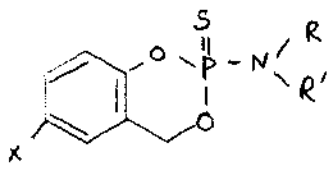
Compound Code No.	X	$-N\begin{matrix}R\\R'\end{matrix}$	LD_{50} (mg/kg) male rats
BD-25	NO_2	Diisobutylamide	250
BD-27	NO_2	Dipentylamide	250
BD-29	NO_2	Dibutylamide	250
BR-6	Br	Diisobutylamide	250
BR-8	Br	Dipentylamide	250
BR-10	Br	Dibutylamide	250

That Data prescribed in table 3.15 show that all phosphoramidothionates have less acute ovral toxicity on rats.

INSECTICIDAL ACTIVITY

The insecticidal activity tests were performed on the American cockroaches, *P. americana* (Linn), according to the method described in section 3.1.1 and the results of the insecticidal activity have been listed in table 3.16.

Table 3.16

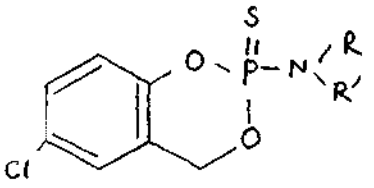


Compound Code No.	X	$-N\begin{matrix}R\\R'\end{matrix}$	Conc. showing 100% mortality (LC_{100} µg/g)
BL-25	NO_2	Diisobutylamide	75
BL-27	NO_2	Dipentylamide	75
BL-29	NO_2	Dibutylamide	75
BR-6	Br	Diisobutylamide	100
BR-8	Br	Dipentylamide	100
BR-10	Br	Dibutylamide	100

The data presented in table 3.16 reveal that all phosphoramidothionates have less oral insecticidal activity on cockroaches (*P. americana*).

GENERAL CONCLUSIONS AND REMARKS

(1) The following 2-alkylamido-6-chloro-4H-1, 3, 2-benzodioxaphosphorin 2-sulphides have been synthesized.



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

= Dipropylamido

= Dibutylamido

= 2,6-Dimethylmorphaline

= 2-Ethylpiperidino

= 4-Benzylpiperidino

= Hexamethylenimido

and examined for oral insecticidal activity on cockroach (*P. americana*) and blow-fly (*Chrysomya megacephala*), acute oral toxicity on rats, anticholinesterase activity in goat whole blood and in blow-fly head homogenate, phytotoxicity on rice seed (Pusa 2-21 variety), chemical hydrolysis in 0.0095(M) NaOH in 50 per cent ethanol (pH 11.85) and fungicidal activity on *Pyricularia oryzae*.

The 6-chloro saligenin cyclic phosphoramidothionates have been prepared by the reaction of the appropriate alkylamidophosphorodichloridothionate with 5-chloro saligenin at

low temperature (0-5°C) in presence of K_2CO_3 as dehydrogen chloride agent.

(ii) All the compounds show common IR bands : 1000-1020 cm^{-1}(s) P-O-C (alkyl); 1235-1260 cm^{-1}(s) and 880-910 cm^{-1}(s), P-O-C(aryl); 800-830 cm^{-1}, P=O(I); 630-670 cm^{-1}, P=O(II).

(iii) In the mass spectra all the compounds show molecular ion peak (M^+) and $(M+2)^+$ ion peak. The $(M+2)^+$ ion peak is approximately one third in intensity of the parent molecular ion peak (M^+). In all cases fragmentation by loss of CH radical from the molecular ions are observed.

(iv) The ^1H NMR spectra of all compounds have signals at δ=4.75-5.75 ppm for the $-CH_2-$protons in the dioxaphosphorin ring and for all the compounds the signal is a eight line multiplet.

From the ^{13}C NMR spectral study of the CL-6, CL-12, CL-14 and CL-17 it has been observed that the coupling (due to ^{31}P) to the CH_2 carbon(C_4) in the dioxaphosphorin ring changes only from 5.18 Hz in CL-12, 5.30 Hz in CL-14, 5.33 Hz in CL-6 and 5.50Hz in CL-17. This probably means that the conformation is almost the same, and this is in accord with the small difference in ^{13}C chemical shifts, 66.45 ppm, 66.47 ppm, 66.35 ppm and 66.20 ppm respectively.

From ^{31}P NMR spectral studies it is fairly evident that the compounds are stable in one conformation.

Further studies including X-ray crystal structure determination are in progress.

(v) 6-Chloro saligenin cyclic phosphoramidothionates are almost non-insecticidal to corkroaches and to blow-flies.

(vi) All compounds are less toxic to rats than salithion.

(vii) All compounds show very poor anticholinesterase activity in blow-fly head homogenate and in goat whole blood.

(viii) None of the compounds is phytotoxic to rice seed (Pusa 2-21 variety) upto the concentration 500 ppm (the highest concentration used).

(ix) From the chemical hydrolysis it has been observed that 6-chloro saligenin cyclic phosphoramidothionates are stable to alkaline hydrolysis (pH 11.85).

(x) The antifungal studies (by growth inhibition) against P. oryzae indicate that 6-chloro saligenin cyclic phosphoramidothionates show very good inhibitory effect on the growth of P. oryzae compared with Hinosan they have greater inhibitory effect. Of all the compounds, 2-(2,6 Dimethylmorpholino-6-Chloro-4H-1,3,2-benzodioxaphosphorin 2-sulphide are most effective against P. oryzae

(xi) Quantitative Structure Activity Relationship shows that a good correlation is obtained between the pED_{50} value (for P. oryzae at 72 hrs) and Structural Information Content (SIC)

and hydrophobic constant (π). The equation is

$$pI_{50} = -0.167\,\pi - 0.318\,MIC + 10.464$$
$$(\pm\ 0.070)\quad (\pm\ 2.200)\quad (\pm\ 1.431)$$

n=12, r=0.78, s = 0.19, $F_{2,9}(Cal) = 6.98$

$F_{tab}(0.05) = 4.3$

From this equation it is clear that the stereo-hydrophobic make up and topology of the bioactive molecule are major determinants of the bioresponse.

(xii) The fungicidal activity and other data justify further examination of these chlorosaligenin cyclic phosphoramidothionates as potential fungicides.

(xiii) From the further studies of biological properties and chemical hydrolysis on some 6-nitro/bromo saligenin cyclic phosphoramidothionates it has been observed that :

(a) all the compounds have less oral insecticidal activity on cockroaches and acute oral toxicity on rats.

(b) the anticholinesterase activity of 6-nitro saligenin cyclic phosphoramidothionates in blow-fly head homogenate and goat whole blood are greater than that of 6-bromo saligenin cyclic phosphoramidothionates.

(c) the 6-bromosaligenin cyclic phosphoramidothionates have good antifungal activity at 72 hrs against *P.oryzae* compared with that of 6-nitro saligenin cyclic phosphoramidothionates.

(d) all the compounds are stable towards alkaline hydrolysis at pH 11.65.